Synthesis and Preclinical Pharmacology of 2-(2-Aminopyrimidinio) Ethylidene-1,1-Bisphosphonic Acid Betaine (ISA-13-1)–A Novel Bisphosphonate

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Received April 1, 1999; accepted June 6, 1999

Purpose. To validate our hypothesis that a bisphosphonate (BP) having a nitrogen-containing heterocyclic ring on the side chain, and with no hydroxyl on the geminal carbon would possess increased activity, and better oral bioavailability due to enhanced solubility of its calcium complexes/salts and weaker Ca chelating properties.

Methods. A novel BP, 2-(2-aminopyrimidinio)ethylidene-1,1-bisphosphonic acid betaine (ISA-13-1) was synthesized. The physicochemical properties and permeability were studied in vitro. The effects on macrophages, bone resorption (young growing rat model), and tumor-induced osteolysis (Walker carcinosarcoma) were studied in comparison to clinically used BPs.

Results. The solubility of the Ca salt of ISA-13-1 was higher, and the log $\beta_{Ca:\,BP}$ stability constant and the affinity to hydroxyapatite were lower than those of alendronate and pamidronate. ISA-13-1 exhibited effects similar to those of alendronate on bone volume, on bone osteolysis, and on macrophages, following delivery by liposomes. ISA-13-1 was shown to have 1.5–1.7 times better oral absorption than the other BPs with no deleterious effects on the tight junctions of intestinal tissue.

Conclusions. The similar potency to clinically used BPs, the increased oral absorption as well as the lack of effect on tissue tight junction of ISA-13-1 warrant its further consideration as a potential drug for bone diseases.

KEY WORDS: bisphosphonates (diphosphonates); calcium-related disorders; bone-related disorders; drug administration; drug absorption tight junctions; mannitol.

INTRODUCTION

Several bisphosphonates (Fig. 1) are in use for the treatment of various calcium-related disorders such as Paget's disease, hypercalcemia of malignancy, tumor osteolysis and, most recently, in osteoporosis (1). The macrophage suppressive effect of BPs has attracted interest recently as a possible approach to pharmacotherapy in rheumatoid arthritis (2). The biological effects of the BPs stem from their incorporation in bone, enabling direct interaction with osteoclasts and/or osteoblasts through a variety of biochemical pathways (3). A significant drawback of the BPs is their very poor oral absorption (less than 1%) which is further diminished by concomitant food intake (4). The oral administration of BPs is associated with GI disturbances (5), and it is believed that they are absorbed via the paracellular route with accompanied disruption of the tight junctions (6,7). Therefore, the development of potent BPs possessing increased oral absorption with no toxic effect is of importance.

It appears that the incorporation of nitrogen-containing heterocyclic rings increases their pharmacological activity (8,9). The capacity of BPs to accumulate in bone is attributed to their high affinity to HAP due to a tridentate interaction of the phosphonic and hydroxyl functions with calcium. In the absence of a hydroxyl group on the geminal carbon only bidentate complexes can be formed resulting in lower affinities to calcium and a better solubility of the calcium/salts complexes (10). The latter characteristics could contribute to better compatibility with food and increased oral absorption. We hypothesized that a BP with a nitrogen-containing heterocyclic ring in the side chain, and with no hydroxyl on the geminal carbon could show increased activity as well as better oral bioavailability.

MATERIALS AND METHODS

Syntheses

Alendronate, [^{14}C]-Alendronate (Sp.Act.1 μCi/mg), Pamidronate, and [^{14}C]-Pamidronate (Sp.Act.1 μCi/mg) were synthesized as described by Alferiev *et al.* (11) with some modifications. Both the "cold" and radioactive 2-(2-Aminopyrimidinio) ethylidene-1, 1-bisphosphonic acid betaine (ISA-13-1) were synthesized by the nucleophilic addition of 2-aminopyrimidine to the activated double bond of vinylidene-1, 1-bisphosphonic acid, using the methodology developed by Alferiev *et. al.* (12) as shown in Fig. 1. Elemental analysis and spectroscopic methods showing the expected results identified the products.

Physicochemical Properties

Dissociation and Stability Constants

The dissociation constants (pKa) of alendronate and ISA-13-1, and the calcium-complex stability constants (logβ) were determined by high precision, potentiometrically controlled titration (SCHOTT Geräte, Hofheim, Germany) and subsequent iteration of titration data with the program ITERAX 2.01. For pKa determinations, 50 ml of a solution consisting of 0.25 mmol BP, 1.5 mmol NaOH and 3.5 mmol NaCl was titrated vs. 0.1 M HCl in equidistant steps of 0.1 ml at 25 ± 0.1°C. The pH was monitored using a glass electrode calibrated by

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the method of blank titration (8 and 2 titrations were performed for pamidronate and alendronate, and for ISA 13-1, respectively).

The stability constants were determined in titrated solutions containing a metal: ligand ratio of 1:1 (Ca:BP) of 0.002 M Ca and BP, and 0.01 M NaOH, ionic strength of 0.1 M (NaCl) with 0.1 M HCl, using combined glass-electrode for pH determinations.

Solubility of the Bisphosphonates

Solubility of the Ca salts of the BPs was determined by analyzing the amount of BP that remained in the supernatant of Tris buffer solutions (pH 7.4) containing 1 mM BP, and 1.2 mM $CaCl_2$ (ionic Ca physiological concentration). The solutions were shaken (100 rpm) and incubated at 37°C. After 1 and 24 h, the solutions were centrifuged (1900 g, 10 min.), and the BPs concentrations in the supernatants were determined at 305 nm for ISA-13-1, and by HPLC for alendronate (13).

Binding Affinity to HAP

The binding affinities of the BPs to HAP were calculated from the BPs adsorption isotherms as described elsewhere (14). The concentration of ISA-13-1 was determined at λ = 305 nm.

Inhibition of HAP Formation and Dissolution

The inhibition of HAP formation and dissolution in the presence of the tested compound were studied as described previously (14). Following preliminary studies, the drug concentrations were chosen to be 0.1 mM and 0.048 M for the inhibition of HAP formation and dissolution, respectively.

Intestinal Permeability

The permeability and oral bioavailability of ISA-13-1 were studied in comparison to pamidronate. Pamidronate's oral absorption in animals and humans as well as its physicochemical characteristics are similar to those of alendronate and other BPs (1,8,10). All experimental animal studies adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23), and the guidelines of the Hebrew University of Jerusalem.

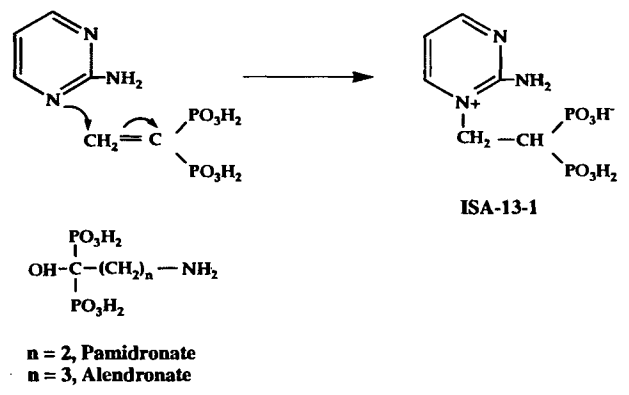

Fig. 1. The nucleophilic addition of 2-aminopyrimidine to the activated double bond of vinylidene-1,1-bisphosphonic acid for the synthesis of 2-(2-Aminopyrimidinio) ethylidene-1,1-bisphosphonic acid betaine (ISA-13-1), and structures of clinically employed BPs.

The permeability of ISA-13-1 and pamidronate across a segment of jejunal tissue were conducted in diffusion cells (1.88 cm^2, 8 ml of bicarbonate-Ringer buffer, 37°C, and gas lift of 95% O_2–5% CO_2). ISA-13-1 and [^{14}C]-pamidronate ([^{14}C] 1 μCi/mg) at concentrations of 1 mM were added to the mucosal bathing solution. Samples (1 ml) were taken from the serosal bathing solutions at 15-min intervals, and were replaced with buffer. Since mannitol is slowly transported via the paracellular route and is used as a marker of the tight junction integrity (15), the permeability of the jejunal tissue to D-mannitol (10 mM and a tracer amount of its ^{3}H isotope, 30 Ci/mmol) was examined in the presence of both drugs.

In Vivo Absorption and Disposition

Absorption and disposition of [^{14}C]-pamidronate and of [^{14}C]-ISA-13-1 (10 mg/kg) were examined in rats following peroral administration of the drugs' solution (via a stomach tube). Male Sabra rats (250–300 g) were acclimated in metabolic cages one week prior to the investigation, with free access to water and food except for 16 h prior to drug administration when only water was made available. The rats were sacrificed 24 h after drug administration, and drug amount in the tibia, femur, kidney, liver, intestine, spleen, muscle, brain, urine and feces were determined by radioactive measurements following digestion of the specimens to CO_2 by means of a SampleOxidizer (Packard, USA).

Effects on Bone Development

In preliminary experiments ISA-13-1 was found more effective than pamidronate. Therefore, the activity of ISA-13-1 was compared to that of alendronate, the most potent BP in clinical use (1). Three-week-old male rats (Sabra) were treated by daily intramuscular injections of ISA-13-1 and alendronate for 14 days at a dosage of 0.01 mg P/kg/day. The control group received normal saline. The bones were analyzed as described previously (14).

Walker Carcinosarcoma (WCS) Model

This model was used to evaluate ISA-13-1 effect on bone resorption in a tumor osteolysis model (14). On day 2 of the experiment, the rats were divided into 3 groups: Control (saline), alendronate, and ISA-13-1 that received 50 μmol/kg, 0.5 ml s.c. injections on days 2, 3, and 4. On days 6 and 9 the rats were weighed. The amount of calcium in the urine were measured on days 2, 3, 4, 6, 8 and 9, as well as the levels of pyridinum crosslinks excreted in urine. Total urinary pyridinoline (PYD) and deoxypyridinoline (DPD) were determined by HPLC as described previously (16).

Effects on Macrophages

The drug was encapsulated in negatively charged DSPG:cholesterol (67:33) liposomes as described in detail elsewhere (17). The growth inhibitory properties of free and liposome-encapsulated ISA-13-1 were studied with murine macrophage cell line, RAW 264 (17). At the end of growth period, cell growth was evaluated using an MTT assay except that serum-containing medium was replaced with medium without serum just prior to the addition of MTT (18). The effect

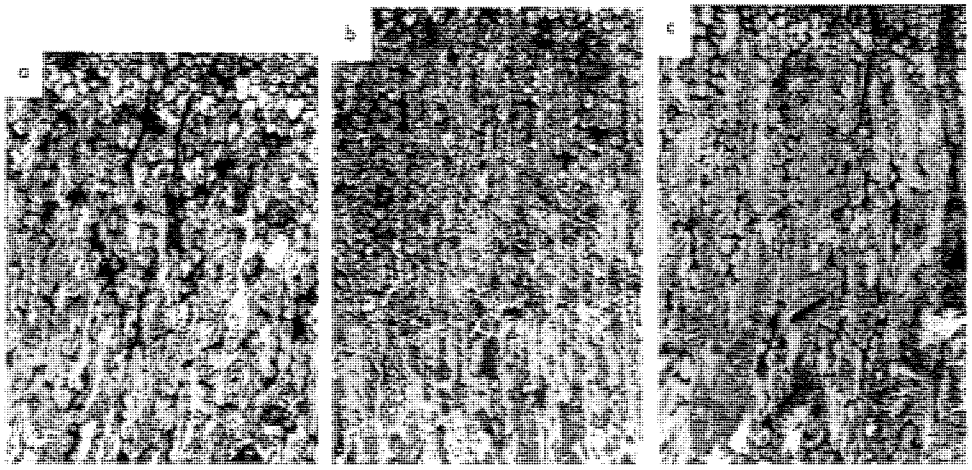

Fig. 2. Representative micrographs of longitudinal sections through the upper tibial metaphysis (the upper part is the growth plate, toluidine blue, ×400). (a), control group; (b,c), rats treated daily for two weeks with 0.01 mg P/Kg/day alendronate (b), and ISA-13-1 (c).

of ISA-13-1 on interleukin-6 (IL-6) from bacterial lipopolysaccharide (LPS) induced RAW 264 cells was studied as described (19,20).

RESULTS

Physicochemical Properties

The first pKa of ISA-13-1 is of the aromatic NH_2, and pKa_2-pKa_4 are of the -POHs (Fig. 2 and Table 1). Thus, the net charge at physiological pH is −2 (−3 of the -POHs and +1 of the ring nitrogen). Similarly, alendronate's net charge at physiological pH is −2.

The stability constant for the calcium complex formation (log $\beta_{Ca:BP}$) of ISA-13-1 was found to be significantly lower than those of the known BPs (Table 1). The solubility of ISA-13-1 Ca salt was more than two-fold than that of alendronate (and pamidronate, data not shown). The obtained N (mol/g) value of alendronate (the maximum amount of BP adsorbed obtained from the slope of the Langmuir isotherm) was slightly higher than that of ISA-13-1.

The inhibition of both HAP formation and dissolution *in vitro* is a basic characteristic of BPs and it is in good correlation with *in vivo* activity (14). ISA-13-1 was insignificantly less active than alendronate in inhibiting HAP formation. Both drugs were found significantly active with similar potency in inhibiting HAP dissolution (Table 1).

In Vivo Effects

Following treatment with ISA-13-1 and alendronate, bone weight and ash quantity were increased in comparison to the control group (Table 2). In addition, higher amounts of Ca (statistically significant only for ISA-13-1) were found in the ash in comparison to the control group. Bone volume was positively affected to the same extent by both drugs (Table 2). Histological examination of longitudinal sections through the upper tibial metaphysis of control and treated rats revealed relatively thin longitudinally oriented metaphyseal bone trabeculae in the control group (Fig. 2a). Wide metaphyseal bone trabecula was observed in rats treated with alendronate and ISA-13-1 (Figs. 2b and 2c, respectively).

Walker Carcinosarcoma Model

Control group urine calcium levels increased during the 9 days period of the experiment (Fig. 3). Both drugs have shown the tendency to maintain the normal urine calcium levels with no significant difference between their potency at all time points. No significant difference was found between the levels

Table 1. Physicochemical Properties of Alendronate and ISA-13-1

	NaCl	Alendronate	ISA-13-1
Dissociation constants pKa_1-pKa_5	—	<1.33, 2.22, 6.36, 10.96, 11.82	<1, 1.10, 1.41, 5.82, 13.78
Calcium-complex stability constant (logβ_1, Ca:BP)	—	7.58 ± 0.11	5.54 ± 0.005*
Solubility# in 1.2 mM Ca solution (% of initial drug concentration)	—	37.5 ± 0.9	94.7 ± 2.5*
Affinity to HAP N (mol/g), amount adsorbed at saturation	—	2.87×10^{-4}	2.56×10^{-4}
Inhibition of HAP formation (% of initial Ca)	17.0 ± 2.2	85.4 ± 6.8	77.7 ± 1.5
Inhibition of HAP dissolution (% of control)	100.0 ± 3.6	82.3 ± 8.3	84.6 ± 9.2

Solubility was determined after 1-h incubation. After 24 h of incubation (steady state) the solubility (% of initial drug concentration) of alendronate and ISA-13-1 was 46.5 ± 2.1 and 55.2 ± 1.8, respectively.

* Differences between alendronate and ISA-13-1 groups were termed statistically significant by the student's t test ($p < 0.05$, mean ± SD).

Table 2. The Effect of Daily IM Injections (14 days) of Alendronate and ISA-13-1 (0.01 mg P/kg/day), on Bone Chemistry, and on the Upper Metaphysis Histology (Computerized Histomorphometry)

	NaCl	Alendronate	ISA-13-1
Bone weight (mg)	219.75 ± 31.75	305.75 ± 26.73*	273.75 ± 23.57*
Ash content (%)	45.07 ± 1.78	52.46 ± 1.54*	52.30 ± 2.24*
Ca in ash (%)	33.49 ± 2.40	34.77 ± 4.38	37.47 ± 2.94*
Cartilage (%)	15.40 ± 0.69	19.17 ± 2.34	20.77 ± 0.76*
Bone volume (%)	16.72 ± 0.78	26.82 ± 0.85*	25.62 ± 1.07*
Bone marrow (%)	57.25 ± 2.05	43.45 ± 2.32*	43.37 ± 1.69*

* Differences were termed statistically significant by the paired t test ($n = 8$, $p < 0.05$, mean ± SD). No significant differences in weight gain, serum calcium, magnesium and phosphorus levels, and alkaline phosphatase activity were found between the control and the treated groups indicating normal bone turnover.

at day 0 and those at all other days post treatment with both drugs. Alendronate treatment resulted in a stronger, but statistically insignificant, hypocalcemic effect than ISA-13-1 on days 6 and 8.

In saline-treated rats, some decline in both PYD (Fig. 4a) and DPD (Fig. 4b) excretion was observed after tumor injection. However, the decline was significant only in DPD levels at day 9 in comparison to time 0. In both PYD and DPD levels, there were no significant differences between all time points after time point 0. In alendronate treated tumor-bearing rats, the excretion of PYD and DPD showed a steady decline until 6 days after tumor injection. However, at day 8, concentrations of both crosslink components started to increase again. The reductions in crosslink excretion of alendronate treated rats at days 4 and 6 were significant in comparison to day 0 and in comparison to the control group at these days. Very similar findings were observed in the ISA-13-1 treated rats, both in regard to the magnitude and time course of change. No statistically significant differences were found between alendronate and ISA-13-1 at all time courses. In both groups, the change in DPD excretion was more pronounced than that observed in PYD excretion.

Effect on Macrophage Cell Line

Encapsulation of ISA-13-1 in negatively charged DSPG liposomes considerably enhanced its growth inhibitory potency on RAW 264 cells compared to free drug (Fig. 5a). Based on the IC_{50} values, liposome-encapsulated ISA-13-1 (IC_{50} = 1.8 μM) was about 110 times more potent than its free counterpart (IC_{50} = 196 μM). Free ISA-13-1 had an inhibitory effect on IL-6 secretion from macrophages (Fig. 5b), but this was attributed to cytotoxic effects of ISA-13-1, since the cell viability was also decreased after the drug and LPS treatment (Fig. 5c). At non-cytotoxic concentrations, liposome-encapsulated ISA-13-1 slightly enhanced the LPS-induced IL-6 production (Fig. 5b).

Permeation, Absorption and Disposition Studies

The permeability of rat jejunum to ISA-13-1 was twice that of pamidronate (Fig. 6a). The transport rate of mannitol was not affected by the presence of ISA-13-1 (Fig. 6b). In contrast, the transport rate of mannitol was increased in the presence of pamidronate.

Significant levels of both drugs were found in the feces, urine, bone, and in the intestinal wall. The bioavailability of ISA-13-1 was 1.5 times higher than pamidronate as evidenced by urine drug levels, or 1.7 times higher by comparing the urine PO/IV concentrations of the drugs. However, drug concentration in the bone was the same for both groups (0.02% of the administered dose).

DISCUSSION

Physicochemical Properties

The solubility of ISA-13-1 Ca salt was found to be significantly higher than that of alendronate, presumably due to bidentate chelation in ISA-13-1 as compared to alendronate (Table

Table 3. Distribution of Pamidronate and ISA-13-1 as % of the Administered Dose in Rats 24 h After IV (1 mg/kg) and PO (10 mg/kg) Administrations

	Pamidronate		ISA-13-1	
	IV	PO	IV	PO
Urine	24.20 ± 8.54	0.48 ± 0.04	21.13 ± 1.27	0.71* ± 0.12
Femur	2.22 ± 0.22	0.02 ± 0.003	1.81* ± 0.06	0.02 ± 0.006
Kidney	0.08 ± 0.01	0.001 ± 0.0008	0.09 ± 0.01	0.001 ± 0.0002
Liver	4.49 ± 1.61	ND	0.69* ± 0.11	ND*
Spleen	0.19 ± 0.05	ND	0.10* ± 0.02	ND*
Intestine	ND	0.24 ± 0.09	ND	0.05* ± 0.01

Note: Neither drug was detected in plasma, muscle, brain, or in the intestinal tissue after IV or PO administration. Neither drug was found in the spleen and liver following PO administration. * Differences were termed statistically significant by the Mann-Whitney test ($n = 6$, $p <$ 0.05, mean ± SD).

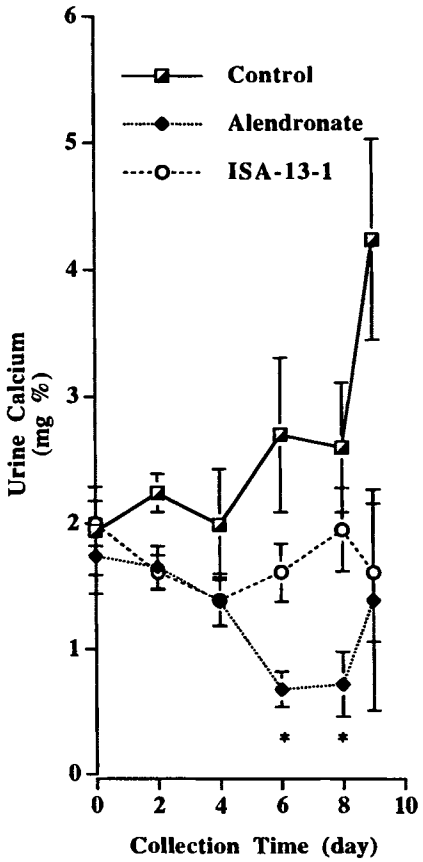

Fig. 3. The inhibition of tumor osteolysis in rats bearing transplanted WCS Calcium amounts in urine. * Differences were termed statistically significant, in comparison to control at the same time point, by the Kruskal-Wallis nonparametric ANOVA test and by the post-hoc Dunn's multiple comparisons test (n = 4–6, mean ± SE, $p < 0.05$).

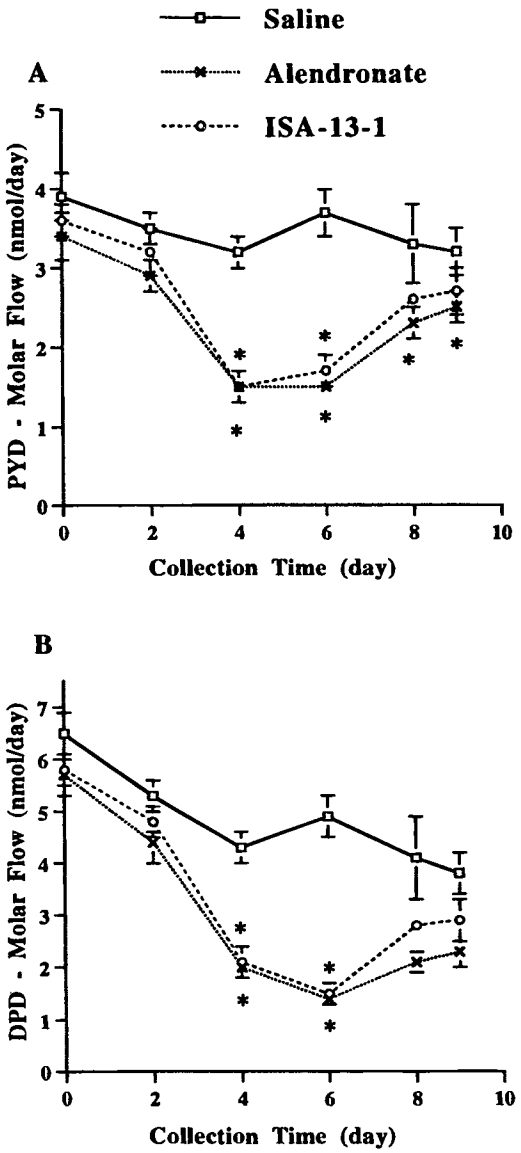

Fig. 4. The inhibition of tumor osteolysis in rats bearing transplanted WCS PYD (4A) and DPD (4B) molar flow. * Differences were termed statistically significant, in comparison to control group at the same time point, by the Kruskal-Wallis nonparametric ANOVA test followed by the post-hoc Dunn's multiple comparisons test (n = 4–6, mean ± SE, $p < 0.05$).

1). From the stability constants of the calcium- BP-complexes it can be concluded that the calcium complex of ISA-13-1 is significantly weaker (by two orders of magnitude) than that of alendronate (Table 1). In addition, the binding affinity of ISA-13-1 to HAP (Table 1) was slightly lower than that of alendronate. This could be expected since only a bidentate rather than tridentate binding to Ca could be formed due to the lack of a geminal hydroxyl group (21,22). Adsorption to HAP affects activity in the *in vitro* model of HAP dissolution (14,23). Indeed, it was found that both compounds significantly inhibited HAP dissolution (Table 1). Inhibition of both HAP formation and dissolution by ISA-13-1 indicates that it has a "crystal poisoning" effect (24), a characteristic of the BP family. The anticalcification effect of ISA-13-1 (via "crystal poisoning") in the subdermal rat model of bioprosthetic heart valve calcification has been documented (14). The crystal poisoning feature can predict to some extent the antiresorption effect of BPs *in vivo* (10,14). Indeed, as shown in this study (see below) both BPs were found as effective antiresorption agents *in vivo* in the experimental models of young intact-rat (Table 2 and Fig. 2), and the WCS (Figs. 3 and 4).

Antiresorption Effect

The treatment by ISA-13-1 resulted in a positive effect on bone development similar to alendronate (Table 2). A nitrogen-containing heterocyclic ring contributes to the high potency of novel BPs such as risedronate and zolendronate (25). The hydroxyl on the geminal carbon has been found to be important for intracellular activity, rather than merely affecting chelation potential (22). This recent study demonstrates that the differences between the inhibitory effects of amino-substituted analogs of hydroxy BPs and hydroxy-BPs tested on bone resorption are due to differences in cellular effects resulting from the substituent on the geminal carbon. Since ISA-13-1 (N-heterocycle having no geminal hydroxyl) and alendronate (containing an aminoalkyl side-chain and a geminal hydroxyl) exhibited similar potency, it is plausible to assume that the contribution of the N-containing heterocyclic ring in ISA-13-1 to potency compensated for the lack of the geminal hydroxyl group. Furthermore, the lack of a geminal hydroxyl group may contribute

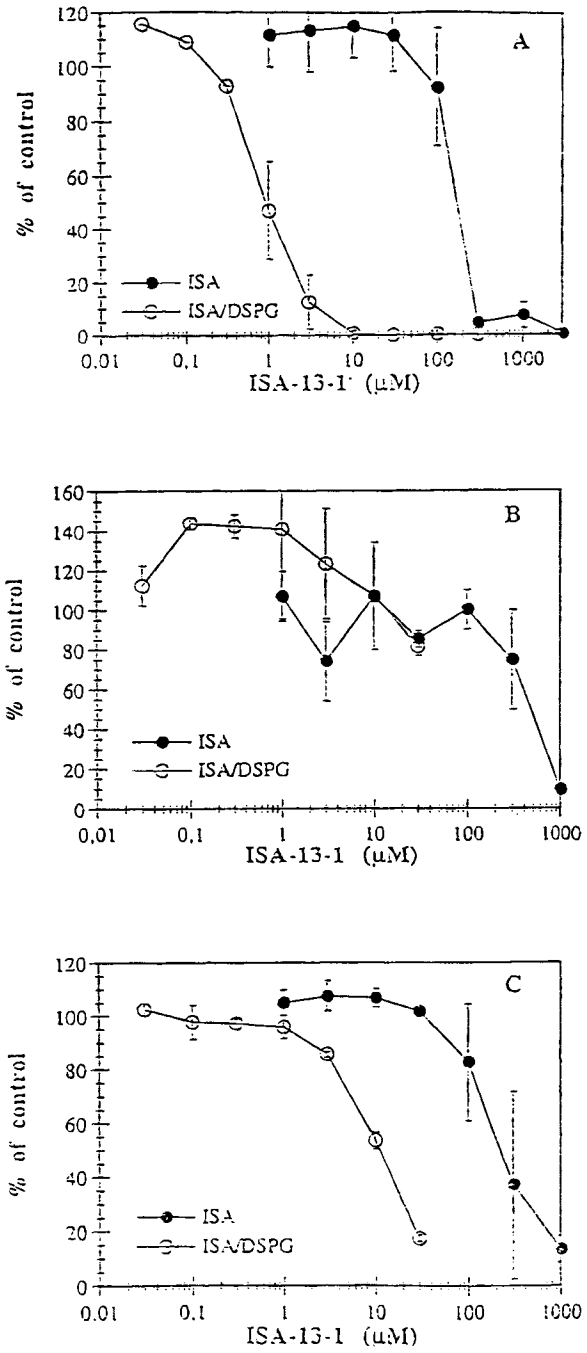

Fig. 5. The effects of ISA-13-1 either as a free drug (ISA) or encapsulated in DSPG liposomes on the (A) growth, (B) LPS induced IL-6 secretion, and (C) viability of RAW 264 cells *in vitro* (mean ± SD, n = 3–5).

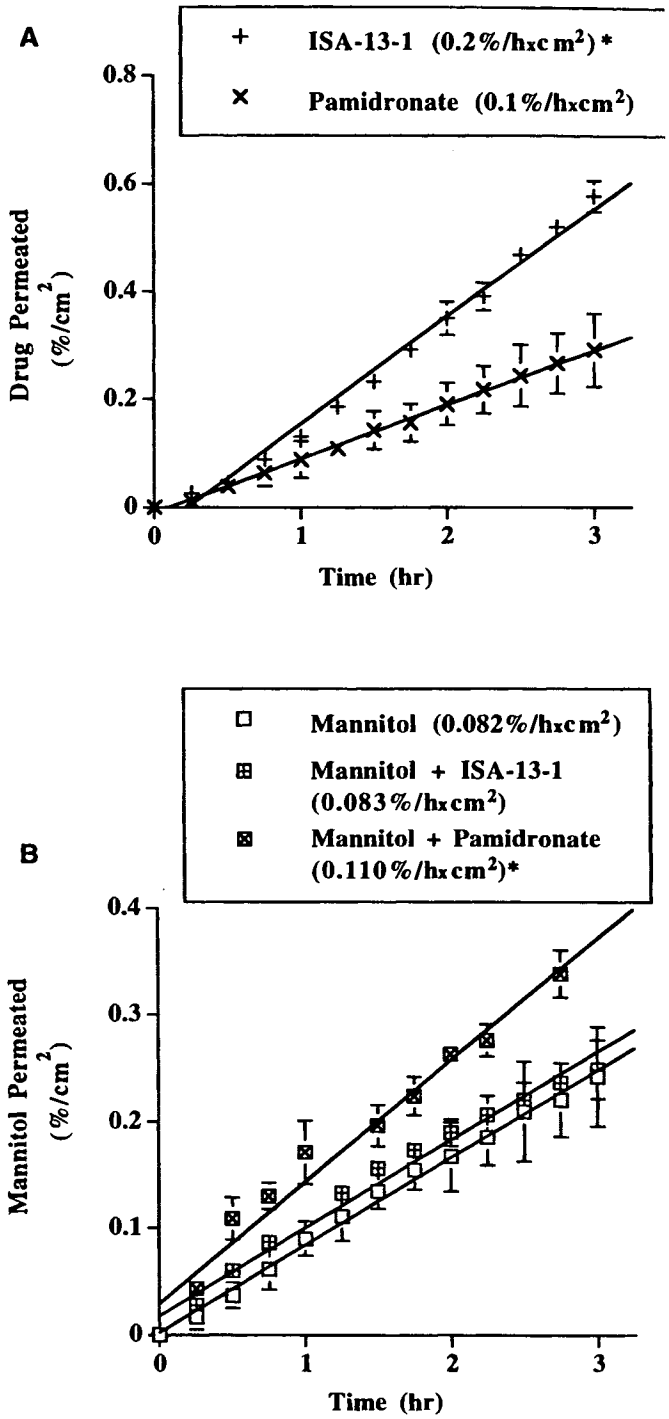

Fig. 6. The rate of the drug (1 mM) permeation across a segment of jejunal tissue mounted between two chambers of a diffusion cell (A), and the permeability of the jejunal tissue to mannitol (10 mM, B) in the presence of ISA-13-1 and pamidronate (1 mM). * Differences were termed statistically significant by the Mann-Whitney test (n = 4, mean ± SD, $p < 0.05$).

to lower amounts of residual BP in bone and avoid concerns of long-term effects (8).

WCS Induced Bone Destruction

In control rats, PYD and DPD values declined continuously, a well-known phenomenon due to skeletal growth and maturation (16). However, between 4 and 6 days after tumor injection a subtle increase was seen for both crosslink components. This increase may well reflect the increased bone resorption due to tumor-induced bone destruction. This explanation is supported by the similar increase in urine calcium values at the same time period (Fig. 3), and the significant osteolysis

observed at the same period by X-rays (data not shown). In both groups of treated rats, a rapid and pronounced reduction in crosslink excretion with a tendency to maintain the normal urine calcium levels was noted. These findings are consistent with a BP-induced inhibition of osteoclast activity and bone resorption. Although DPD is a more bone specific component (PYD also occurs in cartilage and other connective tissues), the BP-induced change was similar for both DPD and PYD crosslinks. The reducing effect of the BPs on pyridinium crosslinks was more pronounced at the earlier time periods. Similar findings have been reported previously following alendronate treatment noting that urinary excretion of the pyridinium crosslinks reflects bone resorption in chronic but not always in acute conditions (26).

Effect on Macrophage Cell Line

Free and liposome-encapsulated ISA-13-1 exhibited growth inhibitory potency on RAW 264 macrophages (Fig. 5) comparable to that of aminobisphosphonates such as pamidronate, indicating that liposome-encapsulation provides a very effective way to deliver the drug to macrophages (17). IL-6 secretion was dose-dependently inhibited by free ISA-13-1 at >300 μM. This action is, however, attributed to the decreased viability of macrophages after the drug and LPS treatment, suggesting that the observed effect may be secondary, and not due to any specific action as produced, e.g., by clodronate (19). Similarly to aminobisphosphonates such as pamidronate (20), and alendronate (Mönkkönen *et al.*, submitted), liposome-encapsulated ISA-13-1 slightly augments the LPS induced IL-6 secretion from macrophages at low concentrations (0.3–1 μM). This increased cytokine secretion from macrophages is suggested to underlie the acute phase response observed in patients treated for the first time with aminobisphosphonates (27).

Permeation, Absorption and Disposition Studies

BPs are not absorbed via the transcellular pathway due to their high hydrophilicity but they are absorbed via the paracellular pathway (6,7) which is sealed by tight junctions. Absorption via the paracellular pathway is restricted to low MW drugs, an obstacle to the absorption BPs of a MW ≥250. An additional obstacle is the BPs negative charge which is repelled by the negatively-charged brush border membrane. It is well known that good solubility in the GI tract is an important determinant of absorption. It is worth noting that elevated transepithelial transport of pamidronate has been observed when minimal calcium and magnesium concentrations have been used in Caco-2 monolayer transport experiments (28). Thus, the better permeability of ISA-13-1 compared to pamidronate across the jejunal tissue (Fig. 6a) can be rationalized by the better solubility of ISA-13-1 Ca salt (14).

The increased transport of mannitol, a paracellular absorption marker (15), in the presence of pamidronate, (Fig. 6b), indicates that pamidronate absorption is associated with opening of the tissue tight junctions. It has been reported that pamidronate caused cytotoxic effects on monolayers of human intestinal epithelial (Caco-2) cells and affected epithelial transport (7). This widening of the intracellular space has been suggested as the mechanism of absorption of pamidronate (7), alendronate (4,29), and tiludronate (30), all acting as self-paracellular absorption enhancers. Indeed, pamidronate has been utilized as a promotor for rectal drug absorption due to its calcium chelating properties and widening of the tight junctions (31). This feature of BPs is similar to the promoting effect of the calcium chelating EDTA on intestinal drug absorption based on Ca depletion (31). It can be assumed that the weaker bidentate complex of ISA-13-1 with Ca and other metal cations in the intestinal epithelium, in comparison to pamidronate and alendronate, resulted in a less impeded permeability of the new BP that had no effect on the tight junctions. This phenomenon of certain BPs may have relevance to the known gastrointestinal side effect of BPs in rodents and humans (32,33), and the avoidance of IM injections due to tissue necrosis at the injection site (1). The lack of ISA-13-1 cytotoxic effect on intestinal epithelium, as well as on tissue necrosis following IM injections is of significance in further evaluating this compound as a potential drug.

The pharmacokinetic data following IV administration of both BPs and PO administration of pamidronate are similar to those obtained in other studies on BPs (4). The better oral absorption of ISA-13-1 (×1.5–1.7) can be attributed to the better solubility of ISA-13-1 in the presence of Ca and other cations. Nevertheless, a similar concentration in the bone was found for both drugs that could be due to pamidronate's better affinity to bone.

The BPs accumulated in the intestinal wall following PO administration most probably as insoluble complexes with tissue constituents including metal cations. The significantly lower concentration of ISA-13-1 trapped in the intestinal wall in comparison to pamidronate (ca. 5 times) is also consistent with weaker complexes and a more soluble ISA-13-1-calcium and other salts. It is conceivable that the lower intestinal-wall uptake could result in less toxic effects of this BP on the GI tissue, than the effects encountered with the PO use of pamidronate and other BPs (32,33). The observed lower ISA-13-1 concentration in the intestinal wall and the lack of effect on the tight junctions could be very important in the process of further evaluation of this potential drug.

Bisphosphonate disposition in noncalcified tissues shortly after IV administration has been documented, and it is markedly lower than in calcified tissue with the exception of the kidney (4). While drug levels in the kidney represent the elimination process, elevated levels in the liver and spleen are believed to be due to the metabolic/elimination process by the RES (macrophages) of BP metal complexes formed especially following rapid high IV dose administration (34). It is important to note that following IV administration markedly higher levels of pamidronate in comparison to ISA-13-1 have been detected in both the liver (×6.5) and the spleen (×2). These findings could be explained again, by the more stable and less soluble complex formation properties of the former BP.

In conclusion, the preclinical pharmacology of ISA-13-1, a novel nitrogen-containing heterocyclic bisphosphonate with no hydroxyl on the geminal carbon, validated our hypothesis that such a derivative would possess potency and increased bioavailability. These characteristics as well as the lower disposition in the intestinal wall and the lack of deleterious effects on the GI tissue warrant its further consideration as a potential drug for bone diseases.

ACKNOWLEDGMENTS

This research was supported in part by grants (I-316-186/93 and I-383-188/94) from the German-Israeli Foundation for Scientific Research and Development (GIF). E. Breuer, A. Hoffman and G. Golomb are affiliated with the David R. Bloom Center for Pharmacy at the Hebrew University of Jewsalem.

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