Evaluation of beneficial health effects of Sikapur® Kieselsäure-Gel on cultured connective tissue fibroblasts

Peter C. Dartsch, Dartsch Scientific GmbH

Besides oxyen, silicon is the most abundant element in the earth’s crust. In humans, it belongs to the most abundant trace elements and is locally associated with bone, skin and blood vessels. Compelling data suggest that silicon is essential for bone formation by affecting cartilage composition and ultimately cartilage calcification. Silicon gel, shown to stimulate collagen type 1 synthesis and osteoblastic differentiation in human osteoblast-like cells in vitro, improve hair tensile strength and positively influences skin surface and its mechanical properties as well as hair and nails. Dietary silicon is predominantly derived from solid plant foods and is readily broken down and absorbed in the gastrointestinal tract in the form of orthosilicic acid. Its bioavailability seems to be strongly influenced by its speciation such as chemical form or matrix.

**Aim of this study**

This study was undertaken to evaluate some health effects such as cellular regeneration or vitality of a formulation consisting of silicic acid with disperse-colloidal silicon dioxide. The questions which should be answered were as follows:

1. Is this specific silicon formulation able to stimulate basal energy metabolism of cultured connective tissue fibroblasts in short-term experiments?
2. Is the formulation able to stimulate vitality and mitotic activity/proliferation in long-term experiments?
3. Is the formulation able to induce cellular and/or cytoskeletal alterations in cultured connective tissue fibroblasts as a sign of cellular uptake and response?

**Silicon formulation**

Sikapur® Kieselsäure-Gel (Silicium Gel F): Silicic acid gel with disperse-colloidal silicon dioxide (MEDOPHARM Arzneimittel GmbH & Co. KG, D-79238 Ehrenkirchen, Germany). Charge 642003; expiry date: 09/2010.

**Cell culture**

This study was carried out with connective tissue fibroblasts (cell line L-929; established from normal subcutaneous and adipose tissue of a male C3H/An mouse; ACC2; Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany). The cells were routinely cultured as mass cultures (cultivated up to 80% confluency before subculturing) in a humidified atmosphere of 95% air and 5% CO2 at 37°C in an incubator. Cells were taken for the experiments at passage 28 to 34.

**Test concentrations**

According to the data sheet, the manufacturer recommends a daily dosage of 15 ml (approximately 15 g). This dosage results in a concentration of 0.25 mg/ml for a distribution within the body fluid (50 to 60 liters) and approximately 5 mg/ml for a distribution within the blood fluid (3.5 liters). Depending on the test assay, the test concentrations varied between 0.1 mg/ml and 20 mg/ml.

**Direct metabolic stimulation of connective tissue fibroblasts**

Experimental design. Cells were grown in 96-well plates for three days to reach approximately 90% confluency. After washing of the cells with phosphate-buffered saline without calcium and magnesium (PBS-), cells were stimulated with phosphate-buffered saline with calcium and magnesium (PBS+) and containing 5 mM glucose at different Sikapur® concentrations. Energy metabolism was measured by the total cellular redox activity resulting in the cleavage and colour change of an externally added orange-red tetrazolium dye (WST-1; Roche Diagnostics, Mannheim, Germany) to yield a yellowish water-soluble formazan. The colour change was continuously monitored (one reading per minute; four sec shaking interval before each reading) by a two-wavelength difference measurement. OD 450 – 690 nm at 37°C ± 0.1°C using a BioTek ELx808 ELSA reader. The double-wavelength measurement proved necessary to eliminate unspecific readings by dye or solution turbidity. For data analysis, OD 450 – 690 nm was corrected by the corresponding blank values. The linear increase in OD 450 – 690 nm during the observation period was taken for the evaluation of the stimulating potential of Sikapur®. All experiments were done in triplicate. Results. As shown in Figure 1, addition of Sikapur® Kieselsäure-Gel resulted in a marked stimulation of basal energy metabolism of cultured connective tissue fibroblasts. The observed stimulatory effect is more pronounced at test substance concentrations in the range from 0.25 mg/ml. However, the stimulation pattern of the test substance showed a Gaussian distribution with a maximum falloff at the lower test concentrations.

**Figure 1:** (A) Typical reading of the changes in optical density (OD 450 – 690 nm) with increasing incubation time demonstrating the linear part of the sigmoidal curve between 40 and 80 min (arrows) as taken in the present set of experiments for quantification and comparison of the results. The curve from 0 to about 30 min demonstrates the uptake of the test substrate until the first effects are achieved and the leveling in the course of the curve beyond 100 min is due to the increase of tetrazolium dye concentration in the reaction mixture. (B) Graphical presentation of the stimulatory effect of Sikapur® Kieselsäure-Gel on total energy metabolism of cultured connective tissue fibroblasts between 40 to 80 min after addition of the test substance (linear part of the measurement curve). Data represent mean value ± S.E.M. (standard error of the mean). BoFl = calculated body fluid concentration = 5 mg/ml; BlFl = calculated blood fluid concentration = 5 mg/ml.
Long-term effect on vitality and mitotic activity/cell proliferation

L-929 cells from 80 to 90% confluent mass cultures were seeded into 12-well plates (cell density: 25,000 cells/well; 1.8 ml of culture medium) and 96-well plates (cell density: 5,000 cells/well; 180 μl of culture medium) and allowed to attach and spread for 24 hours. Thereafter, cells were exposed to Sikapur® Kieselsäure-Gel for 16 hours and methanol fixation. (A) Untreated control cells, (B) 0.25 mg/ml Sikapur®, (C) 5 mg/ml Sikapur®, and (D) 20 mg/ml Sikapur®. Note the alterations after Sikapur® treatment and was in the range of 5 to 9% at body and blood fluid concentrations. At concentrations higher than 10 mg/ml, cell vitality decreased and became even negative (= inhibitory effect) at overdosage concentrations of 20 mg/ml. This activated metabolic state was not related to an increased mitotic or proliferative activity of the cells. A significant difference between the cell numbers of the exposed cells in comparison to the untreated controls was not observed (not depicted).

Morphological and cytoskeletal alterations after Sikapur® treatment
The cytoskeleton of eukaryotic cells is a dynamic three-dimensional structure that fills the cytoplasm and reacts very fast to changes of the internal or external conditions. Basically, the primary types of fibres comprising the cytoskeleton are microfilaments (actin, myosin), microtubules (α- and β-tubulin), and intermediate filaments (vimentin, desmin, cytokeratin). While microfilaments and microtubules are highly dynamic structures and are responsible for movement, cell shape and spindle fibres during mitosis, the intermediate filaments provide tensile strength to the cell and are the cytoskeletal components which are most stable and less dynamic10. Experimental design. Cells from 80-90% confluent mass cultures were seeded on sterile round glass coverslips and allowed to attach and spread for 48 hours. Thereafter, cells were exposed to Sikapur® Kieselsäure-Gel at concentrations ranging from 1 mg/ml to 20 mg/ml for 16 hours. Cells were then fixed by treatment with methanol p.a. for 6 min at -20°C. Fixed cells were examined for morphological alterations. For staining of non-muscle myosin and microtubules, methanol-fixed cells were incubated for 45 min at 37°C in a moist chamber either with anti-pan myosin antibodies or anti-α-tubulin antibodies (both antibodies were from Amersham Buchler, Braunschweig, Germany). After a PBS-wash, the second TRITC-labelled goat anti-mouse IgG + IgM antibody (Sigma Chemie, Deisenhofen, Germany) was applied for 60 min at 37°C in a moist chamber. Then, cover slips were washed in PBS- and embedded in Mowiol 4-8811. As depicted in Figure 3, exposure to Sikapur® Kieselsäure-Gel for 16 hours did not result in marked alterations of cell morphology such as rounding, retraction or flattening of the cells. However, a dose-dependent cytoplasmic vacuolization was observed at test concentrations > 5 mg/ml. The cause for this phenomenon remains unclear, but demonstrates that the cells obviously responded to the exposure to Sikapur® Kieselsäure-Gel. Exposure of cells to Sikapur® Kieselsäure-Gel did not result in marked alterations of the cytoskeleton (Figure 4). Although microtubules seemed to become more abundant with higher Sikapur® concentrations, a marked difference was not observed between all concentrations tested. The intracellular distribution of non-muscle myosin was also unaffected for concentrations up to 5 mg/ml in comparison to untreated controls. Thereafter, non-muscle myosin staining became less prominent with several distinct cytoplasmic vacuoles as already observed at phase contrast (not depicted).

Summary and conclusions
The investigations presented here demonstrate that Sikapur® Kieselsäure-
Circulating insulin levels in the three hours after native whey protein as well as a 43% increase in produced 28% higher peak insulin levels than Optipep (RTD and IRTD) and recovery powders. The research, carried out at the University of Limerick highlights the increased insulinotrophic effects of Carbery’s hydrolysed whey protein, Optipep™, compared to native whey protein. The research, carried out at the Department of Physical Education and Sports Science at the University of Limerick highlights the value of an increased insulin response in aiding recovery and reducing muscle injury post exercise. The market for sports products continues to increase, boosted by growing interest from recreational sportspeople and active consumers. The added performance offered by Optipep will be of interest to manufacturers looking to tap into the lucrative sports nutrition market such as cereal and protein bars, beverages and preventing muscle injury. In addition, the high biological value of the ingredient and the antioxidant effects of its amino acids offer further benefits for professional and amateur sports people. In effect, the enhanced insulinitrophic response of Optipep relative to other proteins means that less protein is required to provide the same rise in insulin.”

Aine Hallihan, head of R&D, Carbery Food Ingredients comments: “The combined effects of increased insulin response and elevated circulating amino acid levels demonstrated by Optipep in the critical post exercise recovery period point to the ingredient’s effectiveness for refuelling muscles and preventing muscle injury. In addition, the high biological value of the ingredient and the antioxidant effects of its amino acids offer further benefits for professional and amateur sports people. In effect, the enhanced insulinitrophic response of Optipep relative to other proteins means that less protein is required to provide the same rise in insulin.”

Paul Donegan, Carbery Food Ingredients’ marketing manager adds: “The opportunities available to manufacturers and marketing companies in sports nutrition are becoming more abundant as the market evolves. Traditionally marketed to hardcore bodybuilders, protein-fortified products are becoming more main stream and must now deliver on nutrition, convenience and taste. The new research proves Optipep’s nutritional suitability for sports nutrition. Carbery’s R&D and applications expertise has also ensured the ingredient’s ability to improve shelf life of fortified products, making it a smart choice.”

Earlier generations of whey proteins presented food manufacturers with a range of technical and processing challenges. Optipep dissolves quickly with excellent heat stability and overcoming issues of native whey by improving shelf life and moisture retention while reducing hardening, bitterness and costs.

www.carbery.com