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Zeolite A Inhibits Osteoclast-Mediated Bone Resorption In Vitro

N. Schütze, M.J. Oursler, J. Nolan, B.L. Riggs, and T.C. Spelsberg

Departments of Biochemistry and Molecular Biology (N.S., M.J.O., T.C.S.) and Endocrine Research Unit (B.L.R.), Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905; Whitby Inc., Richmond, Virginia 23261-5054 (J.N.)

Abstract The effects of Zeolite A on bone resorbing activity of highly purified avian osteoclasts were analyzed. The present study demonstrates that when 100 μg/ml of acid-treated Zeolite A is added to the media the number of pits per osteoclast is reduced 3-fold at 24 h after treatment. Secreted cathepsin B enzyme activity was also reduced 3-fold. A similar reduction in pit number per osteoclast was measured following 48 h of treatment with Zeolite A but there appeared to be less reduction of cathepsin B enzyme activity. The effects on pit number and cathepsin B protein activity were Zeolite dose dependent. The structure of the compound seemed to be responsible for the effects measured since compounds used to represent constituents of Zeolite A (silicon dioxide and aluminum chloride) failed to inhibit bone resorption or reduce the level of secreted cathepsin B enzyme activity. Thus the molecular architecture of Zeolite A or a derivative thereof appears to be important. In conclusion, the data indicate that Zeolite A can inhibit bone resorption. Together with previous data on osteoblasts, this might suggest a potential positive activity of intact Zeolite A or a partial substructure of Zeolite A on bone turnover.

Key words: zeolite A, resorption, osteoclasts, cathepsins, silicon dioxide, aluminum chloride, osteoporosis

In order to prevent bone loss in adults, a balance between osteoblast (OB)-mediated bone formation and osteoclast (OC)-mediated bone resorption is maintained [Puzas and Ishibe, 1992]. Bone remodeling is initiated by the activity of OCs in remodeling units and followed by the activity of OBs [Teitelbaum, 1993]. Disorders of bone remodeling such as osteoporosis result from a disruption of the finely tuned balance between bone formation and bone resorption [Mundy, 1993a]. The activity of OBs and OCs is regulated by a complex network of hormones and locally acting factors [Rifkin and Gay, 1992]. In addition to systemic hormones such as 1,25-dihydroxyvitamin D and parathyroid hormone, other factors, such as interleukins, colony stimulating factors, transforming growth factors, tumor necrosis factors, and more, have been found to be of particular importance [Roodman, 1993; Mundy, 1993b]. Despite our increasing knowledge of the regulation of bone remodeling, there are only limited options for increasing net bone mass. Therefore, additional yet unknown factors or mechanisms are likely to be important for modulating the activity of OBs or OCs.

The trace element silicon has been reported to influence bone metabolism. It has been shown for mice and rats that silicon localized in calcification sites [Carlisle, 1970] and that a silicon deficient diet resulted in changes in collagen synthesis and defects in skeletal structure [Carlisle, 1976]. Also growth promoting effects of silicon in rats have been reported [Schwartz and Milne, 1972]. Recently, the Zeolites, a group of silicon containing compounds, have been developed and submitted to extensive study. Zeolites are aluminosilicates with a cage-like structure that have been used as catalysts for a variety of chemical processes [Kerr, 1989]. The first interest in Zeolites arose when it was found that the addition of Zeolite A to chicken feed increased eggshell thickness [Roland and Dorr, 1989]. Also enhanced proliferation and differentiation of normal human osteoblast-like cells in response to Zeolite in vitro has been reported [Keeting et al., 1991].

In the present study, we have analyzed the effects of Zeolite A on the resorption capacity of

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Address reprint requests to Dr. T.C. Spelsberg, Department of Biochemistry and Molecular Biology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905.

highly purified chicken OCs and lysosomal enzyme activity of these cells.

MATERIALS AND METHODS

Animals and Drugs

Adult white leghorn chicken were used throughout the experiments. Dispase was purchased from Boehringer (Mannheim, Germany). Dynabeads were from Dynal (Oslo, Norway). Zeolite A was a gift from Whitby Research Inc. (Richmond, VA). All chemicals and reagents used were of analytical grade. Stock solutions of Zeolite A and its components were prepared as described by Keeting et al. [1991]. A stock solution containing 4 mg/ml Zeolite A in 0.1 N HCl was prepared fresh for each experiment just before addition to the OC cultures. After 5 min of resuspension in the acid the solution was sterile filtered and this solution or sterile filtered vehicle were immediately added to the cell culture medium at 25 μl/ml. The compounds used to represent components of Zeolite A were $AlCl_3$ and SiO_2 which were similarily prepared in 0.1 N HCl. The addition of this amount of 0.1 N HCl changes the pH of the OC cell culture medium from the preadjusted value of pH 6.50 to pH 6.25. No significant difference in pH was observed between addition of vehicle or Zeolite A (100 μg/ml).

Isolation of Osteoclasts

Two adult hens were routinely used for one experiment. Animals were maintained on a low calcium diet for 7–11 days. For preparation of OCs, hens were euthanized and tibia and humeri were removed. All subsequent steps were carried out on ice or at 4°C. Marrow was removed by flushing bones with a solution containing 1.4 M NaCl, 25 mM KCl, 4 mM NaH_2PO_4, 11 mM Glucose, and 2.5 mM $NaHCO_3$. Thereafter the bones were cut longitudinally and repeatedly shaken vigorously in the flushing solution. The bone fragments were then incubated with Dispase (Boehringer, Mannheim, Germany) for 1 h at 37°C. Released cells were filtered through a 350 μm nylon filter and pelleted (500*g*, 5 min).

Immunomagnetic Purification of Osteoclasts

For isolation of pure viable OC an immunomagnetic procedure as outlined by Collin-Osdoby et al. [1991] and Oursler et al. [1991] was used. Briefly, beads were washed with cell culture medium (DMEM/F12 1:1, plus 10% [w/v] fetal calf serum and antibiotics) and incubated with the antibody for 45 min at room temperature. Following another wash with cell culture medium, the antibody/bead mixture was added to the OC suspension in cell culture medium and incubated at 4°C for another 45 min. The cells were pelleted (500*g*, 5 min) and the OCs magnetically separated from the initial preparation. Finally, OCs were pelleted as above, suspended in cell culture medium and plated in 3–6 wells of 48 well plates together with 7 cleaned and sterilized bone slices. The pH of the culture medium was adjusted to pH 6.5, by addition of 0.1 N HCl.

Osteoclast Bone Resorption Assay

The assay system used was modified from the method of Walsh et al. [1991]. Bone slices were fixed in a solution containing 1% (v/v) paraformaldehyde in a solution containing 1.4 M NaCl, 25 mM KCl, 4 mM NaH_2PO_4, 11 mM Glucose, and 2.5 mM $NaHCO_3$ for at least 30 min. The number of attached OCs per bone slice was determined following staining of the bone slices for TRAP (tartrate resistant acid phosphatase) activity using a conventional immunochemical staining kit (Sigma). TRAP positive multinucleated cells were counted by light microscopy. The cells were then removed mechanically. Resorption pits on the bone surface were visualized by staining with a 1% (w/v) toluidine blue solution (in 1% [w/v] $NaBO_4$) for at least 10 min. Pit size and number of resorption pits was quantitated applying reflected light microscopy using a microscope with a digitizing tablet connected to a microcomputer. Results are expressed as pits per cell for vehicle (0.1 N HCl), Zeolite A, SiO_2, or $AlCl_3$ treated cells as determined from the number of attached cells and the pit number obtained for the 7 bone slices each ±SEM used for individual incubations.

Assay for Lysosomal Enzyme Activity

The activities of secreted cathepsin B and cathepsin L enzyme activity were determined from aliquots of cell culture medium obtained from incubations with OCs. The assay was performed according to Barret and Kirschke [1981] with modifications. Briefly, for determination of cathepsin B a photometric assay was applied using Z ArgArg N Nap as a substrate. Following coupling of released 2-napthylamide to a diazonium salt the change in absorption at 520 nM was used for calculation. Every incubation was done in duplicate. In parallel to each experiment

standards at different concentrations were analyzed. The change in absorption due to 100 nmol of 2-naphtylamide (highest concentration of standard used) corresponds to 10 mU ($10 \cdot 10^{-3}$ μmol/min) [Barret and Kirschke, 1981]. For cathepsin L activity Z Phe Arg N Mec was used as a subtrate and the change in absorption at 460 nM used for calculation. Data obtained for vehicle, Zeolite A, SiO_2, or $AlCl_3$ treated cells were normalized according to the mean number of attached cells per bone slice.

Statistics

Statistical analysis of the pit resorption data was performed using the unpaired Wilcoxon rank sum test. Results are expressed as average ± SEM.

RESULTS

For this study OCs were purified according to the immunomagnetic method as outlined by Collin-Osdoby et al. [1991] and Oursler et al. [1991]. The resulting OC cell population is highly purified to more than 90%. The complex mixture of cells released by enzymatic treatment of bone fragments prior to the magnetic separation beside OCs mainly consists of red blood cells and other mononuclear cells such as macrophages, preosteoclasts, osteoblasts, and preosteoblasts. Thus, after purification of the OC, the number of contaminating osteoblasts in our final cell preparation is very low. Therefore, it is extremely unlikely that the 3-fold inhibitory effect of Zeolite A on OC resorption and the inhibition of cathepsin B activity as described below are mediated indirectly by contaminating osteoblasts.

Effects of Zeolite A on Pit Number per OC

The addition of Zeolite A (100 μg/ml) for a period of 24 h to incubations of purified OC with bone slices resulted in a marked reduction of the OC activity. The reduced level of OC activity was determined in the number of pits per OC. Figure 1 shows that the number of pits per cell for Zeolite A treated cells was 3.3-fold reduced compared to vehicle treated cells (29.8 ± 13.4% vs. 100% as determined from the 21 experiments performed in this study).

In general 10–40 multinucleated TRAP positive cells were identified on each individual bone slice. Up to 50 pits were measured from individual bone slices. After 24 h of incubation the mean number of cells attached to the bone slices

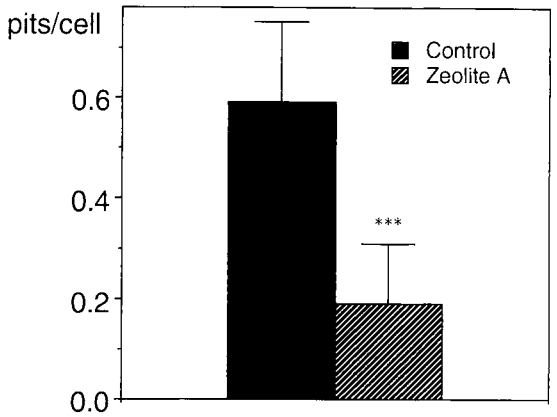

Fig. 1. Zeolite A reduces pit number per OC. OCs were incubated with bone slices as described in Materials and Methods. Vehicle (0.1 N HCl) or Zeolite A at 100 μg/ml were added. After 24 h the pit resorption assay was applied as described. Results are expressed as pit number per OC ± SEM as determined from 7 bone slices per incubation. ***$P < 0.001$.

for vehicle (=100%) treated versus Zeolite A treated cells was not altered (=124 ± 35%, n = 21). In addition the mean pit area was not affected by Zeolite A treatment (100% vs. 85 ± 30%, n = 21).

A similar reduction of pit number per OC by Zeolite A as measured after 24 h of treatment also was obtained after 48 h of treatment as is shown in Figure 2 (inhibition to 29.8 ± 15.4% after 24 h of incubation and to 32.2 ± 13.2% after 48 h, compared to control cells, n = 5). Again no difference was detected in the number of attached cells (100% vs. 99 ± 12%, n = 5) or the mean pit area (100% vs. 93 ± 92%, n = 5). However, the number of pits per OC after 48 h of culture increased 1.6-fold for control cells and 1.7-fold for Zeolite A treated cells compared to 24 h of culture in these experiments. This indicates that the viability of the OC cell population throughout the incubation period is maintained in the absence as well as presence of Zeolite A.

The effect of lower concentrations of Zeolite A on bone resorption was also analyzed (Fig. 3). In these experiments (n = 5), parallel incubations with Zeolite A at a concentration of 100 μg/ml resulted in a reduction in pit number to 32.4 ± 18.0% of control levels. At 10 μg/ml the inhibitory effect was still measurable to 60.2 ± 16.9% of the control levels while at 1 μg/ml there was no reduction of pit number per OC (83.6 ± 13.3% vs. 100%).

To examine the possibility that the inhibition of bone resorption was the result of a depletion of specific serum proteins during culture, the experiments were repeated in BSA containing

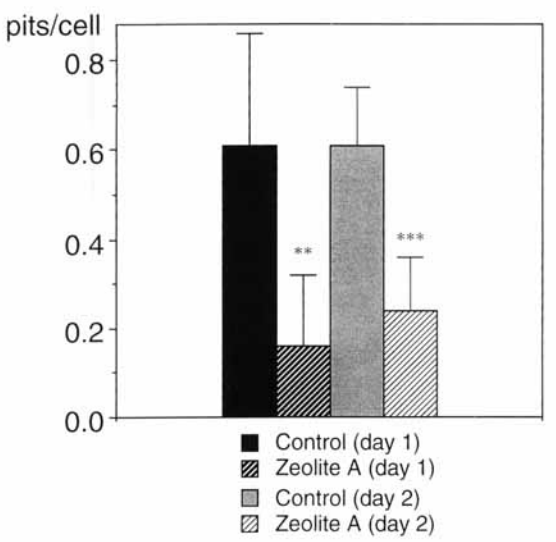

Fig. 2. Time dependent reduction of pit number per OC by Zeolite A treatment. OCs were incubated with bone slices as described in Materials and Methods. Vehicle (0.1 N HCl) or Zeolite A at 100 μg/ml were added. After 24 and 48 h the pit resorption assay was applied as described. Results for Zeolite A treated cells are expressed as pit number per OC ± SEM as determined from 7 bone slices per incubation. ***$P < 0.001$. **$P < 0.005$.

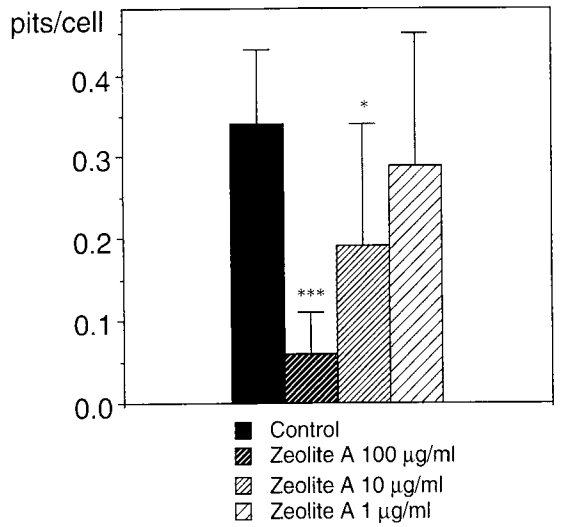

Fig. 3. Dose dependent reduction of pit number per OC by Zeolite A treatment. OCs were incubated with bone slices as described in Materials and Methods. Vehicle (0.1 N HCl) or Zeolite A at 100 μg/ml, 10 μg/ml, or 1 μg/ml were added. After 24 h the pit resorption assay was applied as described. Results for Zeolite A treated cells are expressed as pit number per OC ± SEM as determined from 7 bone slices per incubation. ***$P < 0.001$. *$P < 0.05$.

serum-free medium (Table I). Zeolite A at 100 μg/ml reduced the pit number per OC compared with vehicle control similarily in the BSA-containing medium as it did in the serum-containing medium.

TABLE I. Reduction of Pit Number per OC by Zeolite A Treatment†

Serum-medium		BSA-medium	
Control	Zeolite A	Control	Zeolite A
0.59 ± 0.16	0.19 ± 0.12*	0.54 ± 0.26	0.09 ± 0.14*
0.15 ± 0.04	0.05 ± 0.05**	0.25 ± 0.10	0.08 ± 0.06**

†Cells were isolated and incubated in serum-containing and BSA-containing serum free cell culture medium as described in Materials and Methods. Results are expressed in pit number per OC ± SEM obtained from 7 bone slices per incubation for each of the 2 experiments performed.
*$P < 0.001$.
**$P < 0.005$.

To determine whether a breakdown of Zeolite A releases constituents which represent the active agents which inhibit bone resorption, experiments were performed using Al^{3+}, SiO_2, or combinations of both at concentrations which would correspond to a 100% dissociation of Zeolite A. Table II shows the results obtained from 4 experiments. The data demonstrate that Al^{3+}, $SiO_2 \cdot XH_2O$ or both had no effect on resorption levels, whereas Zeolite A treatment resulted in a reduction in pit number per OC.

To determine whether Zeolite A could alter the bone surface and thereby lead to a reduction in OC resorption activity, bone slices were first incubated with cell culture medium plus acid-treated Zeolite A (100 μg/ml) only for 3 h. The medium was then removed, the bone slices briefly washed with cell culture medium to remove Zeolite A and the slices incubated with the OCs. The number of cells attached after this procedure was not different from parallel incubations with OCs plus vehicle or Zeolite A at 100 μg/ml (Table III). Importantly, the number of pits per cell determined from incubations with these Zeolite pretreated bone slices was not different from those in the control incubations with untreated bone slices where OC were treated with vehicle. Parallel incubations demonstrated that Zeolite A treatment (100 μg/ml) of OC reduced the OC mediated bone resorption levels.

Effects of Zeolite A on Cathepsin B Enzyme Activity

To elucidate part of the mechanism by which Zeolite A modulated bone resorption, secreted cathepsin B enzyme activity was measured in the supernatants obtained from the OC-bone resorption study (i.e., conditioned media). As is shown in Figure 4 cathepsin B activity was reduced after 24 h of incubation with Zeolite A.

TABLE II. Effects of Constituents of Zeolite A on Pit Number per OC†

Assay	Control	Zeolite A	Al^{3+}	SiO_2	Al^{3+} plus SiO_2
1	0.33 ± 0.07	0.04 ± 0.05*	0.21 ± 0.13	0.26 ± 0.15	—
2	0.26 ± 0.06	0.12 ± 0.05*	0.18 ± 0.11	0.19 ± 0.13	—
3	—	0.17 ± 0.08	0.30 ± 0.13	0.37 ± 0.19	0.35 ± 0.12
4	0.38 ± 0.17	0.14 ± 0.10**	0.40 ± 0.16	0.32 ± 0.20	0.35 ± 0.11

†Cells were isolated and incubated as described in Materials and Methods. Results are expressed in pit number per OC ± SEM obtained from 7 bone slices per incubation for each of the experiments performed. In assay 3 control cells became contaminated. Al^{3+} plus SiO_2 were only used in assay 3 and 4.
*$P < 0.001$.
**$P < 0.01$.

TABLE III. Zeolite A Treatment of the Bone Slices Does Not Alter OC-Resorption†

Assay	Control	Zeolite A	Zeolite A wash
A) OC per bone slice			
1	100	90	130
2	100	89	69
3	100	100	90
	100	93 ± 6	96 ± 31
B) Pit number per OC			
1	0.48 ± 0.11	0.11 ± 0.09*	0.44 ± 0.14
2	0.40 ± 0.14	0.16 ± 0.13**	0.50 ± 0.30
3	0.44 ± 0.06	0.13 ± 0.10*	0.34 ± 0.13

†Cells were isolated and incubated as described in the Materials and Methods section. Cells were incubated with vehicle (0.1 N HCl; "control") or Zeolite A (100 mg/ml; "Zeolite A"). In a parallel incubation OC were incubated with vehicle and bone slices which have been pretreated with Zeolite A (100 μg/ml) in cell culture medium under cell culture conditions for 3 h ("Zeolite A wash"). A: The number of cells attached per bone slice is expressed as % of control cells (=100%). B: Pit number per OC ± SEM (n = 7) obtained from 7 bone slices per incubation for each of the 3 experiments performed.
*$P < 0.001$.
**$P < 0.005$.

From the 21 experiments performed for this study Zeolite A reduced the cathepsin B activity to 35.6 ± 27.7% compared to vehicle treated cells (=100%). As is shown in Figure 5, the reduction of cathepsin B enzyme activity after 48 h of treatment was less pronounced (16.1 ± 16.0% vs. 100% after 24 h and 62.3 ± 21.9% after 48 h, n = 5). Dose dependent experiments demonstrate that the reduction of secreted cathepsin B enzyme activity by Zeolite A treatment was measured at a concentration of 100 μg/ml (Fig. 6) (reduction to 41.5 ± 32.3% vs. 100%, n = 5). At 10 μg/ml this effect was less pronounced (56.0 ± 38.0% vs. 100%, n = 5), whereas 1 μg/ml was ineffective (100.9 ± 39.4% vs. 100%, n = 5).

To avoid potential problems with depleted serum factors, cathepsin B enzyme activity from supernatants of incubations using BSA containing medium were studied and found to be similarly reduced by Zeolite A treatment (100 μg/ml) compared to the serum containing medium (Table IV). The constituents of Zeolite A (Al^{3+}, SiO_2, or both) did not reduce the level of secreted cathepsin B enzyme activity (Fig. 7), whereas in parallel incubations with Zeolite A at 100 μg/ml the typical reduction in secreted cathepsin B activity is observed.

Effects of Zeolite A on Cathepsin L Activity

Cathepsin L activity was detectable in the media of 3 of 6 experiments in either control and Zeolite A treated OC incubations (data not shown). In the positive experiments no reduction of cathepsin L activity by Zeolite A treatment was measurable.

DISCUSSION

Inhibitors of bone resorption have the capacity to increase net bone mass. Resorption can be affected in different ways [Roodman, 1993], including differentiation of OC precursors into fully differentiated active OCs [Suda et al., 1992] or the regulation of the resorbing capacity of individual OCs [Baron, 1989].

The experiments described here were aimed at assessing the effect of Zeolite A on the resorbing capacity of isolated highly purified chicken OCs. The results show a 3-fold reduction in pit number per cell by treatment with 100 μg/ml of Zeolite A. The results demonstrate that Zeolite A had no effect on the bone surface by itself. The number of cells attached to the bone slices was identical for vehicle and Zeolite A treated OCs. More importantly, pretreatment of bone slices with Zeolite A prior to the incubation with OCs did not alter this finding. Also the number of

TABLE IV. Reduction of Cathepsin B Enzyme Activity by Zeolite A Treatment†

Assay	Control (serum)	Zeolite A	Control (BSA)	Zeolite A
1	1.56 ± 0.05	0.33 ± 0.09	1.71 ± 0.09	0.46 ± 0.08
2	0.29 ± 0.06	0.02 ± 0.02	0.28 ± 0.02	0.05 ± 0.04

†Cells were isolated and incubated in serum-containing and BSA-containing serum free cell culture medium as described in Materials and Methods. Results are expressed in mU of cathepsin B activity ± SEM of the two determinations.

pits per OC obtained from bone slices treated with Zeolite A prior to the incubation with OCs was not different from the results obtained from bone slices which were only treated with vehicle during the incubation with OCs.

As the number of cells attached to the bone slices was identical for vehicle and Zeolite A treated cells, we conclude that Zeolite A is not toxic to the cells. Further, the cells remain viable throughout the incubation period of up to 48 h as the number of pits per OC increased after 48 h of culture compared to 24 h of culture. The possibility exists that Zeolite A may regulate the time needed for an individual OC to attach to the bone surface and become active. However, the 3-fold inhibition in pit number per OC measured after 24 h of incubation provides strong evidence that the bone resorbing activity of the individual OC is mainly responsible for the Zeolite effect.

Control experiments using BSA in serum free medium clearly eliminated the possibility that this reduction in pit number per cell by Zeolite A treatment is a result of a depletion of certain serum factors.

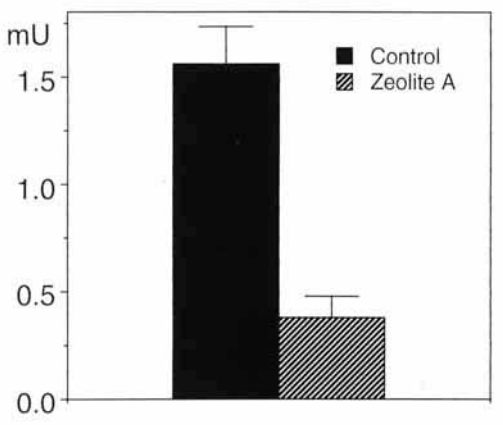

Fig. 4. Inhibition of cathepsin B enzyme activity by Zeolite A. Supernatants of incubations of OCs with bone slices were assayed for cathepsin B enzyme activity as described in Materials and Methods. OCs were incubated with vehicle (0.1 N HCl) or Zeolite A (100 μg/ml) for 24 h. Results are expressed as mU of cathepsin B activity ± SEM of the two determinations.

It is known that the activity of OCs is reduced when the pH of the culture medium is raised from pH 6.5 to pH 7.5 [Arnett and Dempster, 1986; Shibutani and Heersche, 1993]. The reduction in pit number per OC obtained between Zeolite A and vehicle treated cultures can not be explained by differences in pH as the addition of Zeolite A to the cell culture medium does not result in a different pH compared to vehicle treated culture medium. Zeolite A consists of a tetrahedral, cage-like structure. In aqueous acidic solutions as used here, the substance partially dissociates releasing $Si(OH_4)$ and Al^{3+} (Whitby Res. pers. com.). Salicic acid and/or aluminum^{3+} solutions were unable to duplicate the effect of Zeolite A. The results presented in the paper provide evidence that a residual structure of Zeolite A or a component substructure of Zeolite A is responsible for the reduction of OC resorbing activity.

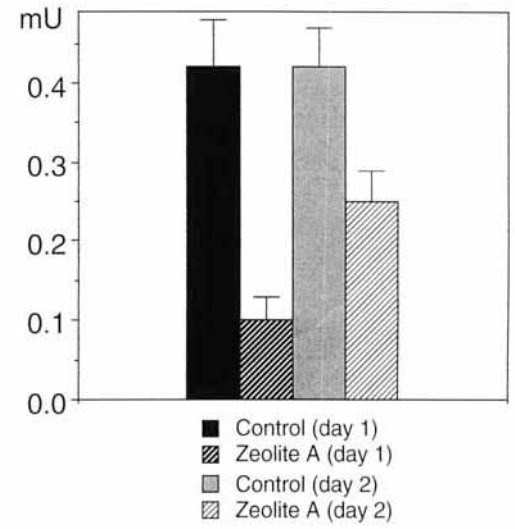

Fig. 5. Time dependent reduction in cathepsin B enzyme activity by Zeolite A. Supernatants of incubations of OCs with bone slices were assayed for cathepsin B enzyme activity as described in Materials and Methods. OCs were incubated with vehicle (0.1 N HCl) or Zeolite A (100 μg/ml) for 24 and 48 h. Results are expressed as mU of cathepsin B activity ± SEM of the two determinations.

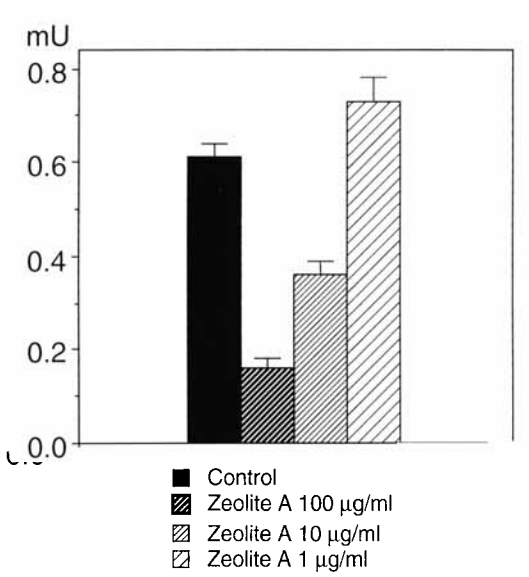

Fig. 6. Dose dependent reduction in cathepsin B enzyme activity by Zeolite A. Supernatants of incubations of OCs with bone slices were assayed for cathepsin B enzyme activity as described in Materials and Methods. OCs were incubated with vehicle (0.1 N HCl) or Zeolite A (100 μg/ml, 10 μg/ml, or 1 μg/ml) for 24 h. Results are expressed as mU of cathepsin B activity ± SEM of the two determinations.

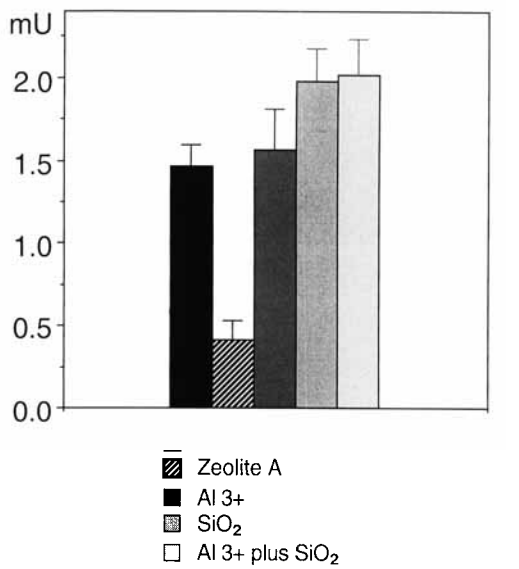

Fig. 7. Constituents of Zeolite A do not reduce cathepsin B enzyme activity. Supernatants of incubations of OCs with bone slices were assayed for cathepsin B enzyme activity as described in Materials and Methods. OCs were incubated with vehicle (0.1 N HCl), Zeolite A (100 μg/ml), SiO_2, Al^{3+}, or both ions for 24 h. Results are expressed as mU of cathepsin B activity ± SEM of the two determinations.

At present we do not know which specific structure of Zeolite A is the active component. However, an attempt to elucidate the mechanism of action of Zeolite A on OC was made by measuring the activities of secreted lysosomal proteases in the media of OC with bone slices. Different lysosomal enzymes have been reported to be of particular importance for bone resorption [Delaisse and Vaes, 1992]. Among different cathepsins and related proteinases, cathepsin B and cathepsin L seem to play a major role in the degradation of the bone matrix [Goto et al., 1993; Blair et al., 1993; Kakegama et al., 1993].

In the present study cathepsin B and cathepsin L were measured in the supernatants of incubations of OCs with bone slices. Whereas cathepsin L activity was uneffected by treatment of OC with Zeolite A, cathepsin B activity was markedly reduced. Paralleling the results of the effects of aluminum and silicon on resorption activity, the cathepsin B enzyme activity seemed to be affected by a residual structure of Zeolite A and not by the molecular components. Moreover, dose and time dependent effects of Zeolite A were observed in the reduction of cathepsin B enzyme activity which paralled the resorption activity. These similarities suggest that this reduction in cathepsin B enzyme activity by Zeolite A treatment, at least in part, may be responsible for the reduction in resorption activity. The possibility that some other contaminant of Zeolite A is active at reducing OC-activity can not been eliminated, but the Zeolite A is highly pure with less than 0.001% contaminants by weight.

In summary, our results demonstrate that Zeolite A or a substructure of Zeolite A reduces osteoclast-mediated resorption activity of mature avian OCs and reduces the secreted levels of cathepsin B enzyme activity. We conclude that this inhibiting effect on bone resorption and the previous data of Zeolite A effects on OB [Keeting et al., 1991] suggest a positive activity of Zeolite A on bone turnover. Future work is necessary to understand the exact mechanism of action of Zeolite A.

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