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Nitric Oxide is a Regulator of Bone Remodelling

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Abstract

Nitric oxide (NO) is known to be implicated in the metabolism of bone, especially as a mediator of cytokine effects on the remodelling of bone tissue. In this study we examine whether NO affects the osteoblast activation or the osteoclast differentiation of primary mouse osteoblast-like and osteosarcoma ROS 17/2·8 cell lines.

Primary osteoblast and ROS 17/2·8 cells released NO upon stimulation of interleukin-1β, tumour necrosis factor-α, and interferon-γ. Sodium nitroprusside, a donor of nitric oxide, increased the activity of alkaline phosphatase in ROS 17/2·8 cells as well as the number of calcified nodule formations in primary mouse osteoblast-like cells. Sodium nitroprusside also completely inhibited 1α,25-$(OH)_2D_3$-induced osteoclast generation in a high concentration (100 μM). However, a low concentration of sodium nitroprusside (3–30 μM) significantly increased the generation of osteoclasts.

These results indicated that NO appears to be an important regulatory molecule in the processes of bone formation and resorption. Hence, NO may be involved in the pathogenesis of bone loss in diseases associated with cytokine activation, such as periodontal disease and rheumatoid arthritis.

Bone remodelling is characterized by the continuing processes of osteoblast-mediated bone formation and osteoclast-mediated bone resorption (Parfitt 1987). Bone metabolism is tightly regulated at the local level by networks of hormones, cytokines, and other factors. In pathological conditions of bone remodelling, including osteoporosis and periodontal diseases, inflammatory cytokines and local mediators are responsible for enhancement of osteoclast resorption and inhibition of repair at the sites of bone resorption. Recently it has been reported that pro-inflammatory cytokines, such as tumour necrosis factor α (TNF-α), interferon γ (IFN-γ) and interleukin 1β (IL-1β), function as modulators of bone remodelling in-vitro and in-vivo (Gowen et al 1983; Bertolini et al 1986). IL-1β and TNF-α are directly implicated in bone resorption by modulating the activities of osteoclasts and osteoblasts. These cytokines are also important for inducing many kinds of cellular effector molecules including oxygen and nitrogen free radicals which modulate the metabolism of bone tissue.

Nitric oxide (NO) is a gaseous molecule regulating many biological functions in nervous, vascular and immune systems (Moncada et al 1991; Lowenstein & Snyder 1992; Nathan 1992). NO-synthase (NOS) catalyses L-arginine to NO which by binding with guanylate cyclase increases cGMP formation. Hence, NO functions as a neurotransmitter, endothelial-derived relaxation factor and immune effector molecule (Stuehr & Nathan 1989; Nathan & Hibbs 1991). Three distinct forms of NOS are identified: a neuronal form (nNOS) in the brain, an endothelial form (ecNOS) in vascular endothelium and an inducible form (iNOS) in macrophages (Bredt et al 1991; Lamas et al 1992; Xie et al 1992). Both ecNOS and nNOS are constitutively expressed at low levels and modulate the entry of Ca^{2+} into cells. However, iNOS is regulated by transcriptional activation of the gene. MacIntyre et al (1991) report that NO augments osteoclast activity in a similar manner with calcium, which induces the retraction of cells through inhibiting the movement of cells to a fresh site of bone resorption. However, NO has a preferential role as an inhibitor of osteoclast activity both in-vitro and in-vivo (Kasten et al 1994; Löwik et al 1994). Primary osteoblast-like cells and osteosarcoma cell lines release NO upon the stimulation of inflammatory cytokines including IL-1β and TNF-α (Damoulis & Hauschka 1994, 1997; Löwik et al 1994; Hukkanen et al 1995; Riancho et al 1995; Ralston & Grabowski 1996). These data suggest that NO produced by osteoblasts may act on either osteoblasts, in an autocrine manner, or on osteoclasts in bone remodelling. Furthermore, there is functional evidence of intercellular collaboration between osteoblasts and osteoclasts. Several studies show that osteoblasts produce a number of factors which stimulate osteoclast bone resorption (Roodman 1993).

We examine the effects of cytokine-induced NO on osteoblast activity and osteoclast differentiation in primary osteoblast-like and ROS 17/2·8 cells. Primary osteoblast and ROS 17/2·8 cells induce the production of NO upon stimulation by IL-1β, TNF-α, and IFN-γ. Sodium nitroprusside increases osteoblast activity and inhibits 1α,25-$(OH)_2D_3$-induced osteoclast generation in a high concentration (100 μM) but increases osteoclast activity in a low concentration (1–30 μM). These results indicate that NO may regulate the metabolism of bone formation and resorption as a functional mediator of cytokine-induced remodelling processes.

Materials and Methods

Animals

The original stock of ICR mice was purchased from the Jackson Laboratory (Bar Harbor, ME) and the mice were

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maintained in the Animal Facility Units of Wonkwang University School of Dentistry (Iksan, Korea).

Reagents

Murine interferon gamma (IFN-γ, 1×10^6 units mg^{-1}) was purchased from Genzyme (Munich, Germany). Lipopolysaccharide (LPS), *N*-(1-naphthyl)-ethylenediamine dihydrochloride, sodium nitrite, and sulphanilamide were obtained from Sigma chemical Co. (St Louis, MO). All reagents and media for tissue culture were tested for the contamination of LPS with a colorimetric limulus amoebocyte lysate assay (detection limit 10 pg mL^{-1}; Whittaker Bioproducts, Walkersvile, MD). All culture wares were purchased from Nunc Inc. (North Aurora Road, IL). Alpha-minimal essential medium (α-MEM) containing L-arginine (84 mg L^{-1}), Hank's balanced salt solution, foetal calf serum (FCS) and other tissue-culture reagents were bought from GIBCO Co. (Gaithersburg, MD).

Cell culture

The primary osteoblast-like and ROS 17/2·8 cells were maintained in α-MEM media with 10% FCS. Primary osteoblast-like cells were obtained by sequential collagenase digestion of mouse calvaria from neonatal mice (10–15 for each group) (Wong & Cohn 1974). Osteoblast-enriched cells derived from the third to fifth digestions were used in all experiments. Cells were cultured in a 10-cm^2 culture dish in α-MEM supplemented with 10% FCS, penicillin (100 int. units mL^{-1}), and streptomycin (100 μg mL^{-1}). Cells were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

Determination of nitrite concentration

Nitrite was measured according to the method described by Green et al (1982). Briefly, 100 μL of culture medium was reacted with the equal amount of Griess reagent, containing 1% sulphanilamide and 0·1% naphthylethylenediamine dihydrochloride in 2% phosphoric acid, for 10 min at room temperature. Absorbance was measured at 540 nm and then nitrite concentration was quantified by using a standard curve of sodium nitrite. Fresh culture medium was used as a control to be the baseline of absorbance.

Determination of alkaline phosphatase activity

ROS 17/2·8 cells were plated onto a 96-well plate at a density of $2\text{-}3 \times 10^3$ cells per well in α-MEM medium and cultivated until cells were confluent. The confluent cells were then treated with appropriate concentration of sodium nitroprusside (0–300 μM) in α-MEM supplemented with 0·4% FCS for 48 h. After removal of culture media from the wells, cells were washed with Dulbecco's phosphate buffered saline and treated with 0·1% Triton X-100/saline for 30 min. To determine the activity of alkaline phosphatase, cell lysate was incubated in 0·1 M glycine-NaOH buffer (pH 10·4) with 100 mM *p*-nitrophenyl phosphate as a substrate at 37°C for 10 min. The optical density of *p*-nitrophenol, a reaction product, was determined at 405 nm using ELISA reader (SLT Labinstruments, SF).

Determination of calcified nodule formation

Primary foetal mouse osteoblast cells were plated onto 24-well plates at a density of 2×10^4 cells per well in α-MEM medium and cultivated to be confluent. The medium was changed with α-MEM supplemented with 50 μg mL^{-1} ascorbic acid and 10 mM β-glycerophosphate. Cultures were maintained up to 21 days without or with sodium nitroprusside (10–100 μM) and the media were refreshed every second day. For the determination of the number of calcified nodules, the cell layer was fixed with neutral buffered formalin, stained with 5% silver nitrate in-situ by the von Kossa technique and washed with 5% sodium thiosulphate for the detection of mineral deposits. The number of mineralized nodules were counted at $\times 40$ magnification under a light microscope.

Bone marrow culture

The mice were sacrificed by cervical dislocation under light ether anaesthesia, and tibiae were aseptically removed. The ends of the bone were cut off and the marrow cavity was flushed with 1 mL of α-MEM. Marrow cells were then collected and washed twice with α-MEM, and plated in α-MEM containing 10% FCS at 3×10^6 cells mL^{-1} in 24-well plates. Sodium nitroprusside and 1α, 25-$(OH)_2D_3$ were added at the beginning and refreshing of the culture. All cultured cells were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air.

Enzyme histochemistry

After culture for the indicated periods, adherent cells were fixed with 10% formalin in phosphate buffered saline for 10 min, then treated with ethanol-acetone (50:50, vol/vol) for 1 min. The cells were then dried and stained with tartrate-resistant acid phosphatase (TRAP) in 0·1 M sodium acetate buffer (pH 5·0) containing AS-MX phosphate and red violet LB salt in 50 mM sodium tartrate. TRAP-positive cells containing three or more nuclei were counted as osteoclast-like multinucleated cells. The results were expressed as the mean $\pm$ s.e. of tetraplicates.

Statistical analysis

Data were expressed as mean $\pm$ s.e. of four experiments. The statistical significance of the difference was determined by a paired Student's *t*-test.

Results

Production of NO by primary foetal mice osteoblast-like and ROS 17/2·8 cells

We tested whether primary foetal osteoblast-like cells and osteosarcoma ROS 17/2·8 cells produce NO. Primary foetal mice osteoblast-like or ROS 17/2·8 cells were stimulated with IL-1β (10 units mL^{-1}), TNF-α (1 ng mL^{-1}), and IFN-γ (50 units mL^{-1}) for 48 h. After stimulation of cells with inflammatory cytokines, NO released into culture supernatant was determined. Single treatment with cytokines did not affect the primary foetal osteoblast-like cells to generate NO. When the cells were stimulated with the three cytokines combined, significant amounts of NO were released compared with control cells in medium only ($P < 0.05$) (Table 1). We also checked the iNOS in the cell lysates of above experimental conditions by Western blot analysis. The results showed that the amount of NO released from primary foetal osteoblast-like cells treated with single or combined cytokines was consistent with iNOS protein in cells (data not shown). We next examined the effect

Table 1. Effects of IL-1β, TNF-α and IFN-γ on the production of nitrite in primary foetal mouse osteoblast-like cells.

Treatment	NO_2^- secretion (μM)
Medium only	10·8 ± 3·4
IL-1β (10 units mL^{-1})	11·5 ± 2·1
TNF-α (1 ng mL^{-1})	14·6 ± 3·9
IFN-γ (50 units mL^{-1})	13·8 ± 2·7
IL-1β + TNF-α + IFN-γ (ALL)	52·6 ± 5·8*
IL-1β + TNF-α + IFN-γ ± L-N^GMMA (1 mM)	16·7 ± 3·4**
IL-1β + TNF-α + IFN-γ + dexamethasone (10^{-8} M)	23·5 ± 3·7**

Nitrite was measured from osteoblast cells (2×10^4 cells) 48 h after stimulation with cytokines. Data showed the means ± s.e. of four experiments. *Significantly different from control, $P < 0.05$; **significantly different from ALL, $P < 0.05$.

Table 2. Effects of IL-1β, TNF-α and IFN-γ on the production of nitrite in ROS 17/2·8 cells.

Treatment	NO_2^- secretion (μM)
Medium only	9·5 ± 3·1
IL-1β (10 units mL^{-1})	11·2 ± 3·2
TNF-α (1 ng mL^{-1})	13·4 ± 2·8
IFN-γ (50 units mL^{-1})	14·2 ± 4·6
IL-1β + TNF-α + IFN-γ (ALL)	51·8 ± 5·7*
IL-1β + TNF-α + IFN-γ + L-N^GMMA (1 mM)	17·4 ± 4·1**
IL-1β + TNF-α + IFN-γ + dexamethasone (10^{-8} M)	22·1 ± 3·9**

Nitrite was measured from ROS 17/2·8 cells (2×10^4 cells) 48 h after stimulation with cytokines. Data were the means ± s.e. of four experiments. *Significantly different from control, $P < 0.05$; **significantly different from ALL, $P < 0.05$.

of a competitive inhibitor of iNOS substrate L-arginine, N^G-monomethyl-L-arginine (L-N^GMMA), on cytokine-induced NO release from primary foetal osteoblast-like cells. L-N^GMMA significantly inhibited the release of NO from the cells. However, iNOS of cell lysate was not changed by L-N^GMMA, as evidenced by Western blot analysis (data not shown). Dexamethasone is a potent anti-inflammatory agent in clinical fields. Interestingly, cytokines-induced release of NO was significantly inhibited in primary foetal osteoblast-like cells that were treated with dexamethasone (1×10^{-8} M) for 48 h. These results indicate that NO may be one of the major soluble mediators in bone remodelling metabolism associated with the inflammatory response.

We also examined the effects of inflammatory cytokines on the production of NO from osteosarcoma ROS 17/2·8 cells (Table 2). Single treatment with each cytokine (IL-1β, TNF-α or IFN-γ), did not stimulate the ROS 17/2·8 cells to produce NO. However, in a similar manner to primary osteoblast-like cells, ROS 17/2·8 cells produced a significant amount of NO when stimulated with the combination of IL-1β, TNF-α and IFN-γ. Both L-N^GMMA and dexamethasone significantly inhibited NO production from ROS 17/2·8 cells upon stimulation with IL-1β, TNF-α and IFN-γ.

Effect of sodium nitroprusside on the activity of alkaline phosphatase in ROS 17/2·8 cells

We next examined whether NO modulates the osteoblast activity of ROS 17/2·8 cells. Alkaline phosphatase is a typical biochemical marker of osteoblast activity in the remodelling process of bone. ROS 17/2·8 cells were exposed to sodium nitroprusside (10–100 μM), a donor of NO, for 48 h, and the activity of alkaline phosphatase was determined (Table 3). Sodium nitroprusside had no influence on alkaline phosphatase at 10 μM and significantly enhanced the osteoblast activity at 30 μM. The activity of alkaline phosphatase elevated by sodium nitroprusside returned to control levels when the cells were treated with L-N^GMMA (data not shown). We also treated ROS 17/2·8 cells with potassium ferricyanide, a structural analogue of sodium nitroprusside, to avoid other chemical effects of sodium nitroprusside other than providing NO to the culture medium. The same concentration of potassium ferricyanide did not show any biological effect on the activity of alkaline phosphatase in ROS 17/2·8 cells (data not shown).

Table 3. Effect of sodium nitroprusside on the activity of alkaline phosphatase in ROS 17/2·8 cells.

Treatment	Alkaline phosphatase (int. units (mg of protein)$^{-1}$)
Medium only	5·14 ± 0·07
Sodium nitoprusside 10 μM	5·81 ± 0·08
30 μM	6·48 ± 0·08*
100 μM	6·88 ± 0·07*

ROS 17/2·8 cells were cultured in the presence or absence of sodium nitoprusside for 48 h. Alkaline phosphatase activity was quantified spectrophotometrically by using *p*-nitrophenyl phosphate (PNPP) as a substrate. One unit of alkaline phosphatase activity was defined as the amount of enzyme which catalysed the transformation of 1 μmol of PNPP per min at 37°C. Data expressed the means ± s.e. of four experiments. *Significantly different from control, $P < 0.05$.

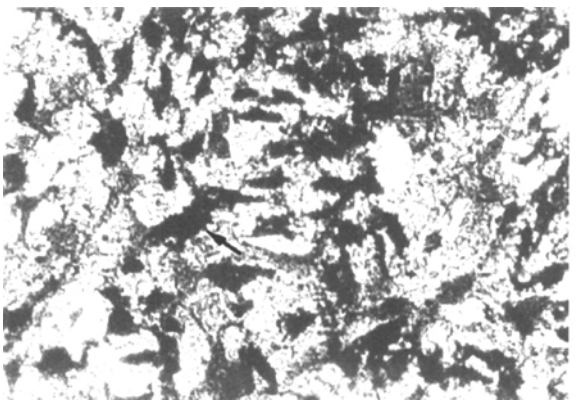

FIG. 1. In-situ von Kossa staining of primary foetal mouse osteoblast-like cells 21 days after culture. Von Kossa-positive nodule is shown as black (× 100, arrow).

Table 4. Effect of sodium nitroprusside on the formation of calcified nodules in primary foetal mouse osteoblast-like cells.

Treatment	No. of calcified nodules
Medium only	88 ± 15
Sodium nitroprusside 10 μM	93 ± 16
30 μM	158 ± 24*
100 μM	100 ± 25*

Primary foetal mouse osteoblast-like cells were treated with ascorbic acid (50 μg mL^{-1}) and β-glycerophosphate (10 mM) for 21 days. Sodium nitroprusside was added simultaneously during the culture period every two days. Data were means ± s.e. of four experiments. *Significantly different from control, $P < 0.05$.

These data suggested that NO may be an effector molecule which regulates osteoblast activity in the process of bone remodelling.

Effect of sodium nitroprusside on the formation of calcified nodules in primary foetal osteoblast-like cells

To determine the effect of NO on osteoblast activity, we examined the formation of calcified nodules in primary foetal osteoblast-like cells treated with the NO donor, sodium nitroprusside. The cells were cultured with ascorbic acid (50 μg mL^{-1}), β-glycerophosphate (10 mM), and sodium nitroprusside (0–100 μM) for up to 21 days. The number of calcified nodules was quantified by the von Kossa technique (Fig. 1). The results showed that sodium nitroprusside significantly increased the number of calcified nodules in osteoblast-like cells treated with ascorbic acid and β-glycerophosphate (Table 4). L-N^GMMA abrogated sodium nitroprusside-induced enhancement of calcified nodule formation in primary osteoblast-like cells (data not shown). We also checked the structural effects of sodium nitroprusside by using potassium ferricyanide, but this did not affect the sodium nitroprusside-induced formation of calcified nodules in foetal osteoblast-like cells (data not shown).

Effect of sodium nitroprusside on the generation of osteoclast-like cells

NO is known to be involved in the regulation of osteoclast activity in the process of bone remodelling. We tested the effect of NO on the formation of osteoclasts from primary

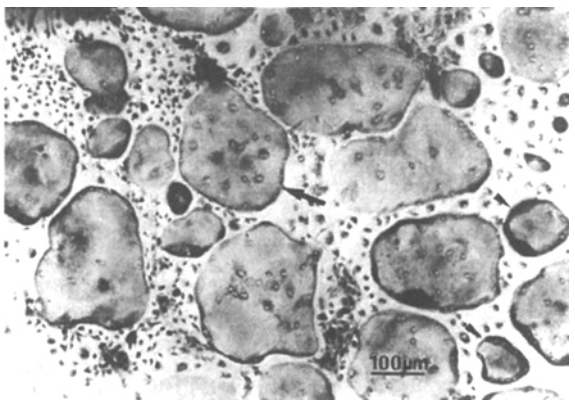

FIG. 2. TRAP-positive multinucleated cells formed in the co-cultivation system of primary foetal mouse osteoblast cells with mouse bone marrow cells (× 100). Primary foetal osteoblast (1.5×10^4 cells per well) and mouse bone marrow cells (3.0×10^5 cells per well) were plated together onto 4-well plates with 10^{-7} M of 1α,25-$(OH)_2D_3$ for 8 days. Cells were then fixed and stained for TRAP. Nuclei located in the centre (arrows) and in the periphery of the cells (arrowhead) were shown in TRAP-positive multinucleated cells.

foetal osteoblast-like cells co-cultivated with bone marrow cells. We quantified the tartrate-resistant acid phosphatase (TRAP)-positive cells as osteoclasts (Fig. 2). Co-cultivation of primary foetal osteoblast cells with bone marrow cells was done in the presence of sodium nitroprusside and 1α,25-$(OH)_2D_3$ (10^{-7} M) for 8 days. Sodium nitroprusside significantly enhanced the TRAP-positive multinucleated cells at 3 μM and increased the number of osteoclasts by up to more than twice at 30 μM (Table 5). However, a higher concentration of sodium nitroprusside (100 μM) dramatically decreased the TRAP-positive multinucleated cells in this co-cultivation system. These results revealed that the effect of NO on the generation of osteoclasts has dose-dependent biphasic characteristics. NO could significantly augment the generation of osteoclasts at a low or moderate concentration of sodium nitroprusside (3–30 μM), but it significantly inhibited the generation of osteoclasts at a high concentration (100 μM) of sodium nitroprusside.

Discussion

Nitric oxide (NO) is a very small lipophilic molecule which rapidly diffuses and reaches the cytoplasmic compartments, and hence results in the activation of diverse biological func-

Table 5. Effect of sodium nitroprusside on the 1α,25-$(OH)_2D_3$-induced osteoclast-like cell generation in co-cultivation of primary foetal mouse osteoblast-like cells with bone marrow cells

Treatment	No. of TRAP-positive multinucleated cells per well
1α,25-$(OH)_2D_3$ only	220 ± 26
Sodium nitroprusside 3 μM	302 ± 46*
30 μM	472 ± 51**
100 μM	112 ± 24**

Primary foetal mouse osteoblast-like cells and mouse bone marrow cells were plated together at a density of 1.5×10^4 cells per well and 3.0×10^5 cells per well onto 4-well plates and cultured for 8 days with 1α,25-$(OH)_2D_3$ (10^{-7} M) and sodium nitroprusside. Data were the means ± s.e. of four experiments. *$P < 0.05$. **$P < 0.01$. TRAP, tartrate-resistant acid phosphatase.

tions. Interestingly, NO is a soluble gas in aqueous medium and its biological functions are confined to adjacent cells since its ultrashort half-life limits the availability of newly synthesized NO to substances or cells nearby (Moncada & Higgs 1993). Recently, it has been reported that pro-inflammatory cytokines such as IL-1β, TNF-α and IFN-γ modulate the metabolism of bone remodelling in-vitro and in-vivo (Gowen et al 1983; Bertolini et al 1986). These cytokines also induce the production of NO in various cell types of bone tissue. These reports suggest that NO may be a modulator in the cytokine-induced processes of bone remodelling; especially it may function in the regulation of bone resorption.

In this study we showed that primary mouse osteoblast-like and ROS 17/2·8 cells did not produce biologically significant amounts of NO upon single treatment with various cytokines including IL-1β, TNF-α and IFN-γ. However, a combination of these three cytokines worked synergistically to generate NO in primary mouse osteoblast-like cells and ROS 17/2·8 cells. These data were consistent with previous studies in several systems, including macrophages (Hibbs et al 1988), neutrophils (McCall et al 1989), mesangial cells (Shultz et al 1991), hepatocytes (Nussler et al 1992), chondrocytes (Palmer et al 1992), mouse osteoblasts (Löwik et al 1994), MC3T3E1 osteoblasts (Hukkanen et al 1995; Damoulis & Hauschka 1997) and human osteoblasts (Ralston et al 1994).

Recent studies have shown that NO plays an important role in the regulation of osteoclast activity. MacIntyre et al (1991) showed that high concentration of NO donors, such as 3-morpholino sydnonimine and sodium nitroprusside, directly inhibited cytoplasmic spreading, one of the resorption processes in osteoclasts. In contrast , both IL-1 and TNF-α stimulated the release of NO to enhance the resorption processes by osteoclasts (Gowen et al 1983; Bertolini et al 1986; Nussler et al 1992). Thus, we tested whether NO has an effect on the metabolism of bone resorption in primary foetal osteoblast-like cells. The results showed that low or moderate concentrations of sodium nitroprusside increased the generation of 1α,25-$(OH)_2D_3$-induced TRAP-positive osteoclast-like cell (Table 5). Inhibition of NO production with NOS inhibitor, L-N^GMMA, partially inhibited bone resorption in the neonatal mouse calvarial organ culture (Ralston et al 1993). However high concentrations of sodium nitroprusside decreased the number of TRAP-positive cells in the same experimental condition. Gowen et al (1986) suggested that NO selectively inhibited the effect of IFN-γ on cytokine-induced resorption of bone tissue. We thought that when IFN-γ was combined with IL-1 and TNF-α, IFN-γ accelerated the machinery of NO generation triggered by IL-1 and TNF-α to produce more NO, enough to suppress bone resorption with a dramatic reduction in osteoclast numbers. These notions suggested that NO could either stimulate or inhibit the osteoclast activity of resorption in a dose-dependent pattern of NO concentration. The results were consistent with the report of Löwik et al (1994) who observed that sodium nitroprusside suppressed the osteoclast activity only at higher concentrations. However, NO may stimulate the generation of osteoclasts, hence subsequently augments the resorption process of bone at low concentration (which is more similar to the physiological level).

Osteoclasts and osteoblasts are closely related in the remodelling processes by which the sequence of resorption and formation of bone is highly ordered. Most previous works have focused on how NO regulates osteoclast activity in bone resorption, whereas little is known about the biological role of NO in osteoblast function. It is now generally accepted that osteoblasts release many soluble factors which modulate osteoclast activity by means of intercellular communication between osteoblasts and osteoclasts. Osteoblasts have receptors for parathyroid hormone on their cell surface (Silve et al 1982) and produce type-I collagen and non-collagenous proteins as well as a wide variety of biomolecules which affect the regulation of cell division, cell differentiation and bone mineralization. We examined the activity of alkaline phosphatase and calcified nodule formation, which are classical markers of osteoblast phenotype. Sodium nitroprusside stimulated the alkaline phosphatase activity and calcified nodule formation of primary foetal osteoblast and ROS 17/2·8 cells in a dose-dependent manner. These results suggested that NO is involved in the osteoblast metabolism of bone. Previously, Fox et al (1995) and Pitsillides et al (1995) had stated that bone formation induced by mechanical stimulation was due to the activation of NOS pre-existing in osteoblasts and showed that NOS inhibitor completely prevented mechanically stimulated bone formation. These data were similar to those we obtained with L-N^GMMA treatment of osteoblasts inhibiting the alkaline phosphatase activity and calcified nodule formation (data not shown). These findings strongly support the theory that NO mediates the intercellular signalling for bone remodelling that exists between osteoblasts and osteoclasts. We also suspect that there may an NO-induced intrinsic activation of bone cells in an autocrine manner because the ultrashort half-life of NO limits its functions only to adjacent cellular components.

Our results indicate that osteoblasts release NO which, in turn, may regulate the activity of cells in the remodelling processes of bone. NO may inhibit or augment osteoclast activity, hence NO induced by cytokines serves as a mediator to control the osteoclastic activity to avoid excess bone resorption as well as to prevent bone loss. However our results suggest that NO may be an important regulator of bone remodelling at local inflammatory sites, especially in cytokine-related diseases, including rheumatoid arthritis and periodontal disease.

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