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Presence of osteoinductive factors in bovine colostrum

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New approaches in the treatment of skeletal defects may benefit from the use of soluble biological factors. We previously standardized a derivative of bovine colostrum (SBCD), deprived of casein and fat and rich in cytokines. In the present study, we tested its possible use as an adjuvant in bone healing. SBCD contained factors involved in stromal cell stimulation and differentiation and induced cytokine production from stimulated mesenchymal stem cells (MSCs). *In vitro*, SBCD promoted proliferation, migration and, in association with osteogenic factors, osteogenic differentiation of osteoblastic and MSCs. In *in vivo* experiments of subcutaneous Matrigel injection in mice, SBCD plus hydroxyapatite, but not hydroxyapatite nor SBCD alone, induced recruitment of macrophages and stromal cells. After 60 days, plugs containing SBCD and hydroxyapatite were densely calcified and diffusely positive for osteocalcin, supporting the occurrence of an early osteogenic process. These results indicate that SBCD is a rich source of factors with osteoinductive properties.

Key words: osteogenesis; colostrum; mesenchymal stem cells; hydroxyapatite

Replacement and regeneration of bone tissue are major topics of interest in several disciplines, traumatology, orthopaedics, neurosurgery, and dentistry being, among others, potentially involved. Standard treatment of bone defects entails grafting bone, which is the second most commonly transplanted tissue after blood, with over 2.2 million bone grafting procedures performed annually worldwide.[1)] Autologous bone grafting is considered the gold standard for reconstruction of bony skeletal defects and for stimulation of bone regeneration. This procedure presents no risk of disease transmission or graft rejection and has the advantage to potentially transfer live osteocytes and osteoblasts to begin new bone formation.[2)] However, it may generate a wound at the harvest site, with possible consequences in terms of extending the surgical time and increasing patient morbidity[3)] and it is limited by the quantity of material available.[4)] Allografts may instead cause immune reactions, have the potential risk of transmitting infectious diseases, and their sterilization procedures may diminish the biomechanical quality of the material.[1,5)] In order to avoid these disadvantages, a broad spectrum of synthetic biomaterials serving as synthetic bone substitutes has been developed during the last years. Especially, calcium phosphate ceramics, such as hydroxyapatite, are increasingly introduced in clinical practice in virtue of their good biocompatibility and bioactivity.[6)]

No matter which is the source of hard, bone-inducing material, an essential role in the process of bone formation is going to be played by stimulating factors, either endogenous or exogenous, favoring proliferation and differentiation of stromal cells. This has spurred extensive research into the potential of growth factors, which have been shown to have the ability to stimulate the differentiation of mesenchymal stem cells (MSCs) into osteogenic cells (osteoinduction). Biofactors have recently been tested for the clinical treatment of musculoskeletal diseases and open fractures with loss of tissue, opening up the considerable potential offered by tissue engineering to orthopaedic and maxillofacial surgeons.[7,8)]

A large number of growth factors are known to be present in human and bovine milk[9)] and particularly in the first milk produced after birth, i.e. colostrum. Non-peptide hormones, peptide hormones, and growth factors have been isolated and quantified in colostrum.[10)] However, the variable source of the product and the lack of standardized composition have so far prevented a reproducible use of colostrum as a natural source of growth factors. We recently generated and characterized

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Abbreviations: MSCs, mesenchymal stem cells; SBCD, standardized bovine colostrum derivative; FGF-b, fibroblast growth factor; TGF, transforming growth factor; BMP-2, bone morphogenic protein-2; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; ASCs, Adipose tissue-derived stem cells; FBS, fetal bovine serum; MTT, 3-(4,5-dimethylthiazole-2-yl)-2.5-diphenyl tetrazolium bromide; ANOVA, analysis of variance; IL, Interleukin.

a lyophilized and standardized bovine colostrum derivative (SBCD), deprived of casein and fat.[11] SBCD contained several bioactive factors, being basic fibroblast growth factor (FGF-b), transforming growth factor (TGF), tumor necrosis factor (TNF), and vascular endothelial growth factor (VEGF), the most represented among growth factors.

In the present study, we evaluated the potential use of SBCD as a possible adjuvant in bone healing. The *in vitro* capacity to stimulate proliferation and migration of MSCs and osteoblasts was assessed, along with experiments on an *in vivo* model of ectopic osteogenesis.

Materials and methods

Colostrum. SBCD, a standardized lyophilized product of bovine colostrum, deprived of lipids and casein, was obtained from Biofer S.p.A. (Medolla, Italy). Preparation and characterization of SBCD, as well as concentration of specific cytokines and of a vast number of growth factors was reported elsewhere.[11]

Cell culturing. The effects of the SBCD on cell proliferation and differentiation were evaluated *in vitro* by using human osteoblastic cell lines (Saos-2, MG-63), murine bone marrow-derived MSCs (D1 ORL UVA), and primary cells obtained from lipoaspirates (human adipose tissue-derived stem cells, henceforth ASC). ASCs were isolated from fat tissue obtained from three different donors as described previously[12] and maintained in 'control medium' consisting of Dulbecco's minimum essential medium enriched with sodium pyruvate and supplemented with 10% fetal bovine serum (FBS, Gibco BRL), 100 U/mL penicillin, 100 µg/mL streptomycin, and 250 ng/mL amphotericin B. The non-adherent cell population was removed after 48 h and the adherent cell layer was washed twice with fresh medium; cells were then continuously cultured since their harvest until sixth passage. D1 bone marrow-derived MSCs were purchased from ATCC (ATCC number: CRL-12424), as were human osteoblastic cell lines Saos-2 (ATCC number: HTB-85) and MG-63 (ATCC number: CRL-1427). D1 cells were kept in Dulbecco's minimum essential medium supplemented with 10% FBS (Gibco BRL), 100 U/mL penicillin, 100 µg/mL streptomycin. Saos-2 and MG-63 cells were, respectively, cultured in McCoy'5A (Gibco, Life Technologies) with 15% FBS, (Benchmark, Gemini Bio-Products) and in Dulbecco's Modified Eagle's Medium (Gibco, Life Technologies) with 10% FBS. Both media were supplemented with 1% penicillin–streptomicin (MD Biomedicals, Thermo Fisher Scientific). Cells were always passaged at subconfluency to prevent contact inhibition and were kept under a humidified atmosphere of 5% CO_2 in air, 37 °C.

Cell vitality assay (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide test. Cells were seeded in 96-well plates (10^3 cells/well) and incubated in suitable media in presence of 2% FBS, 2% FBS + 0.1 mg/mL SBCD, 2% FBS + 1 mg/mL SBCD, 2% FBS + 2.5 mg/mL SBCD, 2% FBS + 5 mg/mL SBCD and 10% FBS, for 1, 3, 5, and 7 days. To evaluate the mitochondrial activity of the seeded cells, i.e. the cell viability at different SBCD concentrations, a test with 3-(4,5-dimethylthiazole-2-yl)-2.5-diphenyl tetrazolium bromide (MTT) (Chemicon International, Billerica, MA, USA) was performed on days 1, 3, 5, and 7 as previously reported.[13] Samples were measured at 570 nm by a microplate reader (BioRad Laboratories, Hercules, CA, USA).

In vitro wound healing scratch plate assay. The *in vitro* wound healing assay was performed according to established protocols.[14] Briefly, cells were seeded in six-well plates and allowed to grow in their media to approximately 90% confluence. *In vitro* wounds were created by drawing lines down the well with a 20 µL plastic pipette. After washing with PBS, cells were maintained in 5 mg/mL SBCD, 10% FBS, and 0.5% FBS (control condition). The digital images were captured after 6 and 12 h of incubation and the area occupied by the cells within the scratch was measured (NIH Image J version 1.42).

Cytokine and growth factors. The presence of specific growth factors in SBCD as well as in ASCs and Saos-2 cells after stimulation with SBCD was evaluated. Cells were seeded at the concentration of 10^4 per well in 96-well plates in control medium. The following day, after washings, cells were incubated for 3 h with RPMI or RPMI containing 5 mg/mL SBCD. After the stimulus, cells were washed and incubated with RPMI alone for further 4 h. Cell media or resuspended SBCD were collected and analyzed using the flexible Bio-Plex system (Bio-Rad Laboratories, Hercules, CA, USA), which is based on a capture sandwich immunoassay, for the simultaneous dosage of different biomolecules. At least three independent repetitions in duplicate were made per experimental condition type. Analyte concentrations were expressed in pg/mL. A standard curve ranging on average from 0.15 to 3700 pg/mL (High Photomultiplier Tube Setting—PMT setting) was prepared and then fitted by Bio-Plex Manager software. The presence of bone morphogenic protein-2 (BMP-2) was evaluated using an ELISA kit (R&D System, Abingdon, UK), following the manufacturer's instructions.

In vitro osteogenic differentiation tests. Cells were seeded in 96-well plates (10^3 cells/well) and incubated in DMEM in the presence of 2% FBS, 2% FBS + 5 mg/mL SBCD, 2% FBS + osteogenic factors (OM) (50 µM Ascorbic Acid, 10 mm Beta Glycerophosphate, and 100 nM Dexamethasone), and 5 mg/mL SBCD + OM (50 µM Ascorbic Acid, 10 mM Beta Glycerophosphate, and 100 nM Dexamethasone) for 1, 3, 5, 7, and 14 or alternatively 21 days. *In vitro* osteogenic differentiation was determined by a series of assays aiming at revealing established bone markers.

(1) Alkaline Phosphatase activity assay. Alkaline Phosphatase activity was determined using a colorimetric end point assay,[15] which measures the conversion of the colorless substrate p-nitrophenol phosphate by the enzyme alkaline phosphatase to the yellow product p-nitrophenol. To measure alkaline phosphatase activity, cells were lysed with 0.05% Triton X-100 and incubated with the reagent solution containing phosphatase substrate (Sigma-Aldrich, Milan, Italy) at 37 °C for 15 min. The rate of color change corresponds to the amount of enzyme present in solution. Optical density was measured at a wavelength of 405 nm (reference 620 nm). Samples were compared against the calibration curve of p-nitrophenol standards. The final alkaline phosphatase concentration was adjusted per total protein content, to avoid biases due to the cell number. Therefore, part of the cell lysates obtained for ALP quantification was incubated with BCA™ (Thermo Fisher Scientific, Waltham, MA, USA) Protein Assay, following to the manufacturer's instructions. Optical density was measured at a wavelength of 570 nm and results were adjusted to a calibration curve made by known number of cells. Alkaline phosphatase values were determined and normalized on whole protein content at day 3 in Saos-2, MG-63, D1 and at day 7 in ASCs.

(2) Calcium content assay. Cell calcium content was determined at day 14 for Saos-2 and D1 or 21 for MG-63 and ASC by Calcium colorimetric assay kit (BioVision Research Products, Mountain View, CA, USA), according to the manufacturer's protocol. The OD was measured at 575 nm within 20 min since preparation. A calibration curve was always made.

(3) Osteocalcin detection in cell-conditioned media. Human osteocalcin was measured in human cell-conditioned media by Osteocalcin Elisa kit (KAQ1381 Invitrogen Corporation, Camarillo, CA, USA) at day 14 for SaOs-2 and at day 21 for MG-63 and ASCs, following the manufacturer's protocol.

(4) Collagen and calcium staining. At the established time points, cells grown in six-plate wells were washed once with PBS and fixed with 4% paraformaldehyde for 10 min at room temperature. The solution was removed and cells were washed with PBS. To stain collagen, Sirius red dye (Direct Red 80, Sigma Aldrich) dissolved (1 mg/mL) in a saturated aqueous solution of picric acid (Sigma Aldrich) was added to the fixed cell cultures. After being kept under mild shaking for 2 h, samples were quickly rinsed in acid water (0.5% acetic acid in pure water) and then abundantly washed with distilled water. Calcium salts were stained after von Kossa following published protocols.[16] For both picro-Sirius Red and von Kossa stains, the cultures were observed under light microscopy and representative pictures were captured by an Olympus camera.

In vivo osteogenesis model in Matrigel plugs plus hydroxyapatite. Animal experiments were performed according to the guidelines for the care and use of research animals and were approved by the local Ethics Committee. Five-week-old male/female Balb-C mice ($n = 35$) were enrolled in this study. Matrigel (8.13 mg/mL, BD, Buccinasco Milan, Italy), in liquid form at 4 °C, was mixed with heparin (64 U/mL, Sigma Aldrich) and SBCD (0.1 mg/mL), in the presence or absence of an injectable nanocrystalline paste (0.3% Ostim®, Osartis, Obernburg, Germany, final concentration 0.6%) and injected (0.4 mL) into the abdominal subcutaneous tissue of mice, along the peritoneal midline, as previously described.[17] Mice were killed at different times (10, 20, or 60 d) and subcutaneous plug samples were formalin-fixed and processed for light microscopy and immunohistochemistry. Briefly, sections from paraffin-embedded blocks were collected onto poly-L-lysine-coated slides and processed with histological and histochemical staining reactions (hematoxylin and eosin, Masson's trichromic and van Gieson stain and von Kossa reaction) using the Artisanlink Apparatus (DAKO). For immunohistochemistry, the following Abs were used: anti-osteocalcin polyclonal Ab (Takara Bio Inc., Shiga, Japan; 1:250 dilution) and anti-collagen IV polyclonal Ab (dilution: 1:500), and anti-F4/80 rat mAb (dilution 1:100) (all from Cambridge Science Park, Cambridge, UK). Endogenous peroxidase activity was blocked with 6% H_2O_2 for 8 min at room temperature. Primary antibodies were applied to slides overnight or for 1 h at 4 °C. Horseradish peroxidase-labeled anti-rabbit Envision polymers (Dako) or anti-rat IgG (Abcam, Cambridge, MA, USA) were incubated for 1 h. The reaction product was developed using 3,3-diaminobenzidine. Omission of the primary antibody or substitution with an unrelated rabbit serum or mouse IgG served as negative control.

Statistical analysis. Data from MTT assays and *in vitro* osteogenic differentiation tests (with the exception of the picro-Sirius Red and von Kossa stains) were all analyzed by GraphPad Prism 5 (GraphPad Software Inc, La Jolla, CA, USA). Each experiment consisting of eight samples per condition was repeated at least three times, for each cell line. As for the primary cells, the experiments were run three times independently with cells from each one of three different donors (thus reaching a total of nine tests for each assay where ASC were used). Statistical analysis was performed by using the one-way analysis of variance (ANOVA) with *post hoc* Dunnett's test or the Student *t* test, as appropriate. A p value of <0.05 was considered significant.

Results

Presence of MSC-stimulating factors in the SBCD

We previously reported that SBCD contains a great number of cytokines and growth factors, possibly involved in MSC proliferation, migration, and differentiation,[11] including FGF-b, TGF, TNF, and VEGF. We here tested whether SBCD also contained the osteogenic protein BMP-2.[18] We found that BMP-2 was present at 53 ± 13 pg/mg SBCD. In addition, after a 3 h

incubation, SBCD induced the release of several cytokines in ASCs, as depicted in Fig. 1. Interleukin (IL)-6, IL-8, monocyte chemotactic protein-1 (MCP-1), and VEGF were increased by SBCD in ASCs as well as in SaOs-2 (not shown), in respect to unstimulated cells or to cells stimulated with 2% FBS (Fig. 1).

Effect of SBCD on proliferation and migration of osteoblasts and MSC

We subsequently evaluate the functional effect of SBCD on two osteoblastic cell lines (Saos-2 and MG-63) and on MSC from bone marrow (D1) and adipose tissue (ASCs) in terms of proliferation and migration. In osteoblasts and MSC cultured in basal conditions, proliferation was increased dose-dependently by the supplementation of SBCD, as shown by MTT analysis (Fig. 2). At 2.5 and 5 mg/mL SBCD, cell proliferation resulted superior to that achieved in conventional maintenance conditions at 10% FBS for all the cell types, but MG-63. For this cell line, 5 mg/mL SBCD only induced a proliferation rate higher than 10% FBS (Fig. 2). In addition, 5 mg/mL SBCD promoted cell proliferation, as evaluated by wound assay (Fig. 3). The effect was comparable to that of 10% FBS (positive control) and significantly higher than the vehicle, with the only exception of SaOs-2 cells (Fig. 3).

In vitro osteogenesis in presence of SBCD

We next evaluated the osteogenic potential of SBCD per se and in association with OM. No differentiation and minimal cell survival were observed when cells were cultured in the presence of OM alone (Figs. 4(A) and 5(A) and (B)). SBCD (5 mg/mL) displayed a significant potential to induce osteogenesis when added to OM, but not alone. This osteogenic effect was comparable to that of 2% FBS plus OM. The osteogenic activity was detected by the early markers alkaline phosphatase activity (Fig. 4(A)) and picro-Sirius Red (Fig. 4(B)), the latter detecting collagen I expression. In addition, this effect was confirmed by the detection of the late osteogenic marker calcium deposition evaluated by calcium content (Fig. 5(A)), osteocalcin release within the culture medium (Fig. 5(B)) and von Kossa stain (Fig. 5(C)).

In vivo osteogenesis model in Matrigel plugs with hydroxyapatite

When SBCD (0.1 mg/mL) was added to Matrigel and injected subcutaneously in mice, a modest effect on cell recruitment was observed in respect to the control plugs treated with Matrigel alone ($n = 6$ for each group, Fig. 6(A) and (B)). In particular, after 10 days, few inflammatory cells and some endothelial cells, organized in vessels containing erythrocytes, infiltrated the Matrigel plug (Fig. 6(A) and (B)). In subsequent experiments, hydroxyapatite crystals (0.6%) were added to the Matrigel preparation and the effect of SBCD (0.1 mg/mL) addition was investigated at time intervals in plugs collected at 10, 20, and 60 days ($n = 4$ for each time). In plugs containing Matrigel and hydroxyapatite alone, only few macrophage-like cells were detected around crystals that maintained their original structure ($n = 3$ plugs for each time interval, Fig. 6(D)). On the contrary, in plugs supplemented with SBCD a marked inflammatory reaction could be detected (Fig. 6(C)). After 20 days, most cells were F4/80$^+$ macrophages and reactive giant cells surrounding the crystals (Fig. 6(E)). Spindle-shaped, fibroblast-like cells were diffusely present. Staining for osteocalcin was weakly positive in these cells, while a heavy deposit was detected in the stromal areas surrounding foci of calcium deposits

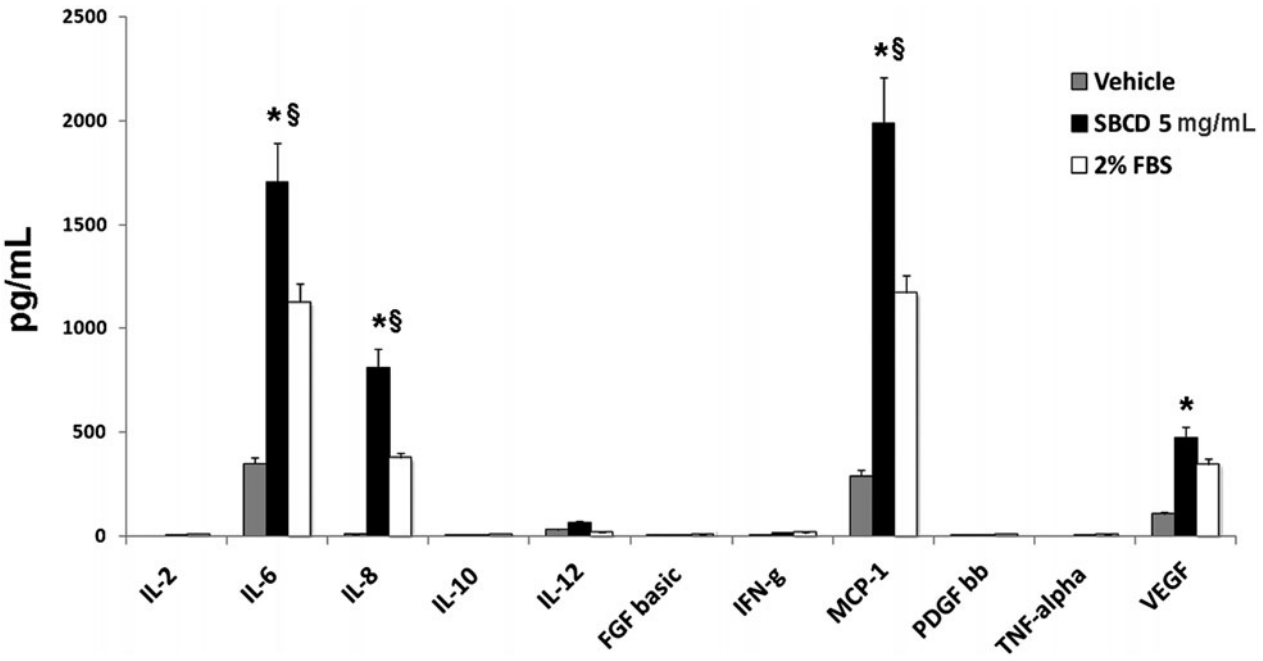

Fig. 1. Growth factor release after cell stimulation with SBCD.

Notes: Cytokines and growth factors present in the media of ASCs after 3 h incubation with SBCD (5 mg/mL) or with vehicle alone (RPMI) or with RPMI plus 2% FBS were detected by Bio-Plex system. Data are mean ± SD of three different experiments performed in triplicate. Three cell lines from different donors were used. Student's t test was performed: * = $p < 0.05$ vs. RPMI; § = $p < 0.05$ vs. 2% FBS.

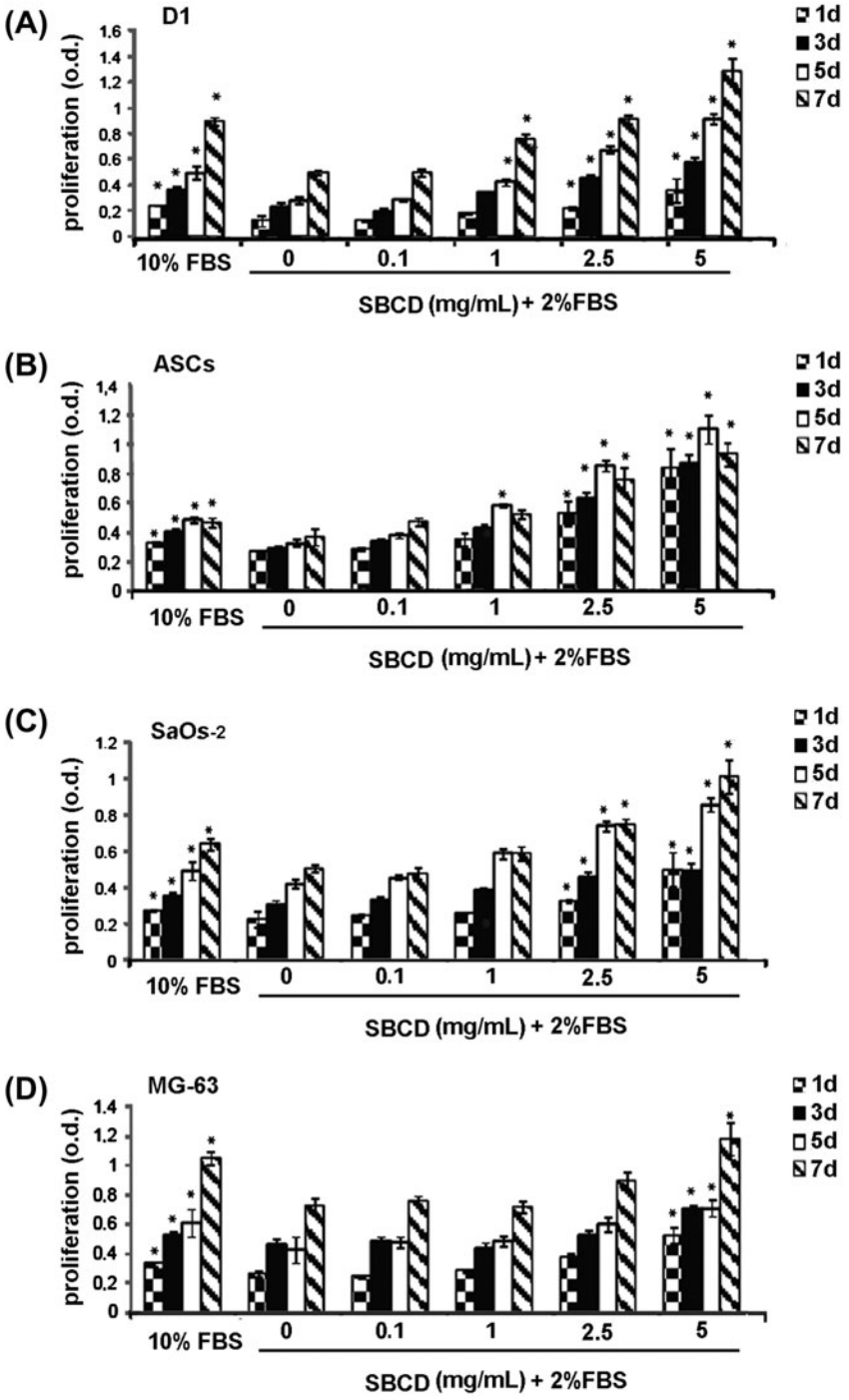

Fig. 2. Proliferative effect of SBCD.

Notes: MTT Assay shows the proliferative effect of SBCD elicited on different cell types (D1, ASCs, SaOs-2, and MG-63) at different concentrations (0.1, 1, 2.5, and 5 mg/mL) and time points (1, 3, 5, and 7 days), when added to media with low serum concentration. Standard 10% FBS medium was used as positive control. Data are mean ± SD of three different experiments performed in eight different tests. Three lines from different donors were used for ASCs. ANOVA with Dunnett's comparison test was performed: * = $p < 0.05$ vs. 2% FBS.

(Fig. 6(F)). At 60 days, the plugs appeared as a hard, subcutaneous plug. Sectioning, in cases requiring decalcification, revealed a poorly cellular stroma, with heavy and extensive von Kossa positive granular calcium deposits (Fig. 7(A) and (C)). They were surrounded by dense stroma, positive at Masson's trichromic and van Gieson staining procedures (Fig. 7(B)). Staining for osteocalcin was heavily and diffusely positive (Fig. 7(D)). Proper formation of lamellar bone could not be appreciated.

Discussion

Several factors and biological preparations have extensively been investigated in the search for bone induction and regeneration, a topic of high biological and clinical interest.[19] The present study demonstrates the osteogenic potential of a natural, extensively available, and fully standardized biological product. In fact, we give evidence of the ability of a standardized bovine colostrum derivative to induce *in vitro*

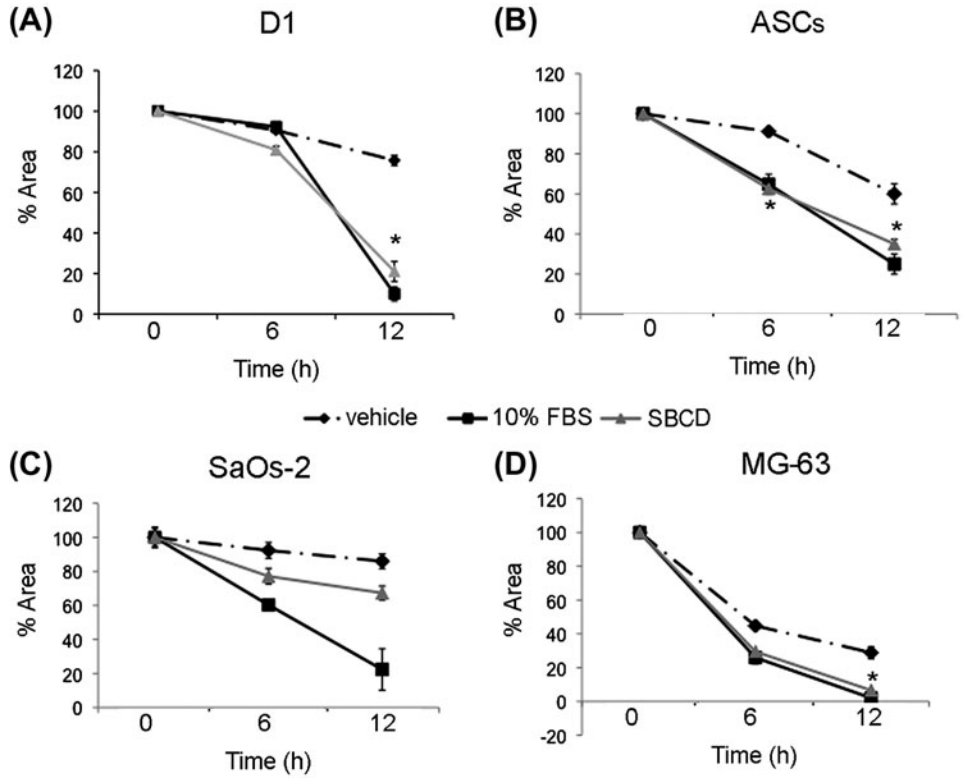

Fig. 3. Effect of SBCD on cell migration.

Notes: Wound healing scratch test was performed at 6 and 12 h on D1, ASCs, SaOs-2, and MG-63 cells. Data are mean ± SD of three different experiments performed in triplicate. Three lines from different donors were used for ASCs. ANOVA with Dunnett's comparison test was performed: * = $p < 0.05$ vs. vehicle.

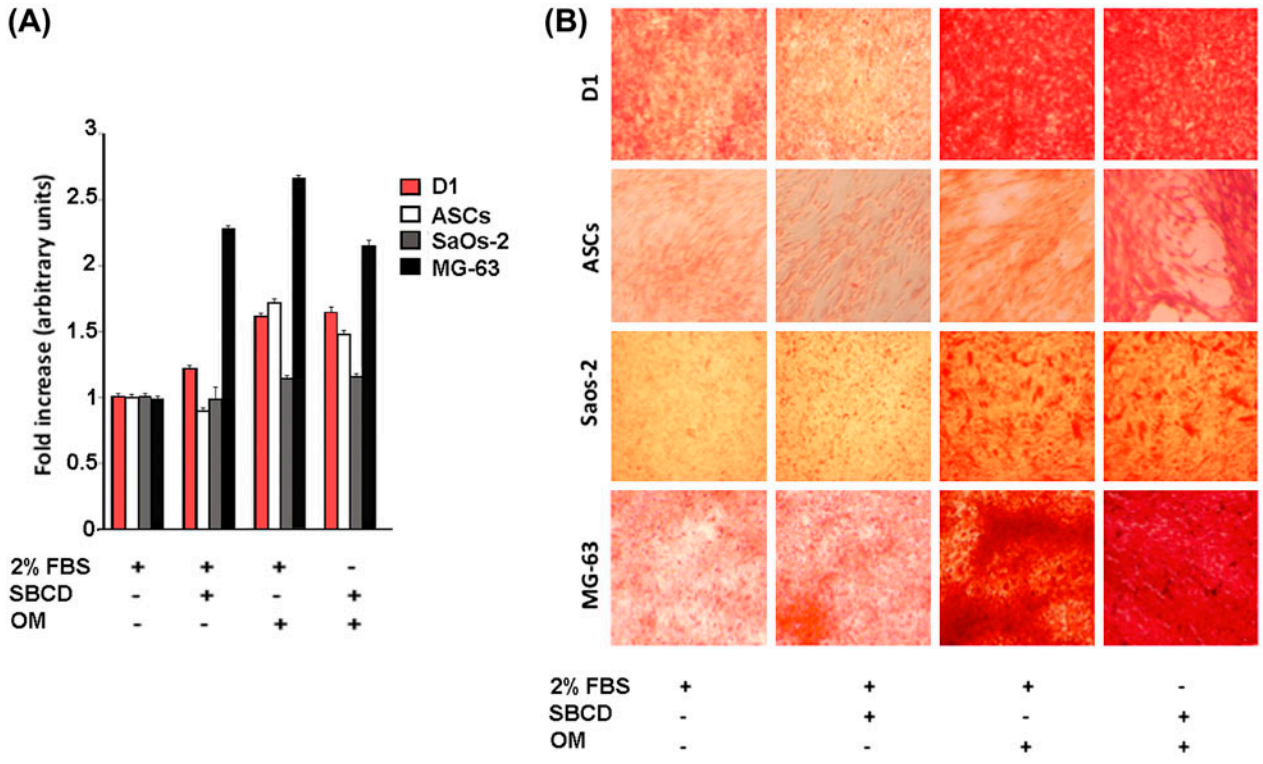

Fig. 4. Induction of early osteogenic markers by SBCD.

Notes: (A) Effect of SBCD and OM in low serum media (2% FBS) on alkaline phosphatase activity measured in D1, ASCs, SaOs-2, and MG-63 cells. Data are expressed as fold increase over control (2% FBS alone) and are mean ± SD of three different experiments performed in eight different samples. Three different cell lines from different donors were used for ASCs. Cells cultured for the same time in the presence of OM alone, omitting both 2% FBS and SBCD, did not survive. (B) Representative micrographs showing the effect of SBCD and OM in low serum media on the collagen deposition detected in D1, ASCs, SaOs-2, and MG-63 cells, as detected by Picro-Sirius Red stain. Original magnification: 100×.

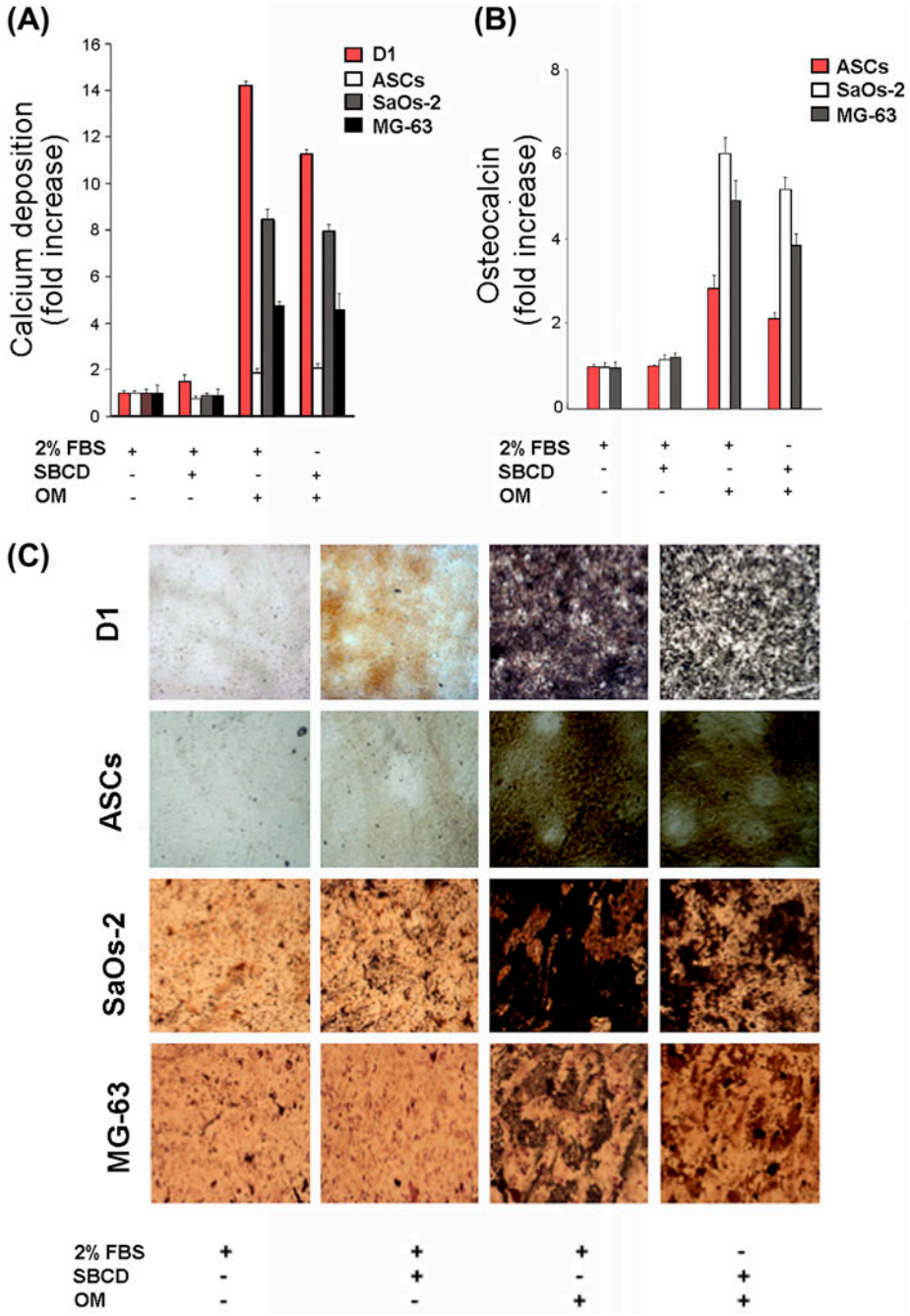

Fig. 5. Induction of late osteogenic markers by SBCD.

Notes: (A) Effect of SBCD and/or OM in low serum media (2% FBS) on calcium deposition as detected in D1, ASCs, SaOs-2, and MG-63 cells based on a quantitative colorimetric assay. Data are expressed as fold increase over control (2% FBS alone) normalized to one. (B) Effect of SBCD and OM in low serum media (2% FBS) on human osteocalcin secretion in ASCs, SaOs-2, and MG-63 cells. Data are expressed as fold increase over control (2% FBS alone) normalized to one. The mean ± SD of three different experiments performed in eight different samples. Three cell lines from different donors were used for ASCs. (C) Representative micrographs showing the effect of SBCD and OM in low serum media (2% FBS) on the calcium deposition as detected by Von Kossa stain, in D1, ASCs, SaOs-2, and MG-63 cells. Cells cultured for the same time in the presence of OM alone, omitting both 2% FBS and SBCD, did not survive. Original magnification: ×100.

proliferation and migration of mesenchymal osteoblast progenitors and to promote osteogenesis *in vitro* and *in vivo* in association with either soluble factors or synthetic bone substitute materials, respectively.

In respect to mature milk, bovine colostrum contains higher amounts of fats, proteins, peptides, fat-soluble vitamins, and various enzymes, hormones, growth factors, cytokines, minerals, and nucleotides. Except for lactose, the levels of these compounds rapidly decrease during the first 3 days of lactation to those typical for mature milk.[20,21] In this study, colostrum was harvested within the fifth hour, as in preliminary experiments this was found the most biologically active fraction.[11] Indeed, after the ultrafiltration and purification process and in fully stable conditions, SBCD contains a number of growth factors and cytokines, which are known to regulate proliferation, differentiation, adhesion, migration, and other functions in many cell types.[11] In addition, we here found that SBCD contained the osteogenic protein BMP-2, known to enhance the activity of osteoblasts and to induce bone formation.[18]

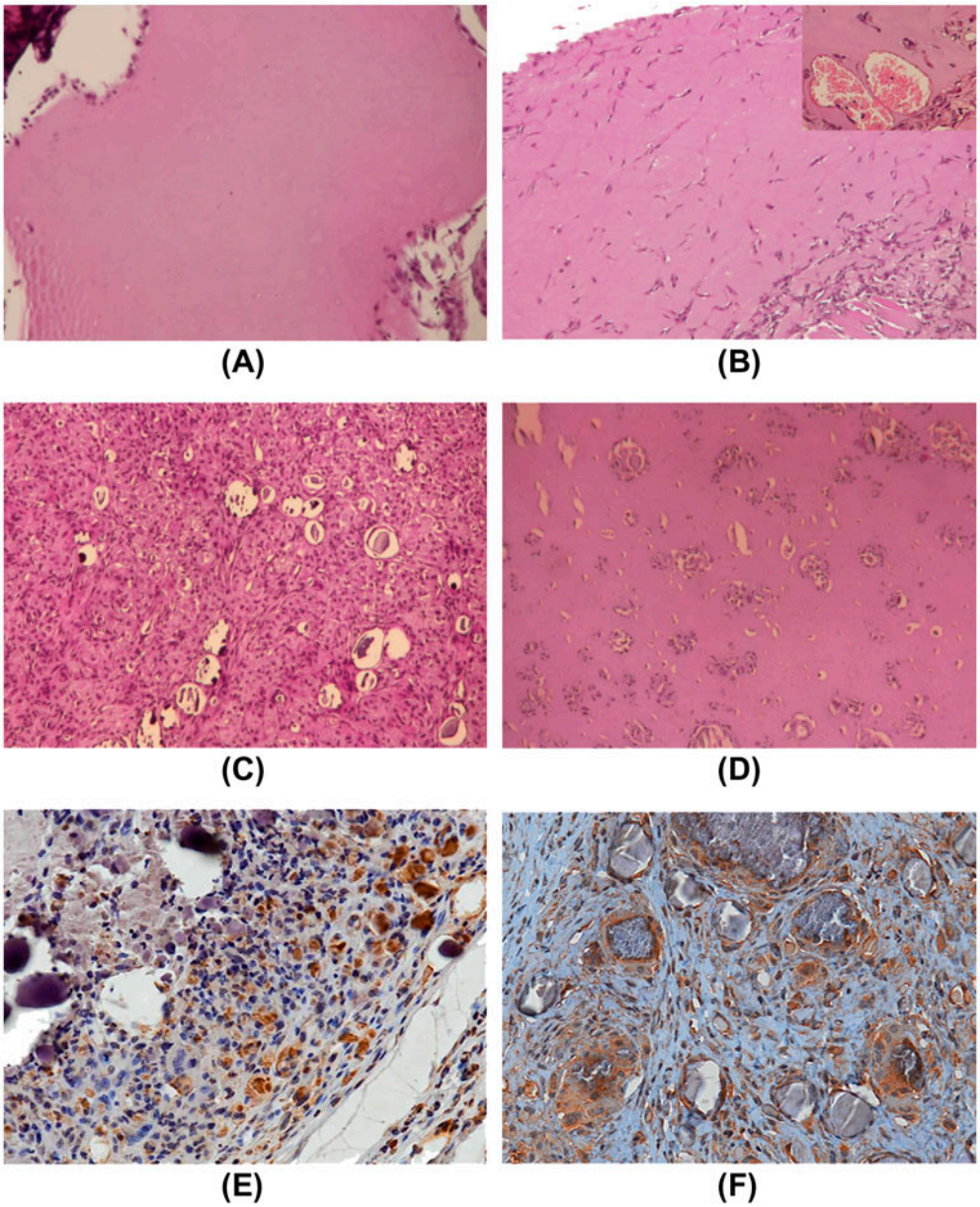

Fig. 6. *In vivo* osteogenic effect of SBCD in Matrigel plus hydroxyapatite at day 20.

Notes: Matrigel was mixed with 0.1 mg/mL SBCD and 0.6% hydroxyapatite and subcutaneously injected in mice. (A–B) Representative hematoxylin/eosin staining showing a modest effect of SBCD alone (B) as compared to vehicle (A). SBCD induced a slight cellular infiltrate (B), with some vessels (B, inset). (C–D) Representative hematoxylin/eosin staining showing a dense cellular infiltrate after 20 days in plugs containing SBCD plus hydroxyapatite (C) and not hydroxyapatite alone (D). (E–F) Representative micrographs showing the immunohistochemical staining of $F4/80^{+}$ macrophages surrounding the crystals (E) and osteocalcin deposition (F) in Matrigel plugs containing 0.1 mg/mL SBCD and 0.6% hydroxyapatite. Original magnification: A–D: ×100; E and F: ×200.

Recently, bovine colostrum was reported to display a wound healing feature that was specifically ascribed to the growth factor-induced activation of tyrosine kinase receptors supporting keratinocyte proliferation and migration.[22] We here reported the ability of an innovative colostrum derivative to promote proliferation and migration of osteoblasts and mesenchymal cells *in vitro*. Similar mitogenic and motogenic effects were observed in fibroblasts treated with mare's colostrum.[23] When added to conventional *in vitro* OM, in the absence of FBS, SBCD sustained the osteoblastic differentiation of mesenchymal cells. Interestingly, SBCD could promote *in vivo* osteogenesis, in the presence of hydroxyapatite, in an ectopic osteogenic model. Neither SBCD nor the hydroxyapatite was able to induce the formation of a properly calcified tissue, when injected separately into mice. Instead, when associated and co-injected, an extensive stromal calcification was observed, associated with presence of osteocalcin, an osteoblast-derived protein expressed only in fully differentiated osteoblasts.[24,25] The generation of a connective tissue within the plugs thus enriched was consistently observed at early times (20 d). The progression toward a calcified tissue was subsequently indicated by calcium deposition and staining for osteocalcin, which is known as a marker of terminal osteoblast differentiation corresponding with matrix deposition and mineralization.[25] In the basic experiments performed in the present study, heavy calcium and osteocalcin deposits in collagenous areas were

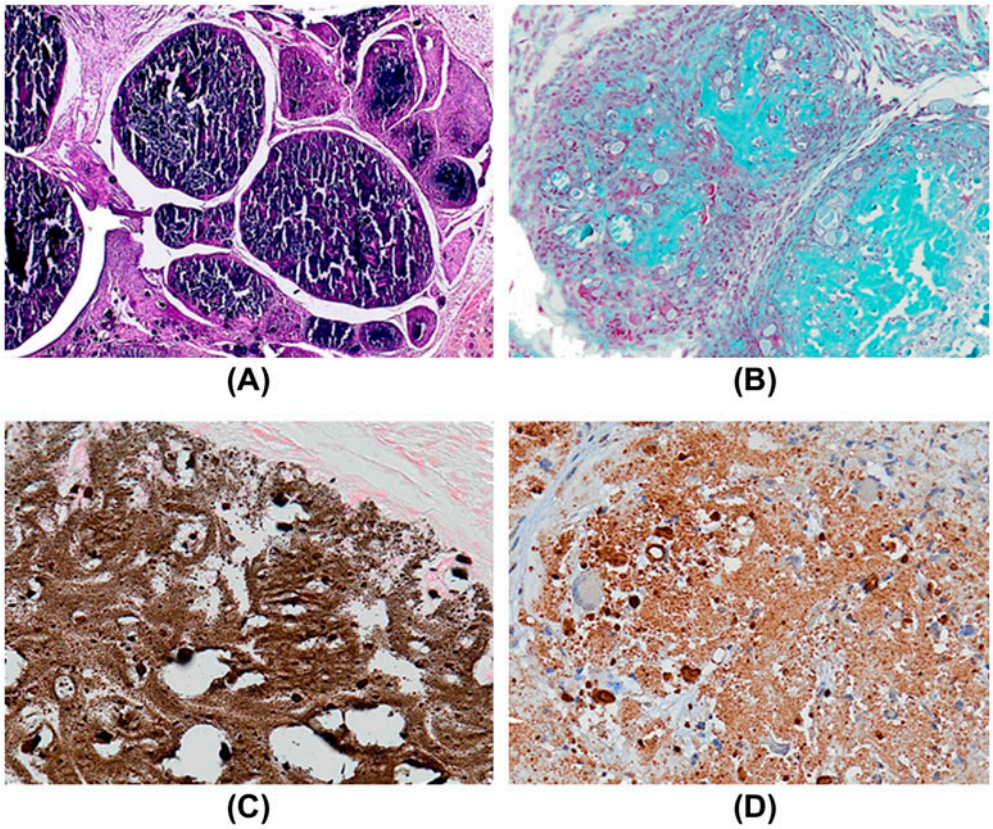

Fig. 7. *In vivo* osteogenic effect of SBCD in Matrigel plus hydroxyapatite at day 60.
Notes: Representative micrographs of Matrigel plugs containing 0.1 mg/mL SBCD and 0.6% hydroxyapatite 60 days after subcutaneous injection in mice. Large calcifications were detected (A, H/E staining), surrounded by stroma (B, Trichromic reaction). Calcium deposits, as shown by von Kossa stain (C) and presence of osteocalcin (D), indicate induction of an osteogenic process. Original magnification: ×40.

interpretable as woven bone formation. This is consistent with previous results showing osteocalcin detection in woven bone in a rabbit model of osteogenesis.[26] We were instead unable to reach evidence of end-stage osteogenesis with lamellar bone formation, possibly due to the absence of additional mechanical stimuli or relatively short time of observation.

The cooperative effect of SBCD with hydroxyapatite on osteogenesis appears as a conductive event in a specifically determined osteogenic microenvironment. In this context, SDBC appears to be mainly involved in mesenchymal precursors' recruitment, proliferation, and to promote differentiation only in with the presence of other osteogenic stimuli. Indeed, hydroxyapatite alone is not able to recruit cells as colostrum, but it is endowed with osteoinductive properties.[27,28] This is also in accordance with the observation that nanosized hydroxyapatite particles,[29–31] mimicking those naturally occurring in bone,[32] increased the alkaline phosphatase synthesis and calcium-containing mineral deposition by osteoblasts.[33] A number of growth factors and cytokines present in SBCD are known to be involved in mesenchymal precursors' recruitment, proliferation, and differentiation *in vivo*.[34] In the present study, we did not determine which of the factors identified might be responsible for the osteoinductive effect observed *in vivo*, but it is conceivable that their simultaneous presence and interaction may lead to a sequence of events favoring the osteogenic process in specifically determined cells. In analogy, dietary supplementation of a growth factor rich fraction of bovine colostrum (1–30 kDa fraction) was shown to promote bone development in juvenile rats.[35]

We are fully aware that SBCD represents a relatively rough approach because of the heterogeneity of its components. Although immunologic reaction to the SBCD was not evident in our experimental model, as shown by the limited inflammatory infiltrate around Matrigel and SBCD plugs in absence of hydroxyapatite, this aspect should be carefully taken into consideration for further clinical studies. Indeed, the use of SDBC totally deprived of immunoglobulins would be regarded as mandatory to prevent immune reaction in humans. SBCD might therefore represent a convenient source of specific agents effective on the osteogenic process, whose identification and separation might lead to purified and highly active osteoinducing biological products.

In conclusion, additional studies and experimental models are needed, as a pre-requisite for future clinical application of the present approach. The data presented here indicate that the association of efficient biomaterials and an adequate supply of cells (MSCs and osteoprogenitors) in presence of a potent source of biological factors, as provided by SBCD, might lead to the goal.

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Allergies and Autoimmunity

Immunomodulatory proteins in colostrum

Plusa T: Department of Internal Medicine, Pneumonology and Allergology, Central Clinical Hospital of the Ministry of National Defense, Military Institute of Health Services in Warsaw, Poland. tplusa@wim.mil.pl: 2009 Mar;26(153):234-8.

The value of bovine colostrums is documented in clinical observations and supported by large database. An antibacterial effect and modulation of the immune response are accepted. The wide spectrum of the activity of a lactoferrin or a proline-rich polipeptide complex was confirmed in experimental and clinical studies. Moreover, a high concentration of immunoglobulins in bovine colostrum gives exceptional opportunity for use as a support in immunodeficiency treatment.

PMID: 19388540 [PubMed – indexed for MEDLINE]

Alzheimer's

Colostral Proline-Rich Polypeptides – Immunoregulatory Properties and Prospects of Therapeutic Use in Alzheimer's Disease.

Curr Alzheimer Res. 2009 Nov 26; Janusz M, Zablocka A.; Department of Immunochemistry, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, 12 R.Weigla, 53-114 Wroclaw, Poland. janusz@iitd.pan.wroc.pl.

A proline-rich polypeptide complex (PRP), subsequently called Colostrinin (CLN), was first isolated from ovine **colostrum**, was shown to possess immunoregulatory properties, including effects on the maturation and differentiation of murine thymocytes and humoral and cellular immune responses, both in vivo and in vitro. PRP seems to restore balance in cellular immune functions and is not species specific. PRP is a complex of peptides of molecular masses ranging from 500 to 3000 Da. The polypeptide contains 25% proline and 40% hydrophobic amino acids. PRP shows a regulatory activity in cytokine (IFN, TNF-alpha, IL-6, IL-10) induction and possesses the ability to inhibit the overproduction of oxygen reactive species and nitric oxide. Besides its immunoregulatory activity, PRP also showed psychotropic properties, improving cognitive activity and behavior of old rats, humans, and chickens. The properties of PRP prompted the authors to propose the complex for the treatment neurodegenerative disorders. Beneficial effects of PRP/Colostrinin were shown for the first time in double-blind placebo-controlled trials and long-term open-label studies. The results were confirmed in multicenter clinical trials. A very important property of PRP/Colostrinin is the prevention of Abeta aggregation and the disruption of already existing aggregates. The same properties were expressed by one of PRP's components, a nonapeptide (NP). Moreover, PRP modulates neurite outgrowth, suppresses uncontrolled activation of cells, reduces 4-HNE-mediated cellular damage, and modulates expression in cellular redox regulation, cell proliferation, and differentiation. Its biological response modifying activity can play an important role in its use in the treatment of Alzheimer's disease.

>PMID: 19939229 [PubMed – as supplied by publisher]

The protective effects of the nutraceutical, colostrinin, against Alzheimer's disease, is mediated via prevention of apoptosis in human neurones induced by aggregated beta-amyloid.

J Nutr Health Aging. 2009 Jun;13(6):522-7.Douraghi-Zadeh D, Matharu B, Razvi A, Austen B.; Neurodegeneration Unit, Basic Medical Sciences, St. George's, University of London, Cranmer Terrace, Tooting, London, UK.

OBJECTIVE: It has previously been demonstrated that oral administration of ovine Colostrinin (CLN), a proline-rich polypeptide isolated from ovine **colostrum**, can effectively treat Alzheimer's disease patients. This study aims to determine whether CLN has effects on the aggregation and toxicity of synthetic beta-amyloid (Abeta), implicated as a causative agent of AD. DESIGN AND MEASUREMENTS: Using cell assays, we examined if pre-treatment of neuronal cells with CLN confers protection. RESULTS: The data from cytotoxicity assays (using MTT and LDH) demonstrated that pre-treatment of human neuronal SHSY-5Y cells with 5 microg/ml CLN, for 24 hours, confers neuroprotection against Abeta-induced neurotoxicity. Twenty-four hour pre-treatment with 5 microg/ml CLN was also shown to reduce Abeta 1-40-induced apoptosis in human neuronal cells as determined via qualitative and quantitative apoptosis assays. CONCLUSION: The neuroprotection conferred with CLN pre-treatment was reduced with the Fas ligand (FasL) binding antibody Nok1, suggesting that the effects of CLN may involve a Fas:soluble FasL interaction. These findings indicate that CLN could possibly play a role in the prevention of AD pathogenesis, though the inhibition of Fas-mediated apoptosis.

PMID: 19536420 [PubMed – indexed for MEDLINE]

Antioxidant

Antioxidants in colostrum and milk of sows and cows.

Reprod Domest Anim. 2009 Aug;44(4):606-11.; Albera E, Kankofer M.; Department of Animal Biochemistry and Physiology, Faculty of Veterinary Medicine, Agricultural University, Lublin, Poland.

On account of the oxidative stress conditions that may appear during parturition, **colostrum** should provide with not only nutritional and immunological components but also antioxidative protection of newborn. There is evidence that apart from well-known antioxidative enzymes like glutathione peroxidase, superoxide dismutase, catalase or low molecular antioxidants, proteins like lactoperoxidase (LPO), lactoferrin (LF) and ceruloplasmin (CP) may exert antioxidative properties in colostrum. The aim of present study was to determine and to evaluate LPO, LF and CP activities in colostrum and milk of sows and cows. Samples were collected from 16 healthy cows five times: immediately after parturition, 12, 24 and 48 h, and 7 days postpartum as well as from 14 healthy sows five times: immediately after parturition, 6, 12, 24 and 36 h later. Examined parameters were determined spectrophotometrically at 412, 560 and 540 nm respectively. LPO activity was higher in sows as in cows and increased significantly within examined time. LF ability to inhibit superoxide radical generation was higher in sows as in cows and increased significantly within examined time. CP oxidase activity was higher in cows as in sows and decreased significantly during experimental period. In conclusion, antioxidative defence system in **colostrum** shows dynamic changes that allow for providing with necessary protection from oxidative stress conditions, which may appear after parturition.

PMID: 19642220 [PubMed – indexed for MEDLINE]

Athletics & Body Building

Bovine colostrum supplementation and exercise performance: potential mechanisms.

Sports Med. 2009;39(12):1033-54. doi: 10.2165/11317860-000000000-00000.Shing CM, Hunter DC,Stevenson LM.; School of Human Life Sciences, University of Tasmania, Launceston, Tasmania, Australia. Cecilia.Shing@utas.edu.au

Bovine colostrum (BC) is rich in immune, growth and antimicrobial factors, which promote tissue growth and the development of the digestive tract and immune function in neonatal calves. Although the value of **bovine colostrum** to human adults is not well understood, supplementation with BC is becoming increasingly popular in trained athletes to promote exercise performance. The combined presence of insulin-like growth factors (IGF), transforming growth factors, immunoglobulins, cytokines, lactoferrin and lysozyme, in addition to hormones such as growth hormone, gonadotrophin-releasing hormone, luteinizing hormone-releasing hormone and glucocorticoids, would suggest that **BC** might improve immune function, gastrointestinal integrity and the neuroendocrine system, parameters that may be compromised as a result of intensive training. A review of studies investigating the influence of **bovine colostrum** supplementation on exercise performance suggests that **BC** supplementation is most effective during periods of high-intensity training and recovery from high-intensity training, possibly as a result of increased plasma IGF-1, improved intramuscular buffering capacity, increases in lean body mass and increases in salivary IgA. However, there are contradicting data for most parameters that have been considered to date, suggesting that small improvements across a range of parameters might contribute to improved performance and recovery, although this cannot be concluded with certainty because the various doses and length of supplementation with **BC** in different studies prevent direct comparison of results. Future research on the influence of **BC** on sports performance will only be of value if the dose and length of supplementation of a well-defined **BC**product is standardized across studies, and the bioavailability of the active constituents in **BC** is determined.PMID: 20030905 [PubMed – as supplied by publisher]

Effects of bovine colostrum supplementation on immune variables in highly trained cyclists.

J Appl Physiol (1985). 2007 Mar;102(3):1113-22. Epub 2006 Nov 9; Shing CM1, Peake J, Suzuki K, Okutsu M, Pereira R, Stevenson L, Jenkins DG, Coombes JS.

Abstract: The aim of this study was to investigate the influence of low-dose **bovine colostrum** protein concentrate (CPC) supplementation on selected immune variables in cyclists. Twenty-nine highly trained male road cyclists completed an initial 40-km time trial (TT(40)) and were then randomly assigned to either a supplement (n = 14, 10 g bovine CPC/day) or placebo group (n = 15, 10 g whey protein concentrate/day). After 5 wk of supplementation, the cyclists completed a second TT(40). They then completed 5 consecutive days of high-intensity training (HIT) that included a TT(40), followed by a final TT(40) in the following week. Venous blood and saliva samples were collected immediately before and after each TT(40), and upper respiratory illness symptoms were recorded over the experimental period. Compared with the placebo group, bovine CPC supplementation significantly increased preexercise serum soluble TNF receptor 1 during the HIT period (bovine CPC = 882 +/- 233 pg/ml, placebo = 468 +/- 139 pg/ml; P = 0.039). Supplementation also suppressed the postexercise decrease in cytotoxic/suppressor T

cells during the HIT period (bovine CPC = -1.0 +/- 2.7%, placebo = -9.2 +/- 2.8%; P = 0.017) and during the following week (bovine CPC = 1.4 +/- 2.9%, placebo = -8.2 +/- 2.8%; P = 0.004). Bovine CPC supplementation prevented a postexercise decrease in serum IgG(2) concentration at the end of the HIT period (bovine CPC = 4.8 +/- 6.8%, P = 0.88; placebo = -9.7 +/- 6.9%, P = 0.013). There was a trend toward reduced incidence of upper respiratory illness symptoms in the bovine CPC group (P = 0.055). In summary, low-dose bovine CPC supplementation modulates immune parameters during normal training and after an acute period of intense exercise, which may have contributed to the trend toward reduced upper respiratory illness in the bovine CPC group.

Bovine colostrum supplementation attenuates the decrease of salivary lysozyme and enhances the recovery of neutrophil function after prolonged exercise.

Department of Sport and Exercise Science, Aberystwyth University, Carwyn James Building, Penglais Campus, Aberystwyth, Ceredigion SY23 3FD, UK; Davison G, Diment BC.

Oral supplementation with bovine colostrum (COL) has been shown to enhance immunity in human subjects. However, there is limited research on the use of bovine COL supplementation to counter exercise-induced immunodepression, as a model of stress-induced immunodepression, and previous research has focused primarily on salivary IgA. The aim of the present study was to determine the effects of bovine COL supplementation on exercise-induced changes in innate immunity (neutrophil function and salivary lysozyme) in addition to salivary IgA. Twenty healthy, active men cycled for 2 h at approximately 64 % maximal oxygen uptake after 4 weeks of daily bovine COL (n 10) or placebo (PLA, n 10) supplementation. Blood and saliva samples were obtained before and after supplementation, before and after exercise. Exercise induced significant increases in markers of physiological stress and stress to the immune system (circulating neutrophils, neutrophil:lymphocyte ratio, immature granulocytes, atypical lymphocytes and plasma cortisol), but there were no differences between the COL and PLA groups. Significant group x time interactions (two-way mixed model ANOVA) were observed for neutrophil function (stimulated degranulation) and salivary lysozyme concentration and release ($P < 0.05$). Significant exercise-induced decreases were observed in these parameters, and bovine COL supplementation either speeded the recovery (neutrophil function) or prevented the decrease (salivary lysozyme) in these measures of innate immunity. These results suggest that 4 weeks of bovine COL supplementation limits the immunodepressive effects induced by an acute prolonged physical stressor, such as exercise, which may confer some benefits to host defence.

PMID: 20030905 [PubMed – as supplied by publisher]

Bone Density

The importance of lactoferrin in bone regeneration.

[Article in Polish]
Pol Merkur Lekarski. 2014 Jul;37(217):65-7; Włodarski KH, Galus R, Brodzikowska A, Włodarski PK, Wojtowicz A.
Abstract
Lactoferrin is an iron-binding protein secreted by mammary gland, thus present in milk and in **colostrum**, which are a cheap and easy to obtain sources of this protein. Lactoferrin is also present in specific granules of neutrophils. Lactoferrin is a multifunctional agent involved, among others in the immune response and in the regulation of bone metabolism. Lactoferrin

actives of osteoblast proliferation and bone matrix secretion, and inhibits apoptosis of osteoblast and osteoclastogenesis. Lactoferrin administered to rodents accelerates bone healing and prevents bone loss induced by ovariectomy. Therefore the use of lactoferrin or milk whey in osteoporosis treatment and prevention is postulated.
PMID: 25154204 [PubMed – indexed for MEDLINE]

Effect of a Growth Protein-Colostrum Fraction on bone development in juvenile rats.

Biosci Biotechnol Biochem. 2008 Jan;72(1):1-6. Epub 2008 Jan 7.Lee J1, Kwon SH, Kim HM, Fahey SN, Knighton DR, Sansom A.

Abstract
Colostrum is a complex mixture of bioactives that promotes neonate growth. Studies show that it contains components capable of promoting bone formation and inhibiting bone resorption. Although many **colostrum**-based nutritional supplements have been developed as growth promotants, few studies have investigated their functional effects. A bovine colostrum 1-30 kDa fraction, Growth Protein-**Colostrum** (GP-C), was administered to juvenile rats as a dietary supplement to determine effects on growth and development. GP-C enhanced the growth and mineralization of the femur as evidenced by increased serum osteocalcin and bone mineral density. Increased levels of serum growth hormone and insulin-like growth factor-1 suggest that the mechanism of enhanced growth is partially controlled by endocrine factors. GP-C was also found to increase osteoblast proliferation in vitro, a finding that indicates a possible mechanism of action of GP-C, but further studies are required. Based on our findings, we hypothesize that a colostrum-based dietary supplement enhances bone growth and development in humans.
PMID: 18175920 [PubMed – indexed for MEDLINE]

Cancer

Potential clinical applications of multi-functional milk proteins and peptides in cancer management.

Curr Med Chem. 2014;21(21):2424-37; Chen HY, Mollstedt O, Tsai MH, Kreider RB1.

Abstract
The progression of cancer involves multiple changes that alter intracellular signaling to promote cell proliferation. Subsequent remodeling of the tumor microenvironment enhances metastasis by manipulating the immune system. Research in the past decade has shown that milk proteins and peptides are often multi-functional, exerting activities such as anti-microbial, immunomodulatory, cancer cell apoptosis, anti-metastasis, and antioxidant effects. Several milk-derived biologics, such as HAMLET (human α-lactalbumin made lethal to tumor cells) and the human recombinant form of lactoferrin, already demonstrated promising results in clinical trials. Lactoferricin peptide analogs are in early clinical development as antimicrobial agents and cancer immunotherapies. In addition, milk proteins and peptides are well tolerated and many exhibit oral bioavailability; thus they may complement standard therapies to boost overall success in cancer treatments. Lactoferrin, colostrum, and specific milk-derived peptide fractions are currently being developed as clinical nutrition for cancer prevention and chemotherapy protection. This review highlights the potential applications of milk proteins and peptides as pharmaceutical drug candidates and clinical nutrition in the overall management of cancer.

PMID:
24524762

Spontaneous regression of low-grade cervical intraepithelial lesions is positively improved by topical bovine colostrum preparations (GINEDIE®). A multicentre, observational, italian pilot study.

Eur Rev Med Pharmacol Sci. 2014;18(5):728-33; Stefani C1, Liverani CA, Bianco V, Penna C, Guarnieri T, Comparetto C, Monti E, Valente I, Pieralli AL, Fiaschi C, Origoni M.

Abstract
OBJECTIVES:
Human papillomavirus (HPV) is the causal agent of cervical cancer. The great majority of abnormal Pap test results – almost 90% – is referrable to either atypical squamous intraepithelial lesion or CIN1. For these lesions, worldwide agreement exists concerning the high rate – ranging from 40% to 70% – of spontaneous regression over a period of 1-5 years. Host's immune response is a key point influencing the natural history of these conditions. Bovine colostrum is a natural agent positively promoting several immune activities against bacterial and viral agents. The aim of this report was to evaluate the potential positive effect of bovine colostrum-containing vaginal tablets administered to CIN1 diagnosed patients in a prospective trial in regards to spontaneous regression rate.
PATIENTS AND METHODS:
A series of 256 consecutive patients with histologically proven CIN1 recruited in a multicentre, observational, Italian study. Patients have been enrolled in a 24-weeks protocol of treatment and re-tested at the end of the study. Rates of regression have been recorded.
RESULTS:
Overall regression rate to a negative histology at the end of the 6 month follow up was 75.5%.
CONCLUSIONS:
Regression to normal histology was observed in a very high rate of cases in a very short period compared to the natural history of these lesions. CIN1 patients could benefit from bovine colostrum topical administration in terms of significantly shortening the regression time.
PMID:
24668716

Chronic Fatigue

Improving fatigue assessment in immune-mediated neuropathies: the modified Rasch-built fatigue severity scale.

Van Nes SI, Vanhoutte EK, Faber CG, Garssen M, van Doorn PA, Merkies IS; PeriNomS Study Group; Bennett D, van den Berg LH, Van den Bergh PY,Cornblath DR, Dalakas M, Devigili G, van Doorn PA, Faber CG, Gorson KC, Hadden RD,Hahn AF, Hartung HP,Hughes RA, Lauria G, Léger JM, Lewis RA, Lunn MP, Merkies IS, Nobile-Orazio E,Notermans NC,Padua L, Reilly

MM, >Smith B.; Department of Neurology, Erasmus Medical Centre Rotterdam, Rotterdam, The Netherlands. s.vannes@erasmusmc.nl

Fatigue is a major disabling complaint in patients with immune-mediated neuropathies (IN). The 9-item fatigue severity scale (FSS) has been used to assess fatigue in these conditions, despite having limitations due to its classic ordinal construct. The aim was to improve fatigue assessment in IN through evaluation of the FSS using a modern clinimetric approach [Rasch unidimensional measurement model (RUMM2020)]. Included were 192 stable patients with Guillain-Barré syndrome (GBS), chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) or polyneuropathy associated with monoclonal gammopathy of undetermined significance (MGUSP). The obtained FSS data were exposed to RUMM2020 model to investigate whether this scale would meet its expectations. Also, reliability and validity studies were performed. The original FSS did not meet the Rasch model expectations, primarily based on two misfitting items, one of these also showing bias towards the factor 'walking independent.' After removing these two items and collapsing the original 7-point Likert options to 4-point response categories for the remaining items, we succeeded in constructing a 7-item Rasch-built scale that fulfilled all requirements of unidimensionality, linearity, and rating scale model. Good reliability and validity were also obtained for the modified FSS scale. In conclusion, a 7-item linearly weighted Rasch-built modified FSS is presented for more proper assessment of fatigue in future studies in patients with immune-mediated neuropathies.

PMID: 20021568 [PubMed – in process]

Digestive Tract Disorders

Bovine colostrum inhibits nuclear factor kappaB-mediated proinflammatory cytokine expression in intestinal epithelial cells.

Nutr Res. 2009 Apr;29(4):275-80. doi: 10.1016/j.nutres.2009.03.011.; An MJ1, Cheon JH, Kim SW, Park JJ, Moon CM, Han SY, Kim ES, Kim TI, Kim WH.

Abstract
Colostrum, a nutrient-rich fluid produced by female mammals immediately after giving birth, is loaded with several immune, growth, and tissue repair factors. However, it remains unknown whether bovine colostrum has anti-inflammatory effects on intestinal epithelial cells (IEC). In this study, we aimed to investigate the anti-inflammatory effects of colostrum on IEC and to elucidate its molecular mechanisms. Human colon cancer HT-29 cells were stimulated with interleukin (IL)-1beta with or without bovine colostrum. The effects of colostrum on nuclear factor kappaB (NF-kappaB) signaling in HT-29 cells were examined using real-time reverse transcriptase-polymerase chain reaction detect IL-8 and intracellar adhesion molecule-1 mRNA expression using a NF-kappaB-dependent reporter gene assay and an electrophoretic mobility shift assay. Furthermore, we assessed the expression levels of inhibitor protein of NF-kappaB-alpha, cyclooxygenase-2, and p65 proteins by Western blotting. Bovine colostrum significantly inhibited IL-1beta-induced IL-8 and intracellar adhesion molecule-1 mRNA expression. Moreover, it suppressed IL-1beta-induced NF-kappaB activation, including NF-kappaB dependent reporter gene expression in a dose-dependent manner. Finally, Western blotting revealed that colostrum decreased the cyclooxygenase-2 protein expression level, inhibited inhibitor protein of NF-kappaB-alpha degradation, and blocked translocation of p65 into the nucleus. These data demonstrated that bovine colostrum might protect against IEC inflammation by inhibiting the NF-kappaB pathway, suggesting

colostrum has a therapeutic potential for intestinal inflammation.
PMID:
19410980&

Effect of specific colostral antibodies and selected lactobacilli on the adhesion of Helicobacter pylori on AGS cells and the Helicobacter-induced IL-8 production.

Scand J Immunol. 2008 Sep;68(3):280-6. doi: 10.1111/j.1365-3083.2008.02138.x.; Rokka S1, Myllykangas S, Joutsjoki V.

Abstract
Helicobacter pylori infection is the most common cause of gastritis, gastric ulcer and adenocarcinoma. It has proven difficult to cure because of its capability to develop strains resistant to antibiotics. The effect of three strains of lactic acid bacteria (LAB) and bovine colostral preparations on the adhesion of H. pylori NCTC 11637 on gastric adenocarcinoma (AGS) cells and on the interleukin (IL)-8 production was studied. Before infection, H. pylori were pretreated with Lactobacillus plantarum MLBPL1, Lactobacillus rhamnosus GG, Lactococcus lactis, or with a colostral preparation with or without specific H. pylori antibodies. The relative number of H. pylori adhered on AGS cells was determined by urease test. IL-8 produced by the cells was studied by enzyme-linked immunosorbent assay. Colostral preparations with and without specific antibodies reduced the adhesion of H. pylori on AGS cells in a dose-dependent manner. Live LAB at a concentration of 10(10) CFU/ml reduced the adhesion by approximately 50% ($P < 0.05$). After the infection of AGS cells by H. pylori, the IL-8 level rose up to about 10-fold (5500 +/- 1600 pg/ml). Pretreatment of H. pylori with colostral preparations or high concentrations of LAB prevented this IL-8 rise. Similar effect was seen with live and heat-killed LAB, the live LAB being more effective. Heat-killed LAB at a concentration of 10(10) CFU/ml rose the IL-8 level of non-infected cells significantly. Suppression of IL-8 production by LAB or colostral products could have a suppressive effect on inflammation in Helicobacter infection.
PMID:
18627549

Bovine colostrum prevents bacterial translocation in an intestinal ischemia/reperfusion-injured rat model.

J Med Food. 2009 Feb;12(1):37-46; Choi HS,Jung KH, Lee SC, Yim SV, Chung JH, Kim YW, Jeon WK, Hong HP,Ko YG, Kim CH, Jang KH, Kang SA.; Department of Emergency Medicine, Kohwang Medical Research Institute, Kyung Hee University, Seoul, Republic of Korea.

This study evaluated whether or not b**ovine colostrum (BC)** is able to treat or prevent intestinal barrier damage, bacterial translocation, and the related systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) in an intestinal ischemia/reperfusion (I/R)-injured rat model. Fifty Sprague-Dawley rats were used. The rats' intestinal I/R injuries were induced by clamping the superior mesenteric artery for 30 minutes. After 3 hours of reperfusion and then twice daily reclamping during the experiment, the experimental group was given BC (4 mL/kg/day) perorally, and the other groups received 0.9% saline and low fat milk (LFM) after intestinal I/R injury. Seventy-two hours later we assessed (1) intestinal damage and intestinal permeability, (2) enteric bacterial count and bacterial translocation, (3) serum albumin, protein, and hepatic enzyme levels, (4) pathologic findings of ileum and lung, (5) activity of oxygen-free radical species, and (6) pro-inflammatory cytokines (tumor necrosis factor-alpha and interleukin-1beta). Intestinal damage, intestinal permeability, and bacterial

translocation to other organs were significantly reduced in rats fed with BC after I/R when compared to rats fed LFM/saline after I/R ($P < .05$). In the evaluation of acute lung injury, neutrophils were found only in the lungs of the saline-fed group after I/R, and the wet/dry ratio of the lung tissue was significantly reduced in the BC-fed group after I/R compared to other I/R groups. A marked difference was found between LFM/saline-fed groups and BC-fed groups regarding malondialdehyde ($P < .05$) and pro-inflammatory cytokines ($P < .01$). In conclusion, **BC** may have beneficial effects in treating and preventing intestinal barrier damage, bacterial translocation and the related SIRS and MODS in the intestinal I/R-injured rat model.

PMID: 19298194 [PubMed – indexed for MEDLINE]

Prebiotic effect of lacto-N-biose I on bifidobacterial growth.

Biosci Biotechnol Biochem. 2009 May;73(5):1175-9. Epub 2009 May 7; Kiyohara M, Tachizawa A, Nishimoto M, Kitaoka M, Ashida H, Yamamoto K.; Graduate School of Biostudies, Kyoto University, Kyoto, Japan. kiyohara@lif.kyoto-u.ac.jp

We demonstrated the prebiotic effect of lacto-N-biose I (Galbeta1-3GlcNAc) on bifidobacteria in vitro. Lacto-N-biose I, a building unit of the type-I milk oligosaccharides, enhanced the growth of many bifidobacteria, especially Bifidobacterium bifidum, B. breve, and B. longum, which are predominant in the intestines of breast-fed infants. It might be a substantial, natural prebiotic in human**colostrums**.

PMID: 19420691 [PubMed – indexed for MEDLINE]

Diet-dependent mucosal colonization and interleukin-1beta responses in preterm pigs susceptible to necrotizing enterocolitis.

J Pediatr Gastroenterol Nutr. 2009 Jul;49(1):90-8; Van Haver ER, Sangild PT,Oste M, Siggers JL; Department of Veterinary Medicine, University of Antwerp, Wilrijk, Belgium.

OBJECTIVES: Intestinal colonization challenges the neonatal innate immune system, especially in newborns with an immature immune response lacking the supportive bioactive components from mother's milk. Accordingly, formula-fed preterm pigs frequently show bacterial overgrowth, mucosal atrophy, and gut lesions reflecting necrotizing enterocolitis (NEC) within the first days after birth. We hypothesized that NEC development is related to a diet-dependent bacterial adherence and a subsequent proinflammatory cytokine response in the gut mucosa immediately after introduction of enteral food. MATERIALS AND METHODS: Premature piglets (92% gestation) received 2 to 3 days of total parenteral nutrition followed by 0, 8, or 17 hours of enteral formula or sow's **colostrum**feeding. RESULTS: Following 8 hours, but not 17 hours, of colostrum feeding, a reduced number of intestinal samples with adherent bacteria (both Gram-negative and Gram-positive bacteria) was counted compared with 0 or 8 hours of formula feeding. Besides a more dense colonization, formula feeding leads to higher intestinal interleukin-1beta (IL-1beta) levels and more NEC-like lesions from 8 hours onward. The load of adherent bacteria was especially high in NEC lesions. Toll-like receptor 4 was detected in enteroendocrine, neuronal, and smooth muscle cells, potentially mediating the increase in IL-1beta levels by Gram-negative bacteria. CONCLUSIONS: Formula feeding facilitates bacterial adherence and the development of a proinflammatory state of the intestine, which may be among the key factors that predispose formula-fed preterm neonates to NEC.

PMID: 19516189 [PubMed – indexed for MEDLINE]

The structural basis for the prevention of nonsteroidal antiinflammatory drug-induced gastrointestinal tract damage by the C-lobe of bovine colostrum lactoferrin.

Biophys J. 2009 Dec 16;97(12):3178-86; Mir R, Singh N,Vikram G, Kumar RP,Sinha M, Bhushan A, Kaur P, Srinivasan A, Sharma S, Singh TP; Department of Biophysics, All India Institute of Medical Sciences, New Delhi, India.

Nonsteroidal antiinflammatory drugs (NSAIDs), due to their good efficacy in the treatment of pain, inflammation, and fever, are among the most prescribed class of medicines in the world. The main drawback of NSAIDs is that they induce gastric complications such as peptic ulceration and injury to the intestine. Four NSAIDs, indomethacin, diclofenac, aspirin, and ibuprofen were selected to induce gastropathy in mouse models. It was found that the addition of C-terminal half of bovine lactoferrin (C-lobe) reversed the NSAID-induced injuries to the extent of 47-70% whereas the coadministration of C-lobe prevented it significantly. The C-lobe was prepared proteolytically using serine proteases. The binding studies of C-lobe with NSAIDs showed that these compounds bind to C-lobe with affinities ranging from 2.6 to 4.8 x 10(-4) M. The complexes of C-lobe were prepared with the above four NSAIDs. All four complexes were crystallized and their detailed three-dimensional structures were determined using x-ray crystallographic method. The structures showed that all the four NSAID molecules bound to C-lobe at the newly identified ligand binding site in C-lobe that is formed involving two alpha-helices, alpha10 and alpha11. The ligand binding site is separated from the well known iron binding site by the longest and the most stable beta-strand, betaj, in the structure. Similar results were also obtained with the full length lactoferrin molecule. This novel, to our knowledge, binding site in C-lobe of lactoferrin shows a good complementarity for the acidic and lipophilic compounds such as NSAIDs. We believe this indicates that C-lobe of lactoferrin can be exploited for the prevention of NSAID-induced gastropathy.

PMID: 20006955&lt

Healing

Bovine colostrum promotes growth and migration of the human keratinocyte HaCaT cell line.

Growth Factors. 2009 Dec;27(6):448-55; Kovacs D, Cardinali G, Aspite N, Picardo M.; San Gallicano Dermatological Institute, IRCCS, Rome, Italy.

Bovine colostrum represents a rich source of growth factors, which are known to play a central role in wound healing. The aim of our study was to investigate the possible mitogenic and motogenic effects induced by colostrum on human keratinocytes. Cell proliferation evaluated by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide test and 5-Bromo-2′-deoxyuridine incorporation revealed that colostrum exerts a growth promoting activity. Scratch assay and immunofluorescence of actin cytoskeleton showed its effectiveness also in inducing cell migration. Furthermore, colostrum treatment increases the levels of tyrosine phosphorylated proteins and the activated forms of the extracellular signal-regulated kinases 1 and 2 and such effects appear to be repressed by the tyrosine kinase inhibitor genistein. Our results indicate that the biological activities of colostrum are specifically mediated by the growth factor-induced activation of tyrosine kinase receptors

and underline the relevance of the synergistic action exerted by the growth factors in stimulating keratinocyte proliferation and migration **essential for tissue repair**.

PMID: 19919532

Mare's colostrum globules stimulate fibroblast growth in vitro: a biochemical study.

J Med Food. 2009 Aug;12(4):836-45; Zava S, Barello C, Pessione A, Garoffo LP, Fattori P, Montorfano G, Conti A, Giunta C, Pessione E, Berra B, Giuffrida MG; Institute of General Physiology and Biological Chemistry, University of Milan, Milan, Turin, Italy.

The **wound repair** function of mare's milk and **colostrum** was investigated. Mare's **colostrum improved wound healing in vivo**; thus fibroblast growth activation by mare's milk and colostrum was examined. **As expected, colostrum was more effective than milk.** To establish the biochemical nature of the bioactive molecules involved, colostrum was fractionated into whey, casein, and fat globules, and the efficacy of these fractions on fibroblast proliferation was studied. **The fat globule fraction provided the strongest stimulation**; its composition was studied and compared with the less-active milk fat globule fraction. The lipid pattern highlighted several differences between mare's colostrum and milk; in particular, total lipid, linoleic acid, linolenic acid, ganglioside, andglycolipid contents were higher in colostrum. A proteomic investigation revealed some differences between the protein composition of colostrum and milk fat globules. Adipophylin and lactadherin were significantly overexpressed in colostrum fat globules. The role of specific lipids on skin wound repair and that of the epidermal growth factor-like domain, embedded within the lactadherin molecule and probably released in conditions stimulating proteolysis, are discussed.

PMID: 19735185 [PubMed – indexed for MEDLINE]

Immunity

Bovine Colostrum: its dietary supplementation role in improvement and modulation of human immune indices

Nutrition and Food Science Department; Home Economics Faculty; Food Hygiene Department; National Institute of Nutrition; Cairo; Sherif S. Ragab and Effat A. A. Afif.

Oral ingestion of **bovine colostrum** lozenges student subjects has been shown to be effective in improvement and modulation of humoral and cell-mediated immune indices during the feeding (two weeks) and post-feeding (two weeks) duration's. Both of circulate serum immunoglobulins IgG, IgM and IgA as a some parameters of humoral immunity and T-lymphocytes CD4 and CD8 as a some parameters of cell-mediated immunity in all of examined human subjects has been significantly influenced by oral supplementation by bovine colostrum lozenges. There was a direct positive correlation between the daily-received **colostrum** dose and the observed influence on the titters of measured immunity indices. A daily dose of 400 mg bovine colostrum supplementation has more improvement and increase effects on estimated parameters rather than 200 mg daily dose

which indicate that the improvement and modulation roles of **bovine colostrum** supplementation on immunity status was carried out through a dose – dependant fashion. All of the student cases of this study were having a low to moderate levels of all measured immunity indices which improved and modulated significantly as a result of daily consumption of **bovine colostrum** supplement.

Factors associated with serum immunoglobulin levels in beef calves from Alberta and Saskatchewan and association between passive transfer and health outcomes.

Can Vet J. 2009 Mar;50(3):275-81; Waldner CL, Rosengren LB; Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, Saskatchewan S7N 5B4, Canada. cheryl.waldner@usask.ca

Inadequate consumption of colostrum can negatively affect calf health and survival. The serum immunoglobulin G (IgG) concentrations of 935 beef calves from 152 herds in Alberta and Saskatchewan have been described, using radial immunodiffusion. The determinants and health effects of serum IgG concentrations were studied in 601 calves sampled between 2 and 8 days of age. Of these calves, 6% had failure of passive transfer and an additional 10% had marginal passive transfer. Serum IgG concentrations were lower in calves born to a heifer, as a twin, or experiencing dystocia. The odds of both calf death and treatment were increased in calves with serum IgG concentrations below 24 g/L; a threshold notably higher than the 16 g/L usually considered as providing adequate passive transfer. The finding of 1/3 of calves with serum IgG concentrations less than 24 g/L suggests that calfhood treatments and mortality could be decreased by ensuring that high risk calves consume **colostrum**.

PMID: 19436479 [PubMed – indexed for MEDLINE]

A novel immunoregulatory protein in human colostrum, syntenin-1, for promoting the development of IgA-producing cells from cord blood B cells.

Int Immunol. 2009 Sep;21(9):1013-23. Epub 2009 Jul 10; Sira MM, Yoshida T, Takeuchi M, Kashiwayama Y, Futatani T, Kanegane H, Sasahara A, Ito Y, >Mizuguchi M, Imanaka T, Miyawaki T.; Department of Pediatrics, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama, Toyama 930-0194, Japan.

Human colostrum contains many bioactive factors that must promote the development of intestinal mucosal immunity in infants. Especially, the presence of certain cytokines such as transforming growth factor (TGF)-beta or IL-10 has been of great interest for IgA production as a function of mucosal immune response. In the present study, we attempted to investigate whether unidentified factors inducing generation of IgA-producing cells from naive B cells might exist in colostrum. For this purpose, colostrum samples were directly added to a culture consisting of naive B cells and dendritic cells from cord blood and CD40 ligand-transfected L cells, comparing with recombinant IL-10 (rIL-10) and/or rTGF-beta. It was noted that most c**olostrum** samples alone were able to induce IgA-secreting cells at higher levels than rIL-10 and/or rTGF-beta. IgA-inducing activity of colostrum was abolished by neither anti-neutralizing mAbs against IL-10 nor TGF-beta, though partially by anti-IL-6 mAb. We prepared partially purified fractions from both pooled colostrums with and without IgA-inducing activity and comparatively performed quantitative proteomic analysis by two-dimensional difference gel electrophoresis followed by liquid chromatography-mass spectrometry. As a result, syntenin-1 was identified as a candidate for IgA-inducing protein in colostrum. Western blot analysis indicated that levels of syntenin-1 in **colostrum**samples were correlated with their IgA-inducing activities. Moreover, we demonstrated that recombinant syntenin-1 could induce preferentially IgA production from naive B cells. These results suggest that syntenin-1 serves as one of IgA-inducing factors for B cells.

PMID: 19592421 [PubMed – indexed for MEDLINE]

Levels and complexity of IgA antibody against oral bacteria in samples of human colostrum.

Immunobiology. 2014 Aug 14. pii: S0171-2985(14)00143-0. doi: 10.1016/j.imbio.2014.08.009; Petrechen LN1, Zago FH2, Sesso ML3, Bertoldo BB4, Silva CB5, Azevedo KP6, de Lima Pereira SA7, Geraldo-Martins VR8, Ferriani VP9, Nogueira RD10.
Author information
Abstract
Streptococcus mutans (SM) have three main virulence antigens: glucan binding protein B (gbpB), glucosyltransferase (Gtf) and antigens I/II (Ag I/II) envolved in the capacity of those bacteria to adhere and accumulate in the dental biofilm. Also, the glycosyltransferases 153kDa of Streptococcus gordonii (SGO) and 170kDa of Streptococcus sanguinis (SSA) were important antigens associated with the accumulation of those bacterias. Streptococcus mitis (SMI) present IgA1 protease of 202kDa. We investigated the specificity and levels IgA against those antigens of virulence in samples of human colostrum. This study involved 77 samples of colostrum that were analyzed for levels of immunoglobulian A, M and G by Elisa. The specificity of IgA against extracts of SM and initials colonizators (SSA, SMI, SGO) were analyzed by the Western blot. The mean concentration of IgA was 2850.2 (±2567.2) mg/100mL followed by IgM and IgG (respectively 321.8±90.3 and 88.3±51.5), statistically different ($p<0.05$). Results showed that the majority of samples had detectable levels of IgA antibodies to extracts of bacteria antigens and theirs virulence antigens. To SM, the GbpB was significantly lower detected than others antigens of SM ($p<0.05$). High complexities of response to Ags were identified in the samples. There were no significant differences in the mean number of IgA-reactive Ags between the antigens ($p>0.4$). So, the breast milk from first hours after birth presented significant levels of IgA specific against important virulence of antigens those oral streptococci, which can disrupt the installation and accumulation process of these microorganisms in the oral cavity.

Inflammation

Bovine colostrum inhibits nuclear factor kappaB-mediated proinflammatory cytokine expression in intestinal epithelial cells.

Nutr Res. 2009 Apr;29(4):275-80; An MJ, Cheon JH, Kim SW, Park JJ, Moon CM, Han SY, Kim ES, Kim TI, Kim WH; Department of Internal Medicine and Institute of Gastroenterology, Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea.

Colostrum, a nutrient-rich fluid produced by female mammals immediately after giving birth, is loaded with several immune, growth, and tissue repair factors. However, it remains unknown whether bovine colostrum has anti-inflammatory effects on intestinal epithelial cells (IEC). In this study, we aimed to investigate the anti-inflammatory effects of **colostrum** on IEC and to elucidate its molecular mechanisms. Human colon cancer HT-29 cells were stimulated with interleukin (IL)-1beta with or without bovine colostrum. The effects of colostrum on nuclear factor kappaB (NF-kappaB) signaling in HT-29 cells were examined using real-time reverse transcriptase-polymerase chain reaction detect IL-8 and intracellar adhesion molecule-1 mRNA expression using a NF-kappaB-dependent reporter gene assay and an electrophoretic mobility shift assay. Furthermore, we assessed the expression levels of inhibitor protein of NF-kappaB-alpha, cyclooxygenase-2, and p65 proteins by Western blotting.

Bovine colostrum significantly inhibited IL-1beta-induced IL-8 and intracellar adhesion molecule-1 mRNA expression. Moreover, it suppressed IL-1beta-induced NF-kappaB activation, including NF-kappaB dependent reporter gene expression in a dose-dependent manner. Finally, Western blotting revealed that colostrum decreased the cyclooxygenase-2 protein expression level, inhibited inhibitor protein of NF-kappaB-alpha degradation, and blocked translocation of p65 into the nucleus. These data demonstrated that **bovine colostrum** might protect against IEC inflammation by inhibiting the NF-kappaB pathway, suggesting **colostrum** has a therapeutic potential for intestinal inflammation.

PMID: 19410980 [PubMed – indexed for MEDLINE]

Weight Loss

Leptin concentrations are a predictor of overweight reduction in a lifestyle intervention.

Int J Pediatr Obes. 2009;4(4):215-23; Reinehr T, Kleber M, de Sousa G, Andler W.; Vestische Hospital for Children and Adolescents, University of Witten/Herdecke, Datteln, Germany. T.Reinehr@kinderklinik-datteln.de

OBJECTIVE: **Leptin** resistance is discussed to be involved in the genesis of obesity. Therefore, we hypothesized that **leptin** levels were negatively associated with degree of weight loss in obese children participating in a lifestyle intervention. METHODS: We studied 248 obese children aged 8-14 years attending the “Obeldicks” lifestyle intervention (mean age 10.6+/-0.2 years, 53% female, 48% pubertal, mean body mass index (BMI) 27.8+/-0.3 kg/m2, and mean standard deviation score [SDS]-BMI 2.43+/-0.03). Baseline leptin concentrations were correlated with change of weight status, waist circumference, and percentage body fat, as calculated from skinfold measurements in the one-year intervention by Pearson correlation and multiple linear regression analyses. Furthermore, the relationship between leptin and cardiovascular risk factors (insulin, insulin resistance index HOMA, blood pressure, lipids, and glucose) were analyzed. RESULTS: A total of 212 children (85%) reduced their overweight, 9 children (4%) dropped out, and 27 children (11%) did not reduce their overweight in the lifestyle intervention “Obeldicks”. The mean reduction of SDS-BMI was 0.34+/-0.02. The reduction of SDS-BMI (r=- 0.27), waist circumference (r=-0.64), and percentage body fat (r=- 0.26) were significantly negatively associated with baseline leptin levels both in univariate analyses and in multiple regression analyses, adjusted to baseline age, BMI, gender and pubertal stage.
Baseline **leptin** concentrations were significantly associated with BMI, pubertal stage, gender, waist circumference, and insulin, but not to any other cardiovascular risk factors in multiple regression analyses. CONCLUSIONS: The finding that baseline leptin concentrations were significantly negatively correlated with the degree of weight loss in a lifestyle intervention supports the hypothesis of **leptin resistance in obesity**. This study is registered at clinicaltrials.gov (NCT00435734).

PMID: 19922035

Leptin is essential in maintaining normal vascular compliance independent of body weight.

Int J Obes (Lond). 2009 Oct 6; Sikka G, Yang R, Reid S, Benjo A, Koitabashi N, Camara A, Baraban E, O’Donnell CP, Berkowitz DE, Barouch LA.; Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

The adipocytokine leptin centrally regulates body weight by enhancing metabolic rate and signaling satiety, but it also has wide-ranging peripheral effects. Leptin receptors are expressed on vascular smooth muscle cells and have a role in maintaining vascular tone. We investigated the vascular effects of leptin repletion or calorie restriction on leptin-deficient mice (ob/ob) and a leptin antagonist on wild-type (WT) mice. Aortic compliance was assessed by the measurement of pulse wave velocity by noninvasive Doppler; blood pressure was measured by left ventricular catheterization. We found that ob/ob mice have much stiffer aortas than WT mice and that reduction in aortic stiffness was greater in ob/ob mice treated with leptin vs calorie restriction, despite similar weight loss. Interestingly, treating WT mice with a leptin antagonist increases aortic stiffness with no change in weight. Thus, we conclude that **leptin** is essential for maintaining normal aortic compliance independent of body weight.International Journal of Obesity advance online publication, 6 October 2009; doi:10.1038/ijo.2009.208.

PMID: 19806156 [PubMed – as supplied by publisher]

Uncategorized

Therapeutic properties of proteins and peptides from **colostrum** and milk

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Colostrum and milk are rich in proteins and peptides which play a crucial role in innate immunity when transferred to the offspring and may accelerate maturation of the immune system in neonates. The immunotropic properties of these proteins prompted investigators research their potential application in prevention and therapy. Lactoferrin (LF) exhibits antibacterial, antifungal, antiviral, antiparasitice, and antitumoral activities. It is protective with regard to intestinal epithelium, promotes bone growth, and accelerates the recovery of immune system function in immunocompromised animals. LF was tried in the treatment of hepatitis C infection and the intestinal form of graft-versus-host disease (GvHD). A proline-rich polypeptide (PRP) demonstrated a variety of immunotropic functions, including the promotion of T-cell maturation and inhibition of autoimmune disorders. PRP, in the form of chewable tablets (Colostrinin) was recently found to improve or stabilize the health status of Alzheimer's disease patients. Casein and casein-derived peptides showed protective activities in enamel demineralization and as caries-preventing agents. The protein hydrolyzates were also protective in diabetic animals, reduced tumor growth, had antihypertensive activity and diminished colicky symptoms in infants. Glycomacropeptide (GMP), a peptide derived from kappa-casein, exhibited various antibacterial and antithrombotic activities. Alpha-lactalbumin (LA) demonstrated antiviral, antitumoral and anti-stress properties. LA-enriched diets were anxiolytic, lowered blood pressure in rats, prevented diarrhea, and led to a better weight gain in malnourished children. HAMLET, a complex of LA and oleic acid, was effective in patients with cutaneous papillomas. Lysozyme found application in infant formulas, the treatment of periodentitis, and the prevention of tooth decay. Milk enriched in lysozyme was used in feeding premature infants suffering from concomitant diseases. Interesting, antibacterial properties were exhibited by lactoperoxidase. Both lysozyme and lactoperoxidase required cooperative action with LF in combating bacteria. *In conclusion, preparations derived from milk and colostrum are effective, easily bioaccessible, and safe, finding wide application in prevention and therapy for newborns and adults.*

PMID: 15995598 [PubMed – indexed for MEDLINE]

Treatment of diarrhea in human immunodeficiency virus-infected patients with immunoglobulins from bovine **colostrum.**

Clin Investig. 1992 Jul;70(7):588-94; Rump JA1, Arndt R, Arnold A, Bendick C, Dichtelmüller H, Franke M, Helm EB, Jäger H, Kampmann B, Kolb P, et al.

Author information

Abstract

Diarrhoea and weight loss are found in more than 50% of patients with the acquired immunodeficiency syndrome (AIDS). In some patients the symptoms can be very severe, leading to death even in the absence of opportunistic infections. In 30% of these patients, enteric pathogens cannot be identified, and approximately only half of the identifiable aetiologic agents of diarrhea in patients infected with the human immunodeficiency virus (HIV) were treatable with antibiotics. Immunoglobulins from bovine colostrum (Lactobin, Biotest, Dreieich, FRG) contain high titers of antibodies against a wide range of bacterial, viral and protozoal pathogens as well as against various bacterial toxins. Lactobin (LIG) is quite resistant to 24-h incubation with gastric juice. In a multi-center pilot study 37 immunodeficiency patients with chronic diarrhea [29 HIV-infected patients, 2 patients with common variable immunodeficiency (CVID), one unidentified immunodeficiency, five patients with graft versus host disease (GvHD) following bone marrow transplantation] were treated with oral LIG (10 g/day for 10 days). Good therapeutic effects were observed. Out of 31 treatment periods in 29 HIV-infected patients 21 gave good results leading to transient (10 days) or long-lasting (more than 4 weeks) normalisation of the stool frequency. The mean daily stool frequency decreased from 7.4 to 2.2 at the end of the treatment. Eight HIV-infected patients showed no response. The diarrhea recurred in 12 patients within 4 weeks (32.4%), while 19 patients were free of diarrhea for at least 4 weeks (51.3%). In 5 patients intestinal cryptosporidiosis disappeared following oral LIG treatment. LIG treatment was also beneficial in 4 out of 5 GvHD patients.(ABSTRACT TRUNCATED AT 250 WORDS)

PMID: 1392428 [PubMed – indexed for MEDLINE]

Oral administration of insulin-like growth factor-I from colostral whey reduces blood glucose in streptozotocin-induced diabetic mice.

Br J Nutr. 2012 Jul 14;108(1):39-45. doi: 10.1017/S0007114511005198. Epub 2011 Oct 10; Hwang KA1, Hwang YJ, Ha W, Choo YK, Ko K.

Author information

Abstract

The aim of the present study was to investigate the effects of oral administration of the insulin-like growth factor-I-rich fraction (IGF-I-RF) from bovine colostral whey on the regulation of blood glucose levels in streptozotocin (STZ)-induced diabetic mice. We obtained a peptide fraction containing IGF-I (10 ng/mg protein) from Holstein colostrum within 24 h after parturition by using ultrafiltration. The blood glucose levels of STZ-induced diabetic mice fed with IGF-I-RF (50 μg/kg per d) were significantly reduced by 11 and 33 % at weeks 2 and 4, respectively ($P < 0·05$). The body weights of STZ-induced diabetic mice increased following the oral administration of the IGF-I-RF. The kidney weights of STZ-induced diabetic mice decreased significantly ($P < 0·05$) following the administration of the IGF-I-RF, and the liver weights of STZ-induced diabetic mice decreased significantly ($P < 0·05$) following the administration of

50 µg/kg per d of the IGF-I-RF. The present results indicate that the IGF-I-RF obtained from Holstein colostrum could be a useful component for an alternative therapeutic modality for the treatment of diabetes in insulin-resistant patients.
PMID: 22018138 [PubMed – indexed for MEDLINE]

Bovine Colostrum Emerges as Immune System Modulator

American Journal of Natural Medicine
ZOLTAN P. RONA, M.D., M.Sc.
Reprint from March 1998.
Bovine Colostrum Emerges as Immunity Modulator is a comprehensive treatise on colostrum, a brief history of its use as a dietary supplement, what it contains, and potential benefits.

Milk-derived proteins and peptides in clinical trials.
Postepy Hig Med Dosw (Online). 2013 Aug 6;67:800-16; Artym J1, Zimecki M.
Author information
Abstract
Clinical trials are reviewed, involving proteins and peptides derived from milk (predominantly bovine), with the exception of lactoferrin, which will be the subject of another article. The most explored milk fraction is α-lactalbumin (LA), which is often applied with glycomacropeptide (GMP) – a casein degradation product. These milk constituents are used in health-promoting infant and adult formulae as well as in a modified form (HAMLET) to treat cancer. Lactoperoxidase (LCP) is used as an additive to mouth hygiene products and as a salivary substitute. Casein derivatives are applied, in addition, in the dry mouth syndrome. On the other hand, casein hydrolysates, containing active tripeptides, found application in hypertension and in type 2 diabetes. Lysozyme is routinely used for food conservation and in pharmaceutical products. It was successfully used in premature infants with concomitant diseases to improve health parameters. When used as prophylaxis in patients with scheduled surgery, it significantly reduced the incidence of hepatitis resulting from blood transfusion. Lysozyme was also used in infected children as an antimicrobial agent showing synergistic effects in combination with different antibiotics. Proline-rich polypeptide (PRP) was introduced to therapy of Alzheimer's disease patients. The therapeutic value of PRP was proved in several clinical trials and supported by studies on its mechanism of action. Concentrated immunoglobulin preparations from colostrum and milk of hyperimmunized cows showed efficacy in prevention of infections by bacteria, viruses and protozoa. A nutrition formula with milk-derived TGF-β2 (Modulen IBD®) found application in treatment of pediatric Crohn's disease. In conclusion, the preparations containing milk-derived products are safe and effective measures in prevention and treatment of infections as well as autoimmune and neoplastic diseases.
PMID:
24018446

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Deep Wounds: Colostrum Dressing Versus Conventional Dressing

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Abstract

Wound dressing plays an important role in wound healing. Newer type of wound dressings includes Biological dressings like collagen granules, colostrum powder, etc. that creates the physiological interface between the wound surface and environment which is impermeable to bacteria. This study was conducted to compare the efficacy of colostrum powder dressing with that of conventional dressing in the management of deep wounds. Hundred consecutive patients with deep wounds (stage II-IV), admitted during the study period were taken for study after considering inclusion and exclusion criteria. Patients were randomly divided into two groups: Colostrum dressing (Group A: n-50) and Conventional dressing (Group B: n-50). The efficacy of both the dressings was compared using following indicators: Reduction in ulcer surface area, rate of granulation, decrease in pain score, percentage wound healing and hospital stay. It was found that the rate of granulation and reduction in ulcer area was significantly more with colostrums dressing ($p< 0.05$). Excellent results with wound healing were observed in 76% patients with colostrums dressing compared to 48% with conventional dressing ($p< 0.05$). Hospital stay for more than 3 weeks was required in 40% patients with conventional dressing compared to 18% with colostrums dressing ($p< 0.05$). Significant reduction in pain was observed in patients with colostrums dressing over subsequent follow ups ($p<0.05$).It was concluded that colostrum powder dressings are safe, promote faster wound healing, and have more patient compliance due to lesser pain. Results indicate that colostrum powder dressings can be used as an adjunct in management of deep wound.

Keywords: Colostrum Dressing, Conventional Dressing, Deep Wounds, Hospital stay, Wound healing

Introduction

Deep wounds are the ones extending deeper, across deep fascia into muscles or deeper structures.[1] Deep wounds are extremely complex and optimal treatment requires an understanding of nutrition, immunology, psychological issues, the physiology and the metabolic interactions among all the major organ systems. Deep wounds that are difficult to treat include diabetic ulcers, venous ulcers, trophic ulcers, pressure sores and necrotizing fasciaitis.[2] These wounds can cause painful lengthy hospital stay, multiple stages of surgeries, permanent disability, prolonged rehabilitation, loss of income and enormous financial burden. Therefore, to tackle these issues, wound dressing plays one of the important roles. It is therefore appropriate that the process and problems of wound healing should be vigorously addressed by all practitioners and investigators involved in the treatment of deep wound patients and in the development and use of new wound repair material.[3]

An ideal dressing used in the wound management should be economical, easy to apply, readily available dressing or method or coverage that will provide good pain relief, protect wound from infection, promote healing, keep moisture, be elastic, and non - antigenic and adhere well to the wound and waiting for spontaneous epithilisation and healthy granulation tissue.[4]

Among newer type of wound dressings - Biological dressings like colostrums powder, collagen create the most physiological interface between the wound surface, environment and impermeable to bacteria.[5] Colostrum powder contains many cells, repair and growth factors which are responsible for healthy cell growth and repair of tissues like the skin, muscle, cartilage and bone. Colostrum powder dressing has certain advantages over conventional dressing like

healthy granulation tissue formation, greater reduction in inflammatory cells, decreased days of healing and decreased pain.[6] This study was conducted to compare the efficacy of colostrum powder dressing with that of conventional dressing in the management of deep wounds.

Materials and Methods

Data was collected from all patients with deep wounds (stage II-IV), who were admitted during the study period after considering the inclusion and exclusion criteria. The Colostrum powder was procured in the form of colostrums capsules from bovine colostrums. Information was collected through a pre-designed, pre-tested proforma. All patients were interviewed as per the proforma and a complete clinical examination was done. Cases were randomly test group (colostrums dressing) and control group (conventional dressing). Conventional dressing was done with betadine and hydrogen peroxide. The efficacy of both the dressings was compared using following indicators: Reduction in ulcer surface area, rate of granulation, decrease in pain score, percentage wound healing and hospital stay. While analysing the wound healing following scoring key was used [6]:

Below 25%: Poor

Between 26-50%: Satisfactory

Between 51-75%: Good

76% & above: Excellent

Sample size

Hundred consecutive patients were randomly divided into two groups: Colostrum dressing (Group A: n-50) and Conventional dressing (Group B: n-50).

Inclusion criteria

- Patients age 20-60 years
- Deep wounds (Stage II-IV)

Exclusion criteria

- Patients who were suffering from arterial disease
- Patients not willing to participate

In the pre-intervention period the measurement of following variable was carried out for all patients: size of wound, ulcer surface area and pain score and stage of wound by using structured observation. After initial assessment, dressing of colostrums powder and betadine/ hydrogen peroxide was carried out for patients of group A and B respectively. Application of colostrum powder was done twice in a day and the observation was done on 3rd, 7th, and 14th day of application.

Statistical Analyses

Data from observation related to wound healing before and after dressing was analysed using SPSS 17.0 software (SPSS, Chicago, IL, USA). The mean values were compared using Student's -test. The frequency distributions were compared using chi-squared test. Statistical significance was assumed when the p value was <0.05.

Results

Baseline demographic variables were similar in both groups ($p>0.05$) (table 1). Rate of granulation and reduction in ulcer area was significantly more ($p< 0.05$) with colostrums dressing (table 2). Excellent results with wound healing were observed in 76% patients with colostrums dressing compared to 48% with conventional dressing ($p< 0.05$) (table 3). Hospital stay for more than 3 weeks was required in 40% patients with conventional dressing compared to 18% with

colostrums dressing ($p< 0.05$) (table 4). Significant reduction in pain ($p<0.05$) was observed in patients with colostrums dressing over subsequent follow ups (graph 1).

Discussion

Deep wounds that are difficult to treat, includes venous ulcers, diabetic ulcers, trophic ulcers, pressure sores and necrotizing fasciitis. Colostrum contains many immune factors, making them suitable for topical use in the wounds. Due to its anti-inflammatory, anti-viral and anti-bacterial properties, it is suitable for oral/ topical applications. There are seven different growth promoters identified in colostrum involved in growth and repair of body cells. Three of the seven factors identified are involved in the healing of wounds. EgF (Epidermal growth factor), Nucleotides, TgF (Transforming growth factors), IgF-I (Insulin-like growth factor) and FgF (Fibroblast growth factors) stimulate skin growth, cellular growth and repair by direct action on RNA and DNA. These growth factors facilitate the healing of tissues of damaged by ulcers, trauma, burns, surgery or inflammatory disease.

In our study most commonly affected age group is 31 to 50 y of age and males are more affected compared to females. In colostrum dressing group 18% patient stayed for 3-4 wk while in conventional dressing group 40% patient stayed 3-4 wk. Rate of granulation and reduction in ulcer area was also significantly more with colostrums dressing ($p< 0.05$). About 76% patients had healing of ulcer more than 75% and 24% had healing between 51-75% in colostrums group while 48% patients had healing of ulcer more than 75% and 46% had healing between 51-75% in conventional group. Colostrum contains many cells and repair factors, which are important for healthy cell growth. Colostrum also decreases the amount of discharge from wound and fastened the healing process. So, in colostrum group there is faster healing and shorter hospital stay. Barry M et al. found that Colostrum is a powerful agent when applied externally[7].

A colostrum powder dressing has another advantage over conventional dressing in terms of non-immunogenic, non-pyrogenic, being natural, easy application, hypo allergic and pain free [8]. A study by Dr. Sporn et al., reported in Science stated that "Polypeptide Transforming Growth Factors (TGF A & B) and Epithelial Growth Factor Isolated from Bovine Colostrum Used for Wound Healing" because growth factors in bovine colostrum were found to be very effective in promoting wound healing. Ashok YK et al. have shown that colostrum is most effective at promoting healing of injuries when it is both taken internally and applied topically to the affected area [6].

A clinical research study by Dr. Bhora et al., found that for promoting wound healing growth factors present in colostrums had certain important part [9]. Noda et al., discovered that TGF A & B present in bovine colostrum were helpful in embryonic development, cell proliferation and tissue repair like cellular activities [10]. Skottner, Arrhenius-Nyberg, Kanje and Fryklund observed that IGF-1 had role in significant body weight gain and significant bone growth. After Topical application to wounds, it resulted in more effective healing [11]. Allen and Rankin, observed that Fibroblast growth factor (FGF), Insulin like Growth Factor (IGF-1) and Transforming Growth Factor (TGF-b), when administered in combination these factors induce growth, proliferation and regeneration of satellite cells. After sometimes these cells will fuse with one another or the adjacent muscle fiber thereby increasing myonuclei numbers for growth and repair. All three Factors are found in Bovine Colostrum [12].

Based on the findings of present study, it can be concluded that colostrum dressing can decrease the hospital stay, promote ulcer healing and decrease pain in cases of deep ulcers. Though at present many different types of dressings like honey dressings, vacuum assisted dressings, hyperbaric oxygen therapy, collagen sheet application and herbal medication like turmeric powder has been tried. Colostrum dressing is cheap, easily available, non immunogenic, easy to apply, provide good pain relief, protect wound from infection and promote healing. So, in future it can be a useful measure for management of deep wounds.

Conclusion

Colostrum powder dressing is non-allergic, safe and promotes faster wound healing. Patient's compliance is more as it causes less pain while the dressing is changed. The above results indicate that colostrum powder dressings may be used as an adjunct in management of deep wound.

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Tables and Figures

Table 1. Distribution based on Patient's Characteristics

Variable		**Group A (n-50)**	**Group B (n-50)**	**p- value**
Gender	**Male**	31	33	**0.83**
	Female	19	17	
Age (years)	**< 30**	6	7	**0.81**
	31- 50	29	26	
	> 50	15	17	
Type of Onset	**Trauma**	33	36	**0.77**
	Spontaneous	16	14	

Table 2. Distribution based on Rate of granulation and Reduction in Ulcer surface area

Variables	Group	N	Mean	SD	p- value
Rate of Granulation (cm2)	A	50	0.76	0.05	< 0.05
	B	50	0.50	0.07	
Reduction in Ulcer Area (cm2)	A	50	29.27	5.02	< 0.05
	B	50	25.13	8.88	

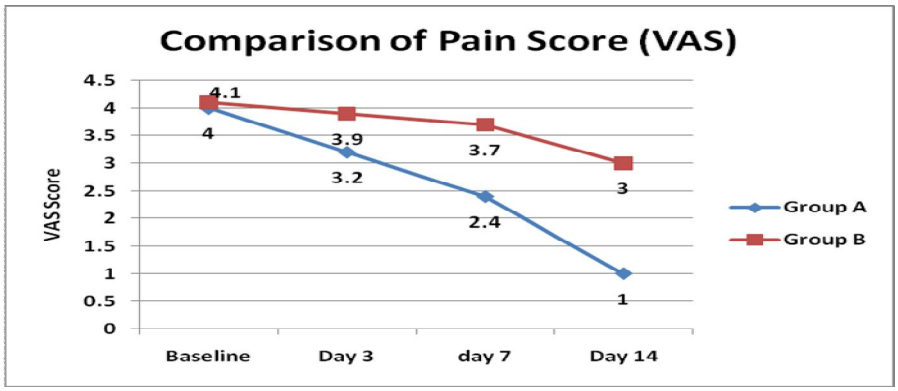
Table 3. Distribution based on assessment of wound healing

Wound Healing	Group A (n-50)	Group B (n-50)	p- value
Poor (< 25%)	0	0	< 0.05
Satisfactory (26-50%)	0	3	
Good (51-75%)	12	23	
Excellent (> 75%)	38	24	
Total	50	50	

Table 4. Distribution based on hospital stay

Hospital Stay (weeks)	Group A (n-50)	Group B (n-50)	p- value
1 - 2	17	10	< 0.05
2 - 3	24	20	
3 - 4	9	20	
Total	50	50	

Graph 1. Comparison of Mean Pain Score between the groups



ENEX PHARMA

TRACKING SHEET: DIABETIC FOOT

Name: Mrs Coulibaly Epse Traoré Date of **birth:** 26 /01/1960 **Profession:** Trader **Contact:** 07 07416324
First names: Salimata Nationality: Ivorian **T2D treatment: ADO then** insulin Third **party contact:** 0101055884
Sex F File No.: DOKUI

Date	Foot grade	Day (J)	Blood sugar or HbA1c	Description Clinical	Description radiological	Antibiotic-therapy	Bandage	
							Dakin	Cream
16 09 2021	II	J1	3 ,10 g/l **9,2%**	Non-necrotic ulceration Of the right leg inflammatory and painful by burning		-Staphypen capsule -Fagyl 500mg -novalgin cp		
Lost to sight								
30 11 2021	II	J71	1.85g/l **10,4%**	Yellowish ulceration +inflammation of the leg	-	Fucidine cp Oflocet cp Dynapar cp	-	**X**
03 12 2021	II	J74		Slightly necrotic ulceration				
06 12 2021	II	J80	0.92g/l	Reddish clean ulceration regression of inflammation	-	DITTO	-	**X**
0712 2021	II	Day81	1.52g/l	Good clinical course Red wound ,bud	-	DITTO	-	**X**
13 12 2021	II	J87		Healing in progress				**X**
23 12 2021		J97		Cicatrization				**X**
31 12 2021		J105		Cicatrization				**X**
09 01 2022		J114		Cicatrization				**X**
14 01 2022		Day119	**0.93 g/l 8,2%**	Cicatrization				**X**

Day1

Day74

Day77

Day80

Day81

Day87

Day97

Day105

Day114

Day119

ENEX PHARMA

<u>TRACKING</u> SHEET: DIABETIC FOOT

Name: Mrs PEHE **Date of birth:** 1957 **Profession: Trader Contact:** 05 05 33 57 55
First names: Opportune **Nationality:** Ivorian Treatment **T2D:** insulin Third party **contact:** 05 45 40 06 45
Gender: F File No.: 53057

Date	Foot grade	Day (J)	Blood sugar or HbA1c	Description clinical	Description radiological	Antibiotic-therapy	Bandage	
							Dakin	Cream
09 12 2021	I	Day1	2.72 g/l	Non-necrotic ulceration of the left big toe from the outer edge to the fixed edge(preciousfoot) + intumescence	No osteitis	-Fucidine Cp -oflocet Cp	-	**X**
14 12 2021	I	J6	1.84g/l **6,7%**	Clean ulceration (red) + intumescence	-	DITTO	-	**X**
16 12 2021	I	J8	1.36g/l	Clean non-swollen healing	-	Staphypen capsule	-	**X**
24 12 2021	I	J16	1.52g/l	Clean healing	-	Staphypen capsule	-	**X**

Day 1 :

Day6 :

Jour8:

Day16:

Observational study conducted in the Oncology Department at the Magodent Hospital at A.E. Fieldorf "Nil" 40 in Warsaw

INTRODUCTION

The primary care issue in patients undergoing oncological therapies, in which drugs are administered intravenously, is to prevent venous complications and perform an early intervention at the time of their occurrence.

The most common form of administration of the cytotoxic drug is the intravenous route. This often leads to the occurrence of an adverse and dangerous phenomenon, which is vein irritation or drug extravasation.

Cytostatics are drugs with a strong irritant effect on the walls of blood vessels, where intravenous administration itself may cause hypersensitivity reactions in patients. The first symptom indicating this is redness in the place of injection.

Some patients may also suffer from phlebitis and irritation of blood vessels.

In addition to the risk resulting from the irritability of cytotoxic drugs, there are additional factors that increase the risk of venous complications in patients undergoing oncological therapies.

They include:

1. The age of the patient (e.g. deposition of atherosclerotic plaques in blood vessels)
2. Comorbidities (e.g. diabetes)
3. Multiple venipunctures.

STUDY DESCRIPTION

The severity of changes was measured on a five-point scale.

0^o – lack of skin lesions

1^0- slight discolouration

2^o- moderate skin discoloration changing into irritation - redness

3^o- irritation and pain in the affected area

4^o- irritation and swelling in the affected area

5^o- a not healing and open wound

Bearing in mind the age of patients and other comorbidities which they suffer from, one should carefully observe the venipuncture to identify lesions that indicate an adverse reaction to the drug.

Additionally, in patients diagnosed with diabetes, an important element is maintaining proper blood sugar levels. Diabetic skin is sensitive to irritation, damages, chemicals and infections. It is associated with a high risk of complications and hence the difficulty in healing skin lesions.

7 women and 13 men aged 51-86 participated in the study.

The observations were conducted from October 9, 2018 to January 7, 2019.

STUDY OBJECTIVE

Determination of the effectiveness of DIABETEGEN in patients undergoing oncological therapies, who have had local skin lesions after administration of cytotoxic drugs.

STUDY MATERIAL AND METHODS

The study covered 20 patients with skin lesions after the administration of 5 Fluorouracil. Each subsequent intravenous administration of chemotherapy results in lesions along the vein. On the site of the venipuncture it is possible to observe red or dark brown lesions. Additionally, they might be accompanied by a burning sensation or pain. Each subsequent administration of chemotherapy causes further irritation of veins which become dark and less visible. There are difficulties in ensuring access to the vein, which may shift the cycle of chemotherapy.

The patients used DIABETEGEN according to recommendations, that is, 3 times a day, applying it on the skin affected by lesions. Lesions appeared on hands and forearms. In the group of study participants, 4 patients suffered from diabetes.

All the patients applied DIABETEGEN on their own.

Check-ups were performed once per 2-3 weeks, depending on the chemotherapy cycle. Each patient had at least 2 check-ups, which were noted down in the observation cards.

In the group of study participants there were no patients with lesions at the 1, 4 and 5 stage.

RESULTS

The stage of lesions before using DIABETEGEN	**The number of patients with lesions at a given stage**
stage 3	9 (45%)
stage 2	11 (55%)

Table 1. The ratio of the number of patients to the stage of their lesions.

The stage of skin regression after using DIABETEGEN	**Ilość osób**
Lack of improvement	6 (30%)
Lesion regression by 1-2 stages	14 (**70%)**

Table 2. The ratio of the number of patients to the stage of lesion regression.

9 patients (45%) suffered from lesions in stage 3, whereas 11 (55%) – in stage 2. 6 patients (30%) did not experience any improvement after using Diabetegen. After regular application of the preparation, it was possible to observe regression of lesions by 1-2 stages in 14 patients (70%), including one person with complete healing of the lesion.

Skin lesions were observed on patients' hands and forearms. They appeared on the site of the insertion of the intravenous cannula. 11 patients (55%) had skin lesions on their hands, whereas 9 patients (45%) – on their forearms. Among these, 4 patients with lesions on their forearms did not observe any positive effects of Diabetegen. 5 patients with lesions on their hands and 5 patients with lesions on their forearms observed the regression of lesions by 1 stage. 4 patients with lesions on their hands experienced complete healing.

There were 4 patients with diabetes type 2 (20%). Lesions on the forearm, stage 3 – 1 patient, stage 2- 3 patients. No improvement observed in 1 patient.

FINDINGS

Patients undergoing oncological therapies require increased supervision of nursing staff, close observation and rapid as well as effective action.

Patients undergoing chemotherapies, who suffered from post-transfusion lesions observed on the injection site, underwent an intensive treatment with DIABETEGEN.

The regression of lesions which appeared after the administration of 5 Fluorouracyl was observed in 70% of patients.

Lesion progression was not observed in any of the patients using DIABETEGEN.

On the basis of the study results, DIABETEGEN received **the best evaluation compared with other products healing wounds available on the Polish market.**

The study was conducted by:

Health Care Manager

Agnieszka Staniak

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Original Research Article

Role of Bovine Colostrum in Healing of Chronic Non-Healing Ulcers – A Clinical Study

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Abstract
This study was conducted to compare the efficacy of colostrum powder as an add on dressing with that of conventional dressing in the healing of chronic non-healing ulcer. Sixty patients with non-healing ulcer were treated with conventional dressing (povidone iodine, hydrogen peroxide) (Group 1) and another 60 patients were treated with add-on Bovine Colostrum (Group 2) for a period of 3 weeks. The healing of ulcer in both groups was determined by using Resvech Ulcer Healing Score (which utilizes 9 ulcer parameters) at weekly intervals for 3 weeks. There was highly significant increase in wound healing in the Bovine Colostrum treated group in comparision to the conventionally treated group, starting from first week upto third week. Bovine colostrum has significant wound healing property.
Keywords: *Resvech Score, Conventional dressing, Diabetic Ulcer.*

Introduction

Non healing ulcer have a significant impact on the health and quality of life of patients and their families, causing pain, loss of function and mobility, depression, distress and anxiety, embarrassment and social isolation, financial burden, prolonged hospital stays and chronic morbidity or even death[1]. Chronic non-healing ulcers could be due to systemic conditions such as diabetes mellitus, tuberculosis, leprosy, venous ulcers, pressure sores, atherosclerosis, and traumatic vasculitis[2]. Complications of chronic ulcer include infection such as cellulitis and infective venous eczema, gangrene, haemorrhage and lower-extremity amputations. Chronic ulcer lead to disability and disability worsens ulcer outcomes resulting in a vicious cycle[3].

The art of suitable and appropriate management of non-healing ulcer remainsa major hurdle in medical science. Inspite of recent advances in aseptic techniques, antimicrobial chemotherapy and ulcer management, several types of ulcer have proved recalcitrant. Conventionally dressing techniques using povidone iodine, hydrogen peroxide, normal saline, debridement of dead and devitalized necrotic slough and tissues, control of

infections by antibiotics, rest, skin care have remained the mainstay of management. But many ulcers take too long to heals with these modalities esp. diabetes mellitus, sickle cell disease, leprosy, Buerger's disease, deep vein thrombosis, atherosclerosis, etc.

Newer biological dressings like PDGF, stem cell and bovine colostrums (BC) dressings which are enriched with growth and immune factors for formation of healthy granulation tissue and early epithelialisation; are recently known to have revolutionized ulcer healing[4].

Thus this study is conducted to compare the efficacy of colostrum powder as an add on dressing with that of conventional dressing in the healing of chronic non-healing ulcer.

Patients and Method

This was an open label quasi experimental study conducted in our tertiary care teaching hospital between 2015 to Oct 2017.

All adult Patients of both gender attending the OPD or admitted to Indoor wards for treatment of their non-healing ulcers were included in the study. The ulcer was of three months duration or longer duration[5] and were of any aetiology. The patients who were hypersensitive to colostrums or having history of lactose intolerance were excluded from the study. Also patients suffering from concurrent illness that may interfere with treatment like carcinoma, tuberculosis, Hansen's disease, connective tissue diseases, severe anaemia, or with ulcers with clinical signs of heavy infection were excluded.

The study protocol, study questionnaire and case record form was designed and approved by the Institutional Ethics Committee before the start of the study. Patients satisfying the inclusion and exclusion criteria were explained about the nature of the study and informed consent was taken from them before enrolment. Data was collected from the study subjects through interview and physical observation regarding their demographic profile, underlying diseases, duration of wound, past history of medication or allergy and drug history. Details regarding the baseline physical examination findings like type of ulcer, site, mode of onset and symptomatology were recorded in the Case Record Form (CRF). The enrolled patients were given either conventional treatment or add on treatment with Bovine Colostrum by the treating physician.

- **Group 1** – Conventional Dressing (cleansing with Normal Saline and wound debridement followed by toileting with various topical agents like Povidone Iodine, Hydrogen Peroxide, Silver Suphadiazine and Rectified spirit on daily basis.
- **Group 2** – Conventional Dressing with Add-on Bovine Colostrum Oral Capsules and Powder (Alchemist, Solan, Himachal Pradesh) applied to margins, edges, floor and base of ulcer and wound area covered by a normal saline soaked gauge.

Assessment of Wound Healing *:* Healing of ulcer was determined by using a Ulcer Healing Score designed by RESVECH SCALE V1.0(5) (Resvech score) utilizing 9 parameters (area, depth, edges, perilesional maceration, tunneling, type of tissue in wound bed, exudates, infection and pain (using VAS score) measured at baseline and at weekly follow-up intervals for 3 weeks.

Statistical analysis: Categorical data was calculated by Pearson's chi square test and continuous data calculated by Student's unpaired t- test using SPSS 21.

Result

A total of 120 patients with chronic non-healing ulcers were enrolled into the study out of which 96 were males and 24 were females; most of these patients were between 31 - 50 years. (Table 1)

These patients had chronic ulcers due to various etiology and were assigned to two treatment groups (Conventional Treatment (Group-1) and Add On Bovine Colostrum (Group-2)) (Table 2)

Resvech Score was measured in all the patients in both groups at baseline and weekly for 3 weeks and the Mean Score in all the 60 patients was calculated and compared statistically. (Table 3)

Table 1 Age and Sex Distribution of Patients with Chronic Non Healing Ulcer

Age	Male	Female	Total
21-30	15	3	18
31-40	28	10	38
41-50	26	4	30
51-60	19	3	22
61-70	8	4	12
Total	96	24	120

Table 2 Type of Chronic Ulcer in Each Treatment Group

Sr no	Type of Ulcer	Group-1 (Conventional Dressing) n=60	Group-2 (Bovine Colostrum Add On Dressing) n=60
1	**Diabetic foot ulcer**	32	32
2	**Trophic ulcer**	10	12
3	**Sickle cell disease ulcer**	8	9
4	**Venous ulcer**	5	5
5	**Necrotising fasciitis**	2	0
6	**Fournier gangrene**	1	1
7	**Post burn**	1	1
8	**Buerger's disease ulcer**	1	0
	Total	60	60

Table 3 Mean Resvech Score at Baseline and Follow-Up (max. score = 40)

Groups	Parameter	Baseline	After 1st Week	After 2nd Week	After 3rd Week
Group 1 (n = 60)	Mean Resvech Score ± SEM	27.87±3.549	25.07±4.133	21.53±4.424	16.88±4.826
	% of Healing from Baseline	-	37.35%	46.18%	57.8%
Group 2 (n = 60)	Mean Resvech Score ± SEM	25.47±4.268	15.78±5.663	9.50±3.981	4.83±2.895
	% of Healing from Baseline	-	60.55%	76.25%	87.93%
Group1 vs Group 2	P value (chi-sq)	0.001	0.000	0.000	0.000

Discussion

Non-healing ulcers that are difficult to treat, includes venous ulcers, diabetic ulcers, trophic ulcers, pressure sores, sickle cell disease ulcer and necrotizing fasciitis. The effect of different topical agents on the healing of the abovementioned ulcers has been subject of extensive research but only few have been properly controlled. Bovine colostrum has attracted the attention of a lot of contemporary research due to immense untapped potential they possess towards healing many recalcitrant ulcers Colostrum contains many immune and growth factors like EGF (epidermal growth factor), TGF (transforming growth factor), IGF(insulin like growth factor), FGF(fibroblast growth factor), stimulate skin growth, cellular growth, and repair by direct action on DNA and RNA[6].

Few researchers have conducted studies on bovine colostrums on wound healing. Thapa (2005) observed bovine colostrum to have chemical debridement action, deodorization of offensive wounds, absorption of edematous fluid around ulcers, antibacterial and anti-inflammatory actions which were of greatest clinical advantage in diabetic ulcers, trophic ulcers, sickle cell disease ulcer, Fournier gangrene and necrotizing fasciitis[7]. According to study by Khirsagar Y et al in 2015 the anti-inflammatory, anti-viral and anti-bacterial properties of bovine colostrums makes it suitable for oral/ topical applications[6].

In our study most commonly affected age group of non healing ulcer is 31 to 50 yr of age and males are more affected compared to females which is corroborated by study of Chaterjee et al (2015)[8] and Khirsagar Y et al (2015)[6].

In our study there was highly significant ($p<0.001$) improvement in healing rate in bovine colostrum add on group at the end of first, second and third week of treatment as evident from Resvech score. The healing rate improved from 60.55% at end of first week to 87.93% in third week of treatment in bovine colostrum add on group in comparison to conventional dressing group which showed healing rate from 37.35% to 57.8% in same time period. Studies by Chaterjee et al (2015) and Khirsagar Y et al in 2015 confirmed that bovine colostrum improves healing rate appreciably in non healing ulcers in comparison to conventional dressing[8][6].

Conclusion

Bovine colostrum produces very significant increase in healing of chronic non-healing ulcers due to varying aetiology and has a remarkable potential in revolutionizing management of non healing ulcers.

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Original Article

Surgery Section

A Comparative Study of Colostrum Dressing Versus Conventional Dressing in Deep Wounds

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ABSTRACT

Introduction: Deep wounds are extending deeper, across deep fascia into muscles or deeper structures. Understanding of nutrition, immunology, psychological issues, the physiology and the metabolic interactions require for optimal treatment of deep wounds. Wound dressing plays one of the important roles in wound healing. Newer type of wound dressings - Biological dressings like colostrum powder, collagen granules create the physiological interface between the wound surface and environment which is impermeable to bacteria.

Aim: To compare the efficacy and safety of colostrum dressing and conventional dressing in deep wounds.

Materials and Methods: Data was collected from all patients with deep wounds (stage II-IV), admitted during the period of April 2013 to March 2014, considering the inclusion and exclusion criteria.

Results: Less number of dressings, short healing time, rapid healing and decrease pain seen in colostrum dressing group compared to conventional dressing group.

Conclusion: Colostrum powder dressings are safe, promoter of wound healing, more patient compliance in terms of less pain, less number of dressing required. This treatment though found to be more expensive than conventional dressings; results indicate that colostrum powder dressings may be used as an adjunct in management of deep wound.

Keywords: Discharge, Healing time, Pain

INTRODUCTION

Deep wounds extend deeper, across deep fascia into muscles or deeper structures. Understanding of nutrition, immunology, psychological issues, the physiology and the metabolic interactions are required for optimal treatment of deep wounds. These deep wounds can cause various morbidities in the form of prolonged hospital stay, multiple surgeries, permanent disability and deformity, prolonged rehabilitation and enormous economical problems. Therefore, to tackle these issues, wound dressing plays one of the important roles. It is therefore appropriate that the process and problems of wound healing should be vigorously addressed by all practitioners and investigators involved in the treatment of deep wound patients and in the development and use of new wound repair material [1].

The properties of ideal dressing used in the wound management are that, it should be economical, easy to apply, readily available, a dressing or method or coverage that will provide good pain relief, protect wound from infection, promote healing, maintain moisture, be elastic, and non - antigenic and adhere well to the wound and untill spontaneous epithelisation occurs and healthy granulation tissue is formed [2]. In 150 A.D the Greek surgeon, Galen of Pergamum had first addressed the fact that the wound should be kept moist to ensure adequate healing [3]. Among newer type of wound dressings - Biological dressings like colostrums powder, collagen create the most physiological interface between the wound surface, environment and impermeable to bacteria [4].

Colostrum powder contains many cells, repair and growth factors which are responsible for healthy cell growth and repair of tissues like the skin, muscle, cartilage and bone. Colostrum powder dressing has certain advantages over conventional dressing, like healthy granulation tissue formation, greater reduction in inflammatory cells, decreased days of healing and decreased pain.

This study is conducted to compare the efficacy of colostrum powder dressing with that of conventional dressing in the management of deep wounds.

AIM

To compare the efficacy and safety of colostrum dressing and conventional dressing in deep wounds in terms of reduced pain, healing time, number of dressings healing quality and complications.

MATERIALS AND METHODS

Source of data

Data was collected from all patients with deep wounds (stage II-IV), who were admitted during the period of April 2013 to March 2014, for study considering the inclusion and exclusion criteria. In this study experimental research method was used to assess role of colostrums powder dressing on wound healing. The Colostrum powder was commercially procured in the form of colostrums capsules from bovine colostrums (Immurich). Information was collected through a predesigned pretested proforma prepared by investigators for each patient.

All patients were interviewed as per the proforma and a complete clinical examination was done. Cases were randomly selected and allocated into test group and control group, Cases allocated in test group treated with colostrums powder dressing and Cases allocated in control group treated with conventional dressings. Conventional dressings were done with betadine and hydrogen peroxide. In this study, effect of colostrums powder dressing was studied by evaluating and comparing the dependent variables within experimental and control group. The dependent variables were process of wound healing in the form of size of wound, soakage of wound, amount and colour of exudates, pain experienced by patient and decrease in stages of wound during the days of application of colostrums powder on wound for 15 d.

Sample size

In this study samples were the patients admitted in the hospital during study period having ulcers and pressure sores ranging from

stage II-IV. Two hundred patients were selected randomly after meeting inclusion and exclusion criteria. Which were further divided into two groups. One group with Colostrum dressing (n=100) and other with conventional dressing (n=100).

Inclusion criteria

Patients of age 20-60 y with deep wounds of stage two to four and willing to participate in study were included in the study.

Exclusion criteria

Patients of age below 20 y with ulcer in stage one and patients who were suffering from arterial disease and not willing to participate in the study were excluded from the study. In the pre-intervention period the measurement of dependent variable was carried out for all patients of both the groups. The measured dependent variables were size of wound, soakage of wound, amount and colour of exudates, pain, sepsis, type and stage of wound by using structured observation.

After initial assessment, the manipulation of independent variable i.e. intervention of treatment of colostrums powder on wound carried out for the samples of experimental groups. For the samples of experimental group the observations (O) and application of colostrum powder was done twice in a day 7am and 7pm (stage 3 and 4) the observation was done on every 3rd day. i.e. 3rd, 6th, 9th,12th and 15th day of application.

RESULTS

For the patients of conventional dressing group, the series and timing of observation was same as that of experimental group [Table/Fig-1]. Observation checklist to assess rate and process of wound healing for 15 d areas included were size of wound, temperature, soakage of wound, amount and colour of exudates, sepsis, pain, margins of ulcer etc.

While analysing the scores of wound healing following scoring key was used [5]:

Sr.no.	Percentage	Grade
1	Below 25%	Excellent
2	26-50%	Good
3	51-75%	Satisfactory
4	76 & above	Poor

Data from observation related to wound healing before and after was analysed in frequency and percentage.

DISCUSSION

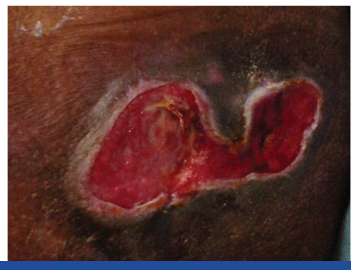
Deep wounds that are difficult to treat, includes diabetic ulcers, venous ulcers, trophic ulcers, pressure sores and necrotizing fasciitis. Colostrum contains many immune factors which make them suitable for topical use in the wounds. Due to its anti-viral, anti-bacterial, and anti-inflammatory properties, it is suitable for oral and/or topical applications. There are seven different growth promoters identified in colostrum involved in growth and repair of body cells. Three of the seven factors identified are involved in the healing of wounds. Nucleotides, EgF (Epidermal growth factor), TgF (Transforming growth factors), FgF (Fibroblast growth factors) and IgF-I (Insulin-like growth factor) stimulate skin growth, cellular growth and repair by direct action on DNA and RNA. These growth factors facilitate the healing of tissues of damaged by ulcers, trauma, burns, surgery or inflammatory disease.

In our study most commonly affected age group is 41 to 50 y of age and males are more affected compared to females [Table/Fig-12-14]. In colostrum dressing group 20% patient stayed for 3-4 wk while in conventional dressing group 40% patient stayed 3-4 wk. Which is almost double than the colostrum dressing group [Table/Fig-15]. Colostrum contains many cells and repair factors, which are important for healthy cell growth. So, in colostrum group there is fast healing and short stay at hospital.

Eighteen patients had percentage reduction of ulcer between 91-99%, 50 patient had percentage reduction between 81-90%. 4

[Table/Fig-1]: Preintervention Day-0

[Table/Fig-2]: Intervention Application of Colostrum powder



[Table/Fig-3]: Postintervention Day-3

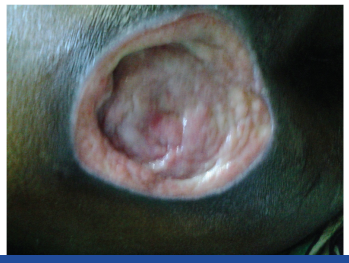
[Table/Fig-4]: Postintervention Day-6

[Table/Fig-5]: Conventional dressing group Day-0

[Table/Fig-6]: Post Intervention Observation (Day-15) Colostrum group

[Table/Fig-7]: Observation (Day-0) conventional dressing group

[Table/Fig-8]: Postintervention Day-15



[Table/Fig-9]: Conventional dressing group Day-6

[Table/Fig-10]: Postintervention Day-12

[Table/Fig-11]: Observation (Day-15) conventional dressing group

Sr.no	Age (Years)	No. of patient	Percentage (%)
1	21-30	28	14
2	31-40	40	20
3	41-50	74	37
4	51-60	58	29
	Total	200	100

[Table/Fig-12]: Age distribution

Sr.no	Sex	No. of patient	Percentage (%)
1	Male	136	68
2	Female	64	32
	Total	200	100

[Table/Fig-13]: Sex distribution

Sr.no	Type of Onset	No. of patient	Percentage (%)
1	Traumatic	140	70
2	Spontaneous	60	30
	Total	200	100

[Table/Fig-14]: Onset of ulcer

Period of stay (weeks) (A)	No. of patients	Percentage (%)
1-2	35	35
2-3	45	45
3-4	20	20
Total	100	100

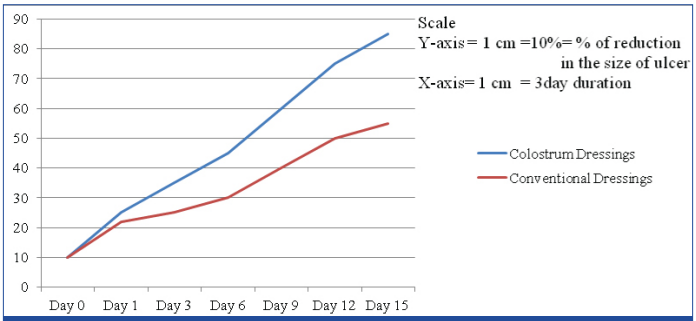
Period of stay (weeks) (B)	No. of patients	Percentage (%)
1-2	25	25
2-3	35	35
3-4	40	40
Total	100	100

[Table/Fig-15]: Average duration of hospital stay (a) colostrum powder dressings (b) conventional dressings

Reduction in size of ulcer (%) (a)	No. of patients
61-70	12
71-80	20
81-90	50
91-99	18
Total	100

Reduction in size of ulcer (%) (b)	No. of patients
61-70	24
71-80	48
81-90	24
91-99	04
Total	100

[Table/Fig-16]: Percentage of reduction of ulcer size (a) Colostrum dressings (b) Conventional dressings



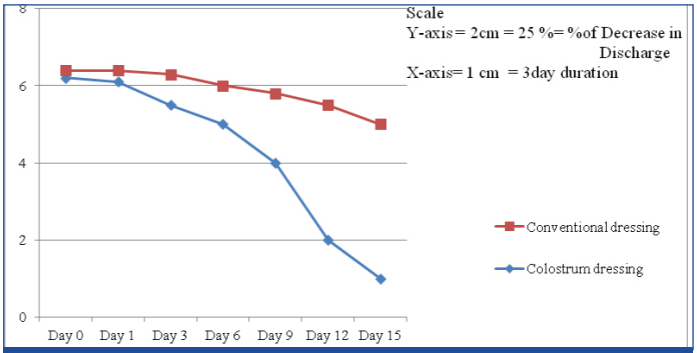
[Table/Fig-17]: Percentage of reduction in the size of ulcer

patients had percentage reduction of ulcer between 90-99% and 24 patients between 81-90% and 48 patients between 71-80% [Table/Fig-16,17].

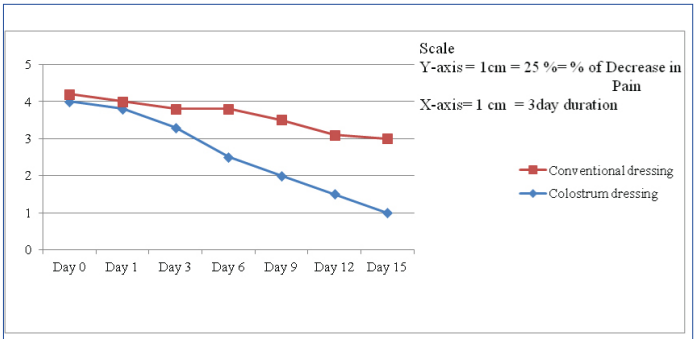
No. of dressings (A)	No. of patients	Percentage (%)
1-5	00	00
6-10	35	35
11-15	31	31
16-20	19	19
21-25	15	15
26-30	00	00
Total	100	100

No. of dressings (B)	No. of patients	Percentage (%)
1-5	00	00
6-10	00	00
11-15	02	02
16-20	34	34
21-25	45	45
26-30	19	19
Total	100	100

[Table/Fig-18]: No. of dressings required (a) Colostrum dressings (b) Conventional dressings



[Table/Fig-19]: Decrease in discharge after colostrum application



[Table/Fig-20]: Decrease in pain after colostrum application

Thirty five patients required colostrum dressing between 6-10, while only 15 patients required between 21-25. 45 patients required conventional dressing between 21-25, which is almost triple time than colostrum dressing. Maximum number of conventional dressing required is 30 [Table/Fig-18]. So, conventional dressings required much more. This is because colostrum decrease the amount of discharge from wound and also fastened the healing leads to decrease in number of dressings [Table/Fig-19]. Barry M, had find that Colostrum proves to be a powerful agent when applied externally [6] [Table/Fig-20]. A colostrum powder dressing has another advantage over conventional dressing in terms of non-immunogenic, non-pyrogenic, being natural, easy application, hypo allergic and pain free [3].

A study by Dr. Sporn et al., reported in Science stated that "Polypeptide Transforming Growth Factors (TGF A & B) and Epithelial Growth Factor Isolated from Bovine Colostrum Used for Wound Healing" because growth factors in bovine colostrum were found to be very effective in promoting wound healing. Our study have shown that colostrum is most effective at promoting healing

of injuries when it is both taken internally and applied topically to the affected area [7]. A clinical research study by Dr. Bhora et al., found that for promoting wound healing growth factors present in colostrums had certain important part [8].

Noda et al., discovered that Transforming growth Factors A and B (TGF A & B) present in bovine colostrum were helpful in embryonic development, cell proliferation, and tissue repair like cellular activities. They also reported it promoted the synthesis and repair of DNA -the master code of the cell [9]. Skottner, Arrhenius-Nyberg, Kanje and Fryklund observed that IGF-1 had role in significant body weight gain and significant bone growth. After Topical application to wounds, It resulted in more effective healing [10].

Allen and Rankin, observed that Fibroblast growth factor (FGF), Insulin like Growth Factor (IGF-1) and Transforming Growth Factor (TGF-b), when administered in combination these factors induce growth, proliferation and regeneration of satellite cells. After sometimes these cells will fuse with one another or the adjacent muscle fiber thereby increasing myonuclei numbers for growth and repair. All three Factors found in Bovine Colostrum [11].

Based on the findings of this study, it can be concluded that colostrum dressing can decrease the hospital stay, promote ulcer healing and decrease pain in cases of deep ulcers. Though at present many different types of dressings like honey dressings, vacuum assisted dressings, hyperbaric oxygen therapy, collagen sheet application and herbal medication like turmeric powder has been tried. Colostrum dressing is cheap, easily available, non immunogenic, easy to apply, provide good pain relief, protect wound from infection and promote healing. So, in future it can be a useful measure for management of deep wounds.

CONCLUSION

Colostrum powder dressing is non-allergic, safe, promotes wound healing. Patient compliance is more as it causes less pain while the dressing is changed, also in terms of less number of dressing required. This treatment however is found to be more expensive than conventional dressings. The above results indicate that colostrum powder dressings may be used as an adjunct in management of deep wound.

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