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Orthosilicic acid stimulates collagen type 1 synthesis and osteoblastic differentiation in human osteoblast-like cells in vitro

D.M. Reffitt,[a] N. Ogston,[b] R. Jugdaohsingh,[a] H.F.J. Cheung,[b] B.A.J. Evans,[c] R.P.H. Thompson,[a] J.J. Powell,[a] and G.N. Hampson[b,*]

[a] *Gastrointestinal Laboratory, The Rayne Institute, St. Thomas' Hospital, London SE1 7EH, UK*
[b] *Department of Chemical Pathology, St. Thomas' Hospital, London SE1 7EH, UK*
[c] *Department of Child Health, University of Wales College of Medicine, Cardiff CF4 4XN, UK*

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Abstract

Silicon deficiency in animals leads to bone defects. This element may therefore play an important role in bone metabolism. Silicon is absorbed from the diet as orthosilicic acid and concentrations in plasma are 5–20 μM. The *in vitro* effects of orthosilicic acid (0–50 μM) on collagen type 1 synthesis was investigated using the human osteosarcoma cell line (MG-63), primary osteoblast-like cells derived from human bone marrow stromal cells, and an immortalized human early osteoblastic cell line (HCC1). Collagen type 1 mRNA expression and prolyl hydroxylase activity were also determined in the MG-63 cells. Alkaline phosphatase and osteocalcin (osteoblastic differentiation) were assessed both at the protein and the mRNA level in MG-63 cells treated with orthosilicic acid. Collagen type 1 synthesis increased in all treated cells at orthosilicic acid concentrations of 10 and 20 μM, although the effects were more marked in the clonal cell lines (MG-63, HCCl 1.75- and 1.8-fold, respectively, $P < 0.001$, compared to 1.45-fold in the primary cell lines). Treatment at 50 μM resulted in a smaller increase in collagen type 1 synthesis (MG-63 1.45-fold, $P = 0.004$). The effect of orthosilicic acid was abolished in the presence of prolyl hydroxylase inhibitors. No change in collagen type 1 mRNA level was seen in treated MG-63 cells. Alkaline phosphatase activity and osteocalcin were significantly increased (1.5, 1.2-fold at concentrations of 10 and 20 μM, respectively, $P < 0.05$). Gene expression of alkaline phosphatase and osteocalcin also increased significantly following treatment. In conclusion, orthosilicic acid at physiological concentrations stimulates collagen type 1 synthesis in human osteoblast-like cells and enhances osteoblastic differentiation.

Keywords: Orthosilicic acid; Silicon; Bone; Osteoblasts; Collagen type 1; Osteoblast differentiation

Introduction

Silicon (Si) is a ubiquitous environmental element found mainly as insoluble silicates, although small amounts of soluble Si are also present in natural waters, chiefly as orthosilicic acid [$Si(OH)_4$] [1]. Around neutral pH, orthosilicic acid polymerises at concentrations much above 2 mM, forming a range of silica species from soluble dimers to colloids and solid phase silica [2]. Some plants and lower animals may promote this reaction, as they use polymeric silica for structure and growth [3,4,5]. The normal diet contains (a) orthosilicic acid present in water or following hydrolysis of foods in the gastrointestinal tract, (b) nonhydrolyzed polymeric silica from plants [6], and (c) silicates due mainly to soil and dust contamination or as food additives [7,8,9]. Absorption studies have shown that only orthosilicic acid is in a bioavailable form with uptake in humans exceeding 50% of the ingested dose [10,11]. Fasting concentrations of Si in plasma are 2–10 μM, rising to 20–30 μM after meals, and approximately 700 μmol/day is normally excreted in urine.

In 1972, Carlisle [12] and Schwarz and Milne [13] first reported that silicon deficiency in chicks and rats led to

* Corresponding author. Department of Chemical Pathology, St. Thomas' Hospital, Lambeth Palace Rd., London SE1 7EH, UK. Fax: +00-44-207-928-4226.

E-mail address: geeta.hampson@kcl.ac.uk (G.N. Hampson).

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abnormally shaped bones and defective cartilagenous tissue, both of which were restored upon the addition of soluble Si to their diet. This led to the suggestion that Si may play an important role in connective tissue metabolism especially in bone and cartilage. The element's primary effect in bone and cartilage is thought to be on matrix synthesis rather than mineralization, although its influence on calcification may be an indirect phenomenon through its effects on matrix components [14].

Many of these earlier studies and more recent ones on extracellular matrix formation have been mainly carried out in animals such as chicks [10], rats [15,16,13], and calves [17]. The effects of soluble Si on bone matrix synthesis has not been confirmed in humans and species differences may exist. There is also a paucity of studies on the effects and the mechanisms of cellular action of soluble Si on human osteoblasts. Studies of the effects of soluble Si on human osteoblasts *in vitro* have been done using Zeolite A (ZA) which is a silicon-containing compound [18]. Keeting et al. [18] showed that ZA stimulated the proliferation and differentiation of cultured cells of the osteoblast lineage but they failed to demonstrate any effect of ZA on matrix synthesis. Furthermore, ZA hydrolyzes to release both silicic acid and aluminium salts, and thus the active component of this compound is not established. An important aspect of bone formation is the synthesis and deposition of collagen type 1, which constitutes 90% of the total organic extracellular matrix in mature bone, by preosteoblasts or early undifferentiated osteoblast-like cells [19]. In the present study we examined the effects of soluble Si (orthosilicic acid) on collagen type 1 synthesis in the more early osteoblastic cells. We used (1) the human osteosarcoma cell line MG-63, which represents a homogeneous clonal cell population derived from a specific stage of the osteoblastic lineage [20], (2) primary osteoblast-like cells derived from human bone marrow stromal cells, and (3) a near homogeneous preparation of an osteoblast precursor cell line. This cell line is an immortalized clonal human bone marrow cell line (HCCl) which has been shown to differentiate along the adipogenic and osteogenic lineages with manipulation of culture conditions [21]. As type 1 collagen is also a major constituent of skin, we assessed the effects of soluble Si on human skin fibroblasts too. We also determined the effects of soluble Si on osteoblastic differentiation and sought to ascertain its cellular mechanisms of action in the MG-63 cells.

Materials and methods

Study design

The study was divided into two parts. In the first series of experiments we investigated the effects of varying concentrations of soluble Si in the form of orthosilicic acid ($Si(OH)_4$) on collagen synthesis in (a) human osteoblastic cells and (b) skin fibroblasts. In the second set of experiments we examined the influence of soluble Si on (a) osteoblastic differentiation and (b) two of the mechanistic pathways involved in collagen type 1 synthesis (mRNA expression and proline hydroxylation of collagen type 1) in the MG-63 cells.

Cell culture

(a) The MG-63 cells were grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO BRL, Life Technologies, Paisley, Scotland, UK) supplemented with 5% fetal calf serum (FCS, GIBCO BRL), penicillin (100 IU/ml), streptomycin (100 μg/ml), and amphotericin B (2.5 μg/ml) in 24-well plates (GIBCO BRL) at 37°C in a humidified 5% CO_2 incubator. The cells were seeded at a density of $5 \times 10^4/cm^2$ and grown to confluence. Cells of passage number between 67 and 84 were used in the experiments described below. All plastic ware was obtained from Corning Costar Corp. (Cambridge, MA, USA).

(b) Human bone marrow stromal cells were grown as detailed below. An aliquot of bone marrow aspirates was obtained from five subjects (3 M, 2 F, age range 2–18 years) undergoing a percutaneous aspiration of bone marrow from the iliac crest for investigation of various hematological conditions. Only samples from patients who had normal hematopoietic cellular morphology were used in the present study. Ethical approval had been obtained from the University Hospital of Wales and St. Thomas' Hospital ethics committees and all patients or their parents gave informed consent. The bone marrow aspirates were suspended in 10 ml alpha-minimum essential medium (α-MEM, GIBCO BRL) containing 20 units/ml heparin and centrifuged at 1500 rpm for 10 min. The cell pellet was resuspended in 20 ml α-MEM and 15 ml lymphocyte separating medium (GIBCO BRL) was added to the bottom of the marrow suspension and centrifuged as previously described [22]. Mononuclear cells present at the interface were harvested and seeded in culture flasks and allowed to attach undisturbed for 7 days. At confluence, the cells were subcultured in 24-well plates and only cells of the first three passages were used in the further studies detailed below. To determine whether these cells were of the osteogenic lineage, they were assessed for the expression of osteoblastic markers, namely collagen type 1, alkaline phosphatase, parathyroid hormone (PTH)/PTH-related peptide receptor (PTHR1), osteocalcin, and the transcription factor cbfa1 [23]. Dexamethasone (Dex, 10^{-7} M) was added to the cells and alkaline phosphatase activity and cAMP response to PTH (1–34) were assessed at basal, day 3, 7, and 14. Alkaline phosphatase activity and cAMP production following stimulation with 10^{-7} M PTH (1–34) was enhanced in all cell lines tested following incubation with 10^{-7} M Dex with maximum response observed in 7–14 days (Fig. 1a). RT-PCR amplification of mRNA, described below, isolated from all cell lines demonstrated expression of the bone-

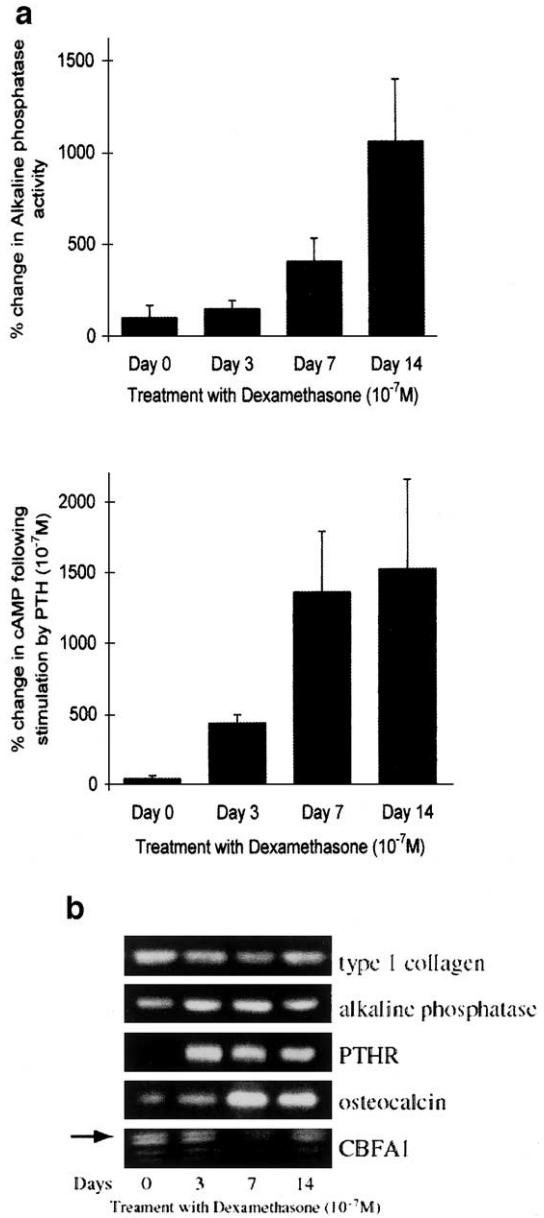

Fig. 1. (a) Percentage change in alkaline phosphatase activity from basal (Day 0) and following treatment with Dex (10^{-7} M) for 3,7, and 14 days. Percentage increase in PTH (1–34) (10^{-7} M)-induced cAMP production at baseline (Day 0) and following treatment with Dex (10^{-7} M) for 3, 7, and 14 days. The results (mean ± SD) are representative of three different human bone marrow stromal cell lines measured in duplicate. (b) Expresson of the osteoblastic related genes by RT-PCR at the different time points.

related genes detailed above (Fig. 1b). The primer sequences were as previously described [23,24].

(c) The conditionally immortalized HCCl [21] was grown in MEM (GIBCO BRL) containing 10% FCS and antibiotics as described above. Cells were grown in 24-well plates to confluence. These cells also express the bone-related markers mentioned above when grown in osteogenic medium. HCCl cells of passage number between 44 and 48 were used.

(d) Human skin fibroblasts were cultured from normal skin samples obtained at surgery. The fatty layer was removed and the skin specimen cut into small pieces of 1 mm^2 and placed individually into a 12-well culture plate. DMEM containing 20% FCS and antibiotics was added and the cultures were left undisturbed for 14 days. The medium was then changed every 3–4 days until the cells reached 70% confluence. They were then subcultured in 24-well plates and grown to confluence.

Addition and measurement of silicon

A 1.0 mM stock solution of orthosilicic acid was prepared from sodium silicate or a standard solution containing 9.8 mg/ml Si (both Aldrich Chemical Co Ltd, Gillingham, UK) using ultrahighly purified water. The stock solution was sterilized by γ-irradiation (Gammacell 1000 Elite, Nordion Netechnology, Reading, UK). Aliquots of the sterile stock solution were added to the culture medium at concentrations of 10, 20, and 50 μM. The final concentrations of Si in the media were measured in duplicate wells by inductively coupled plasma optical emission spectrometry (ICPOES) (Jobin-Yvon JY 24, Instruments SA, Longjumeau, France) in a clean air room (class J) as previously described [11]. At confluence, all the cell lines were washed twice with phosphate buffered saline (PBS) and changed to serum-free DMEM with either vehicle or soluble Si (10, 20, and 50 μM). Following incubation for 72 h, the medium was harvested and stored at −20°C until quantification of collagen type 1 as described below. The cells were washed twice with PBS and lysed in 0.3 M NaOH for 30 min at room temperature. Protein concentrations were determined by the method of Bradford [25].

Measurement of collagen type 1

Quantification of collagen type 1 synthesis was performed by measuring the amount of carboxy-terminal propeptide of type 1 procollagen (CICP) liberated into the culture medium during the synthesis and deposition of type 1 collagen by the preosteoblastic cells using the Prolagen-C kit (Metra Biosystems Ltd, Great Haseley, UK). Results were expressed per microgram of protein.

Alkaline phosphatase activity and osteocalcin production

For assessment of alkaline phosphatase activity and osteocalcin, the MG-63 cells were grown in medium containing, in addition, $1,25(OH)_2$ vitamin D (10^{-7} M) (Sigma Chemical Co.), ascorbic acid (50 μg/ml) (Sigma), and β-glycerophosphate (10 mM) (Sigma). At confluence orthosilicic acid was added in serum-free medium as described above. Alkaline phosphatase activity in the cell layer was measured colorimetrically by monitoring the release of *p*-nitrophenol from *p*-nitrophenyl phosphate (Sigma) at 37°C as previously described [22]. The amount of osteocalcin

secreted into the culture medium was assayed by an enzyme immunoassay (Metra Biosystems Ltd). Results were normalized to protein.

RNA isolation and semiquantification by reverse transcription–polymerase chain reaction (RT-PCR)

Total cellular RNA was isolated from the MG-63 cells following incubation with orthosilicic acid for 12 and 36 h as described above. RNA was extracted using the Trizol reagent (GIBCO BRL) and purified according to the manufacturer's instructions. The samples were analyzed by RT-PCR. Two micrograms of total RNA was reverse-transcribed in the presence of random hexanucleotides as random primers and Moloney murine leukemia virus reverse transcriptase (GIBCO BRL). The reaction mixture was incubated at 37°C for 1 h and then at 95°C for 5 min to terminate the reaction. In PCR amplification, 2.0 μl of the resultant first-strand cDNA mixture was used per PCR in a 25-μl reaction mixture containing 1.0 mM $MgCl_2$, 100 μM each dNTP, 50 ng of each primer, and 1 U of Amplitaq DNA polymerase (GIBCO BRL). Reaction mixes were overlayed with mineral oil and PCR was carried out using a Hybaid Omnigene thermal cycler (Hybaid Ltd, Teddington, Middlesex, UK). Typical cycling conditions were as follows: 94°C/2 min, 60°C/30 s, 72°C/1 min between 20 and 40 cycles depending on the optimization procedure as detailed below. Oligonucleotides used to amplify collagen type 1 [23], alkaline phosphatase, and osteocalcin genes were as previously described [24]. Primer sequences for S14 gene (ribosomal RNA) were 5′: ATCAAACTCCGGGCCACAGGA and 3′: CCCCATCCCCTCTGACAGCAC. The PCR product size was 133 base pairs. Optimization of RT-PCR cycle number in order to allow semiquantitative assessment of product levels was carried out by generating saturation curves of the level of the PCR product against cycle numbers 20–40. The optimum number of cycles was chosen following assessment of the linear amplification curve obtained for collagen type 1, alkaline phosphatase, osteocalcin, and S14 and was 30, 35, 35, and 25, respectively. Following amplification, the products were electrophoresed in 3% agarose gels and visualized by ethidinium bromide staining. Normalization was made to S14 mRNA (the housekeeping gene) present in each sample. All samples from the different culture conditions were analyzed simultaneously and each individual sample was analyzed at least three times and quantified by densitometric scanning. The results for all the PCR products were in the linear range.

Treatment of the MG-63 cells by inhibitors of prolyl hydroxylase

To assess whether silicon influences prolyl hydroxylase activity, we investigated the effects of orthosilicic acid on collagen type 1 synthesis in the presence of 2 prolyl hydroxylase inhibitors [26]; *cis*-4-hydroxy-L-proline (cis-HP) (Sigma) and protocatechuic acid (PA) (Sigma).

cis-HP (1.0 mM) and PA (2.0 mM) were added to serum-free culture medium. In some wells orthosilicic acid was added at 10 μM in the presence of the prolyl hydroxylase inhibitors and left to incubate for 72 h as described above. The concentrations of CICP were determined in the medium.

Statistical analysis

The data were analyzed using Student's *t* test and significance was assigned at $P < 0.05$.

Results

Si concentrations in the culture medium

The baseline Si concentration in serum-free DMEM kept in plastic containers was (mean ± SD) 1.60 ± 0.5 μM. Following addition of 10, 20, and 50 μM orthosilicic acid, the final concentrations measured in triplicate were 11.7 ± 1.8, 21.9 ± 2.2, and 50.9 ± 3.1 μM Si, respectively.

Effect of soluble Si on collagen type 1 synthesis

Collagen type 1 synthesis increased in all cell lines tested following treatment with orthosilicic acid at 10 and 20 μM. Treatment of the MG-63 cells with orthosilicic acid at 10 and 20 μM increased the amount of collagen type 1 in the culture medium by a factor of 1.75 ($P < 0.001$ vs untreated cells) (Fig. 2). However, addition of orthosilicic acid at supraphysiological concentration (50.0 μM) resulted in a smaller increase in collagen type 1 synthesis (MG-63 : 1.45 at 50 μM compared to 1.75 at 10 and 20 μM, $P = 0.004$). Similar effects were observed in the HCC1 cell line (Fig. 2). Orthosilicic acid also stimulated collagen type 1 synthesis in the bone marrow stromal cells, although the effect was less than in the clonal cell lines (1.45 and 1.3 at 10 and 20 μM compared to untreated cells). The results were reproducible between cell populations isolated from the different patients. Collagen type 1 was also significantly increased in cultures of skin fibroblasts treated with orthosilicic acid at 10 and 20 μM, albeit to a smaller degree than in the osteoblastic cells (Fig. 2). Treatment of the skin fibroblasts with 50 μM orthosilicic acid did not significantly increase collagen type 1 synthesis.

Effect of soluble Si on alkaline phosphatase activity and osteocalcin in the MG-63 cells

Alkaline phosphatase activity and osteocalcin synthesis were significantly increased at the added orthosilicic acid concentration of 10 μM (1.5-fold vs untreated cells, $P < 0.01$). However, the increase in alkaline phosphatase activ-

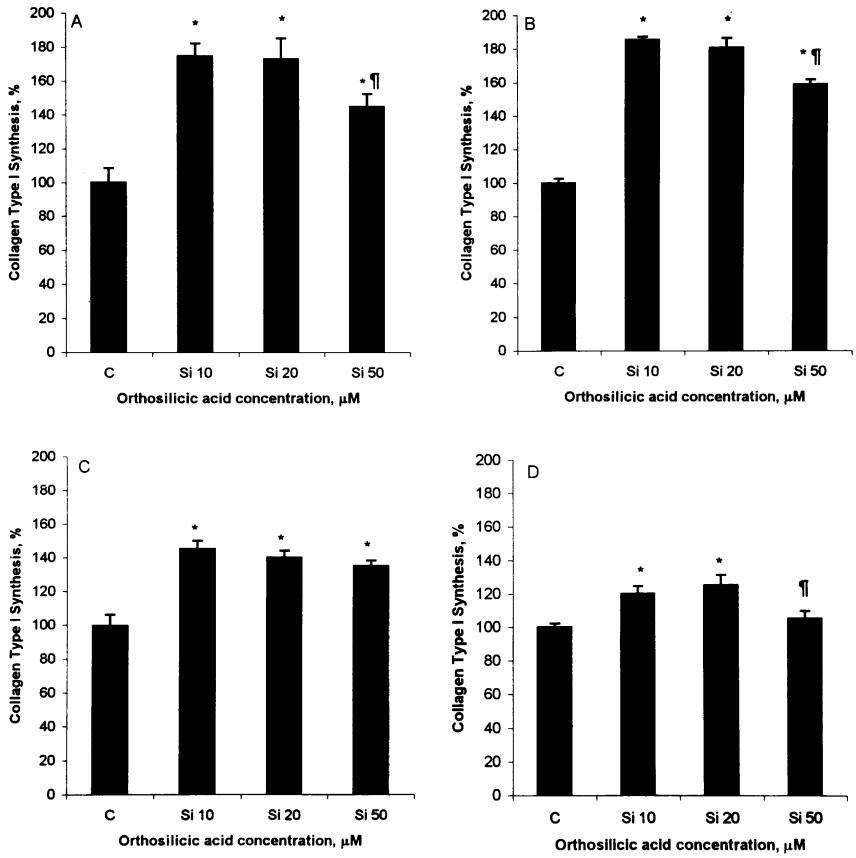

Fig. 2. Collagen I C-terminal propeptide (CICP) (ng/μg protein) in serum-free medium following addition of orthosilicic acid (10, 20, 50 μM) expressed as percentage of control in (A) MG-63 cells; (B) HCC1 cells (results are mean ± SEM of two different experiments done in triplicate); (C) human bone marrow stromal cells, (D) skin fibroblasts (results are mean ± SEM of experiments on three different cell lines carried out in triplicate. *$P < 0.05$ compared to control, ¶$P < 0.05$ compared to orthosilicic acid concentrations of 10 and 20 μM.

ity and osteocalcin in cells treated with 20 μM orthosilicic acid was less compared with the increase observed at 10 μM (1.2-fold only vs untreated cells, $P < 0.05$). No stimulatory effects were observed at concentrations of 50 μM (Fig. 3).

MRNA expression of collagen type 1, alkaline phosphatase, and osteocalcin

Comparable levels of collagen type 1 mRNA were detected in untreated MG-63 cells and cells treated with orthosilicic acid at the different concentrations (10, 20, 50 μM) at 12 and 36 h (Fig. 4). In contrast, treatment with orthosilicic acid for 36 h significantly increased the relative abundance of alkaline phosphatase and osteocalcin mRNA (Fig. 4).

Collagen type 1 synthesis following treatment with proline hydroxylase inhibitors

Inhibition of prolyl hydroxylase activity by *cis*-HP (1.0 mM) and protocatechuic acid (2.0 mM) reduced collagen synthesis by 50% (Fig. 5). The stimulatory effect of soluble Si (10 μM) on collagen type 1 synthesis was abolished in the presence of the prolyl hydroxylase inhibitors.

Discussion

Our results demonstrate that physiological concentrations of Si in the form of orthosilicic acid stimulate collagen type 1 synthesis in human osteoblast-like cells and skin fibroblasts. Treatment with Si also enhanced osteoblastic differentiation. Orthosilicic acid did not alter collagen type 1 gene expression but our results suggest that it may modulate prolyl hydroxylase activity. Further studies are required to confirm this.

The human osteoblastic cell lines were treated with orthosilicic acid since dietary silicon compounds are hydrolyzed into orthosilicic acid prior to gastrointestinal absorption. Silicon is thus almost entirely present as orthosilicic acid in human serum and the bioavailability of silicon in orthosilicic acid is high [27,10,11].

Maximal stimulation of collagen type 1 synthesis was observed within the physiological range while at the supraphysiological concentration (50 μM), the observed effect of

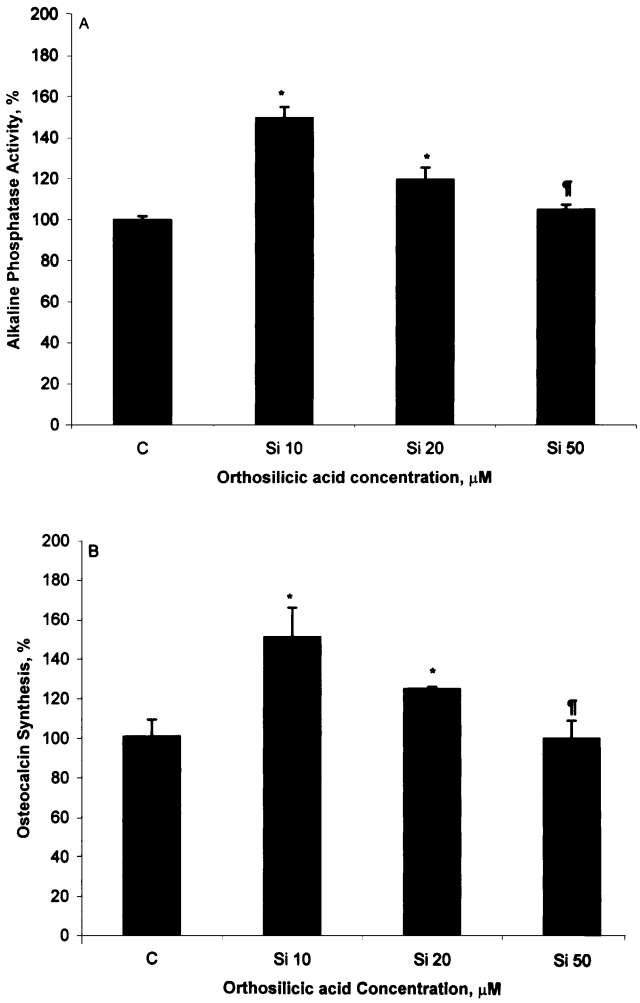

Fig. 3. Alkaline phosphatase activity (A) and osteocalcin secreted in medium (B) following addition of orthosilicic acid (10, 20, 50 μM) to the MG-63 cells, expressed as percentage of control. Results are mean ± SEM of two different experiments done in triplicate. *$P < 0.05$ compared to control, ¶$P < 0.05$ compared to orthosilicic acid concentrations of 10 and 20 μM.

soluble Si was diminished. A similar pattern was seen in all the early osteoblastic cell lines although the results just failed to reach statistical significance in the primary bone marrow stromal cell cultures. This dose-dependent effect on bone cell function has previously been reported with other trace metals, such as copper. Trace amounts of copper are essential for collagen cross-linking but any beneficial effects on bone matrix formation is abolished at pharmacological concentrations [28].

The increase in collagen type 1 synthesis was more marked in the MG-63 and the clonal HCC1 cells compared to the primary bone marrow cell cultures. One explanation for this may be the heterogeneity and multiclonality of our primary cultures as previously documented [24]. The smaller, but significant, increase in collagen type 1 seen in the skin fibroblasts is likely to underestimate the effects on total collagen, as another fibrillar collagen, type III collagen, is also a major constituent of skin but virtually absent in bone. This would be compatible with the findings of Calomme and Vanden Berghe [17], who observed an increase in hydroxyproline concentration in the dermis of calves following supplementation with orthosilicic acid.

Other markers of bone formation such as alkaline phosphatase activity have also been shown to be reduced in

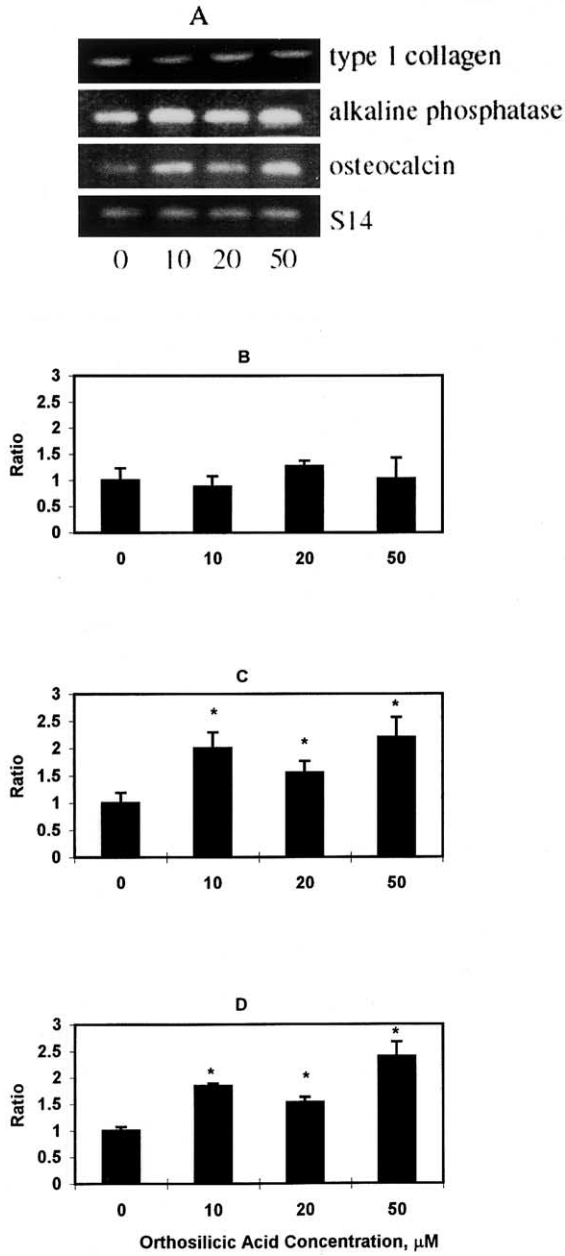

Fig. 4. (A) RT-PCR of mRNA expression of collagen type 1, alkaline phosphatase, osteocalcin, and the housekeeping gene S14 (ribosomal RNA) in the MG-63 cells following treatment with orthosilicic acid at 10, 20, 50 μM for 36 h. Ratio of mRNA of (B) collagen type 1, (C) alkaline phosphatase, (D) osteocalcin relative to control (O) determined by densitometry in MG-63 cells. The bands were scanned and expression was normalized to S14 (ratio of gene product/S14). The results are mean ± SEM of three separate experiments. *$P < 0.05$ compared to control.

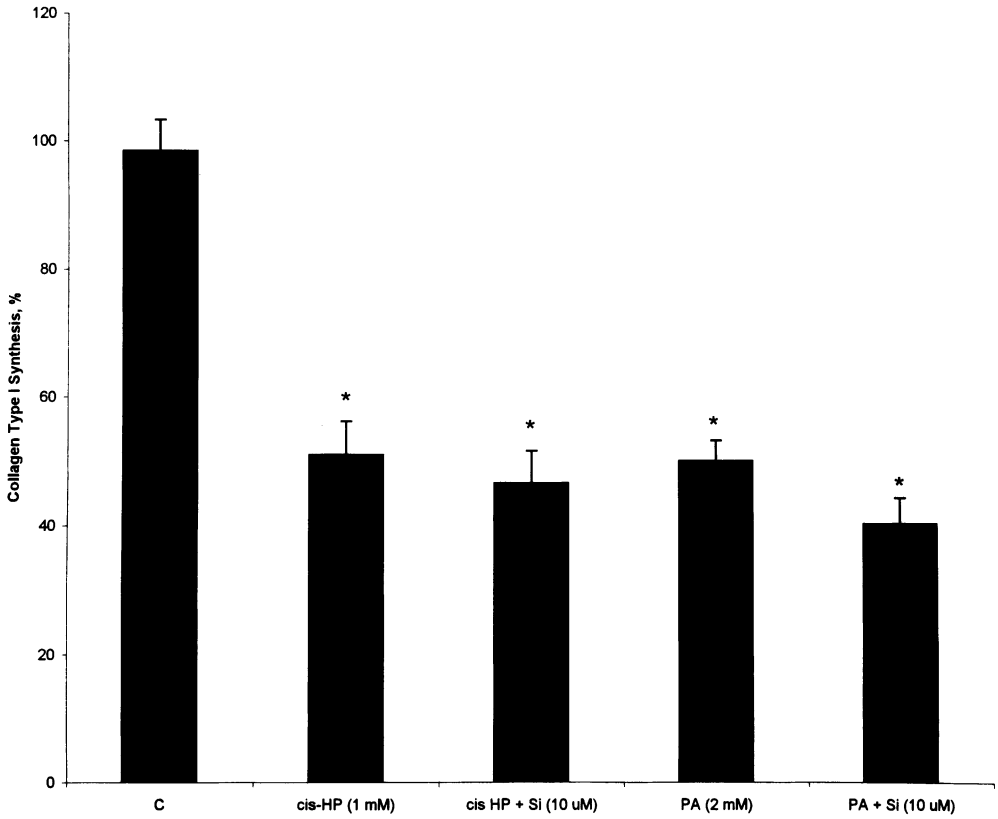

Fig. 5. Collagen I C-terminal propeptide (CICP) synthesis expressed as percentage of control following treatment of MG-63 cells with prolyl hydroxylase inhibitors (*cis*-4-hydroxy-L-proline (*cis*-HP) 1.0 mM and protocatechnic acid (PA) 2.0 mM) and orthosilicic acid 10 μM. *$P < 0.05$ compared to control.

Si-deficient animals [14]. In the present study, Si enhanced alkaline phosphatase activity and osteocalcin synthesis in the MG-63 cells. Whether Si has a direct effect on osteoblastic differentiation and commitment to mineralization is unclear. In Si-deficient animals, the formation of organic matrix, whether in cartilage or bone, is affected more severely than the mineralization process [14]. We can therefore speculate that soluble Si may influence these processes indirectly through its effects on bone matrix synthesis. Indeed type 1 collagen/extracellular matrix has been shown to enhance the expression of the differentiated osteoblastic phenotype as assessed by increases in alkaline phosphatase activity, osteocalcin synthesis, and mineralization [29]. We used the MG-63 cell line, as previous studies [30] with this relatively undifferentiated cell line which is phenotypically similar to mesenchymal osteoprogenitor cells have shown that they behave as osteoblast precursors in that induction of alkaline phosphatase activity is coupled to collagen matrix synthesis and/or accumulation. The pattern of regulation of alkaline phosphatase activity and osteocalcin synthesis and their respective mRNA species suggests that both changes in mRNA expression and posttranscriptional mechanisms may be implicated, as previously described [29]. Alterations posttranscriptionally such as in the rate of translation or as a result of posttranslational modifications may explain the discordance between the mRNA levels and alkaline phosphatase activity and osteocalcin synthesis observed at orthosilicic acid concentrations of 50 μM. This could also have contributed, at least in part, to the lack of a dose-dependent effect of Si on the mRNA expression of alkaline phosphatase and osteocalcin.

Collagen type 1 synthesis involves a series of closely coordinated physiological processes. After being transcribed and translated, the resulting proteins undergo extensive posttranslational modifications before being assembled in a triple helix and released in the extracellular space [31]. Several factors including hormones, cytokines, growth factors, vitamins, and trace elements can influence these processes [32]. A specific effect of soluble Si on collagen type 1 gene expression is unlikely, as collagen type 1 mRNA abundance in the MG-63 cells remained unchanged following treatment with soluble Si. A critical step in collagen type 1 synthesis and its secretion into the extracellular space is the hydroxylation of the proline residues of the collagen chains, a reaction catalyzed by prolyl hydroxylase. Indeed it has previously been reported that optimal activity of prolyl hydroxylase appears to depend on the presence of adequate concentrations of Si [33,34]. However, the increase in prolyl hydroxylase activity observed following the addition of soluble Si to the diet of Si-deficient animals may reflect the

rate of collagen biosynthesis where soluble Si is generally implicated. However, our results would imply that soluble Si may be involved in this particular step of the collagen type 1 biosynthetic pathway, since no stimulatory effect of Si on collagen type 1 synthesis was observed when prolyl hydroxylase activity was inhibited. The mechanism for an interaction between soluble Si and prolyl hydroxylase is unknown. Prolyl hydroxylase requires several cofactors such as ferrous ions, α-ketoglutarate, and ascorbic acid to be active [33]. Si may be an additional cofactor. It has also been proposed that Si binds endogenous toxic metals such as aluminium, thus leading to optimal enzyme activity [35]. However, this is unlikely, as only the oligomeric form of Si and not the monomeric form, used in the present study, binds to aluminium significantly [10]. Si is also known to bind to glycosaminoglycan macromolecules and has been shown to play a role in the formation of cross-links between collagen and proteoglycans [36], thus resulting in the stabilization of bone matrix molecules and preventing their enzymatic degradation. This may be another possible mode of action of soluble Si. It is likely that the mechanism of action of Si on matrix synthesis may involve a complex biochemical set of interactions [37] with biological molecules and merits further investigation. Such studies may also shed light on the molecular and cellular regulation of bone matrix synthesis in general.

In conclusion, the present study indicates that physiological concentrations of soluble Si stimulate collagen type 1 synthesis in human osteoblast-like cells and promote osteoblastic differentiation. The precise cellular mechanism by which silicon affects collagen type 1 synthesis requires further investigation, although our results suggest that it may be related to a possible role in the regulation of prolyl hydroxylase activity. Our in vitro results of a stimulatory effect of orthosilicic acid on collagen synthesis and osteoblast differentiation suggest that this compound may have a stimulatory effect on bone formation in vivo. To date, there have been no studies looking at the potential effect of orthosilicic acid on bone formation in osteopenic animal models *in vivo*. This therefore warrants further investigations. The impact of soluble Si, on bone metabolism, in humans also needs to be studied further, as it may prove to be useful in the treatment of osteopenic bone disorders.

Acknowledgments

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