Bisphosphonate's effect in hepatic rat cells: An electron microscopy study

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ABSTRACT: Purpose: Bisphosphonates (BPs) are selective inhibitors of osteoclast mediated bone resorption, used for the treatment of bone disorders as well as for tumors, whereas long-term bisphosphonate use is associated with adverse gastrointestinal effects. The objective of the study is to investigate the possible effects of BPs in hepatic structure. **Materials and methods:** Specimens from the liver of ten female 12-month old Wistar rats were used as control group and of ten female 12-month old Wistar rats to which Alendronate (Fosamax, Merck) was administered per os for 13 weeks, were used as experimental group. Samples were observed under a Transmission Electron Microscope. **Results:** In the experimental group, extensive depletion of the glycogen, different sized vacuoles and enlarged sinusoids were found in hepatic cells. Furthermore, there was lack of microvilli of hepatocytes in the Disse's space. The same findings were reported in all sections of the experimental group. **Conclusion:** This is the first study of liver structure after the administration of bisphosphonates, with electron microscopy. This report, indicate the presence of mild hepatic damage in liver tissues studied. Our study demonstrates a possible correlation between alendronate administration and hepatic cell function, nevertheless due to the small specimen further research is needed.

INTRODUCTION

Bisphosphonates (BPs) are selective inhibitors of osteoclast-mediated bone resorption. They promote bone mineral secretion while at the same time considerably reduce bone turnover by inducing osteoclast apoptosis[1-13]. The first generation of non nitrogen-containing BPs inhibit osteoclasts by inhibiting ATP-dependent enzymes and the nitrogen-containing BPs by inhibiting the Farnesyl Diphosphate (FPP) synthase[14,15]. They are absorbed not only into active bone mineral surfaces but also into other cell types such as macrophages, epithelial, endothelial cells, circulating monocytes and neoplastic cells[14,15].

Long-term bisphosphonate use is associated with adverse gastrointestinal effects. Specifically, adverse events of the gastrointestinal tract include abdominal pain, epigastric pain, nausea, dyspepsia, vomiting, constipation, diarrhea, bleeding, esophageal ulceration, esophagitis, esophageal erosions, esophageal adenocarcinoma, Barrett's esophagus, gastric ulcer, cancer of gastrointestinal tract and hepatotoxicity[4,5,7,11, 12,14-21]. In general, bisphosphonates are poorly absorbed by the gastrointestinal tract as a result of their poor lipophilicity[1]. Oral bioavailability is about 0.9 to 1.8%[2,3,22] leading to very low peak values in the plasma[22]. Systemically available bisphosphonates disappear very rapidly from plasma, and they are either taken up by bone tissues or excreted by the kidneys which is the only route of elimination[1,2,3]. Renal clearance appears to involve both glomerular filtration and an especially secretory pathway[3]. To date, all bisphosphonates studied show no evidence of metabolism[1]. The drug which is not excreted within 24 hours after dosing is believed to be sequestered in the skeleton. Afterwards, it is released slowly into the circulation until to be eliminated from the kidneys[2]. The elimi-

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nation from bone tissue is slow, ranging from 200 days in rats to 12 years in humans[2]. The bone (tibia) contains the highest concentration of all tissues[24] and the disposition of the BPs in soft tissues (liver, kidney and spleen) is dependent on route and rate of drug administration and the osmolality of the vehicle[23,24,25].

In our report, we have studied the effects of bisphosphonate use on liver. In liver, bisphosphonate use has been associated with hepatotoxicity confirmed by liver biopsy, severe acute hepatitis and chronic hepatitis. However, data concerning the side effects of BP use on the liver, are still limited.[15, 26-30]

MATERIALS AND METHODS

This study was approved by the Bioethics Committee of the Medical School of the Aristotle University of Thessaloniki. Twenty female Wistar rats, 12-month old, weighing approximately 500 g, were used in the experiment. Rats were housed in stainless steel cages, with one rat per cage, 12h light-dark cycle and relative humidity and temperature control.

The animals were randomly allocated into two groups: Group A, the experimental group, that consisted of 10 animals, and Group B, the control group, that also consisted of 10 animals. Alendronate (Fosamax, Merck) was administered per os to animals of Group A at a dose of 0.05 mg/kg body weight/week dissolved in 3cc normal saline for a period of 13 weeks, as previously described. The drug was administered thirty minutes prior to breakfast. The dose was calculated according to the usual human dose[31,32]. The duration of the study was limited to 13 weeks and after euthanasia, the liver of the animals was removed and specimens were processed for electron microscopy examination.

Transmission Electron Microscopy

Liver tissue samples were sectioned into $<1cm^3$ pieces. They were placed into glutaraldehyde 3% for 2 hours, followed by 1 hour into osmium tetroxide (OsO_4) 1%. Staining was performed with uranyl acetate 1% for 16 hours and then samples were dehydrated with increased ethanol concentrations. Samples were embedded into Epon resin and ultra-thin sections (60-90 nm) were taken. Finally, sections were stained with Reynolds's stain. Samples were observed under a TEM JEOL 1011.

RESULTS

Electron microscopy examination did not reveal pathologic changes within the hepatocytes of the control group, however the presence of significant amount glycogen was observed. It was also found that the smooth endoplasmic reticulum, the lysosomes, the sinusoids, the space of Disse as well as the bile canaliculus were normal (Fig. 1).

In the experimental group, evidence of mild dysfunction of the hepatic cells was found in all sections and there was an extensive depletion of the glycogen in the hepatocytes (Fig. 2). Dilated sinusoids (Fig. 3) and increased number of vacuoles in the cytoplasm has also been seen. Moreover, the microvilli of hepatocytes on the Disse's space surface were missing in many areas (Fig. 3) and finally, collagen fibers were detected in extracellular matrix (Fig. 4).

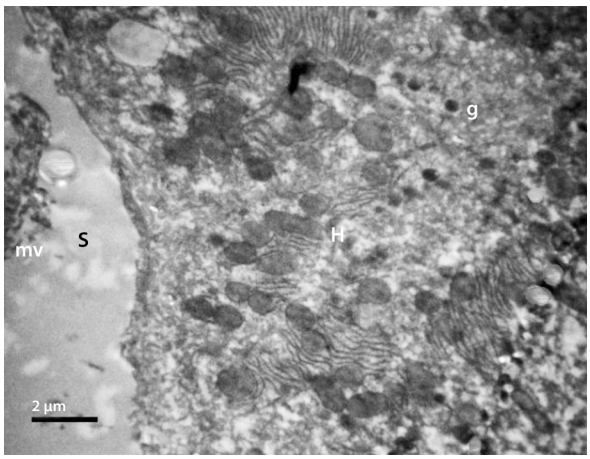

Figure 1: Control group. S: sinusoid, mv: microvilli, H: hepatocytes, g: glycogen.

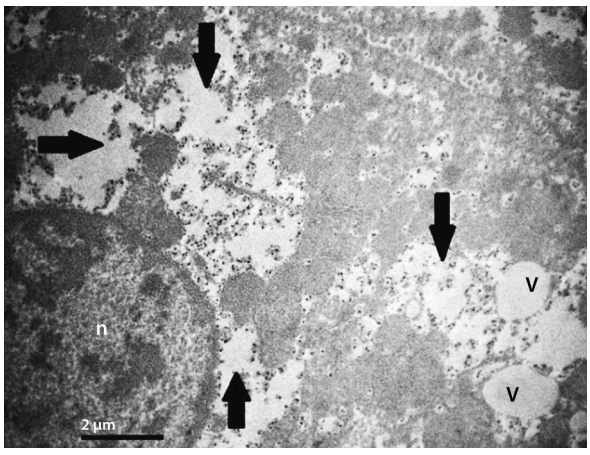

Figure 2: Experimental Group. Depletion of glycogen ( ), vacuoles (v), nucleus of hepatocyte (n).

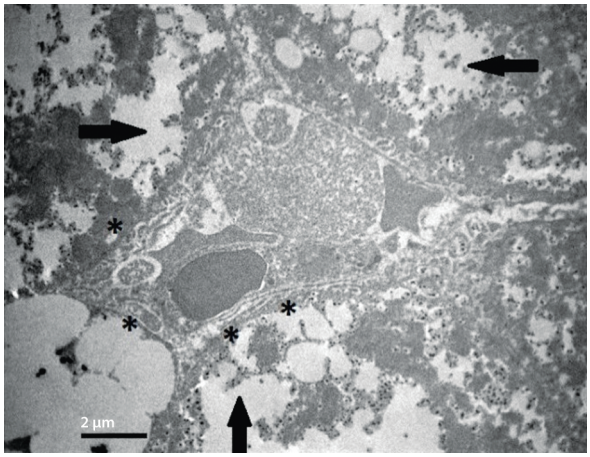

Figure 3: Experimental Group. Extensive depletion of glycogen ( ). Absence of microvilli in the space of Disse (*). Dilated sinusoids.

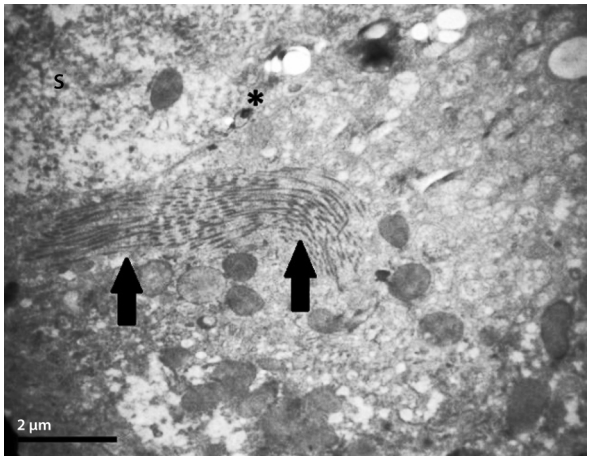

Figure 4: Experimental Group. Presence of collagen in extracellular matrix ( ). Sinusoids (S). Absence of microvilli in the space of Disse (*).

DISCUSSION

Bisphosphonates are selective inhibitors of osteoclast mediated bone resorption and they are used for the treatment of bone disorders as well as for tumors. Their adverse effects have been studied and reported in many previous studies and case reports. Our report tries to elucidate the possible effects of BPs in hepatic structure and consequently in hepatic function.

In various case reports, alendronate has been associated with hepatic dysfunction[26,27,29]. Besides alendronate, there were other bisphosphonate drugs associated with hepatic dysfunction or lesion[33-37,15]. According to Lieverse et al (1998), alendronate medication was the cause of severe hepatitis in a 77 year-old woman as no other obvious cause was found and the hepatitis resolved after alendronate was no longer administered[28]. Furthermore, Halabe et al (2000), reported that alendronate may affect liver function, by inhibiting hepatic synthesis of cholesterol. However, the histologic findings suggested that the patient had inflammatory, rather than toxic liver injury[30]. Our study agrees with previous reports, as the histological findings in our research indicate mild hepatic damage (extensive depletion of the glycogen, dilated sinusoids and increase number of vacuoles, and absence of microvilli in the peri-sinusoidal space). According to Panebianco et al (2016), hepatic stellate cells (HSCs), also known as perisinusoidal cells, are pericytes found in the perisinusoidal space of the liver and are the major cell type involved in liver fibrosis. Liver fibrosis is the formation of scar tissue in response to liver damage. When liver is damaged, stellate cells can shift into an activated state and secrete collagen scar tissue, which can lead to cirrhosis. Therefore, the presence of collagen in the extracellular matrix may indicate hepatic damage[41]. A correlation seems to exist between alendronate administration and hepatic cell dysfunction. Conclusively, bisphosphonate use may be the cause of hepatic damage[38-41].

Our study demonstrates a possible effect of alendronate administration on liver's function and morphology. As far as we know, our study is the only one in the literature that examines the possible effects of bisphosphonate use in hepatic tissue by electron microscope. However, there were limitations, such as the small number of animals and the short-term administration of the drug due to the fast accumulation of alendronate in bone tissue[1]. Further research is needed, in order to detect the possible effect of bisphosphonate drugs in hepatic cell function and also the mechanisms of action.

Η επίδραση των διφωσφονικών στα ηπατικά κύτταρα ποντικιών: Μελέτη με ηλεκτρονικό μικροσκόπιο

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ΠΕΡΙΛΗΨΗ: Σκοπός: Τα διφωσφονικά αποτελούν εκλεκτικούς αναστολείς της οστικής απορρόφησης και η μακροχρόνια χρήση τους έχει συσχετισθεί με ανεπιθύμητες επιδράσεις στο γαστρεντερικό σύστημα. Σκοπός της εργασίας είναι η διερεύνηση των πιθανών επιδράσεων των διφωσφονικών στη δομή των ηπατικών κυττάρων. **Υλικά και μέθοδος:** Ελήφθησαν δείγματα ηπατικού ιστού από δέκα θηλυκά ποντίκια Wistar ηλικίας δώδεκα μηνών που έλαβαν Αλενδρονάτη από του στόματος για 13 εβδομάδες και από δέκα θηλυκά ποντίκια Wistar ηλικίας δώδεκα μηνών που δεν έλαβαν το φάρμακο και χρησιμοποιήθηκαν ως μάρτυρες. Τα δείγματα μελετήθηκαν με το ηλεκτρονικό μικροσκόπιο. **Αποτελέσματα:** Στα ηπατικά κύτταρα των ποντικιών που έλαβαν Αλενδρονάτη βρέθηκαν εκτεταμένη απώλεια γλυκογόνου, διαφόρου μεγέθους κενοτόπια, διευρυμένα κολπώδη τριχοειδή, καθώς και απουσία των μικρολαχνών σε πολλά σημεία του χώρου του Disse. **Συμπέρασμα:** Πρόκειται για την πρώτη μελέτη με ηλεκτρονικό μικροσκόπιο ηπατικών δειγμάτων μετά τη χορήγηση διφωσφονικών, η οποία υποδεικνύει ήπια ηπατική βλάβη. Μια πιθανή συσχέτιση μεταξύ της χρήσης της Αλενδρονάτης και της ηπατικής λειτουργίας καθίσταται εμφανής. Ωστόσο, εξαιτίας του μικρού αριθμού δειγμάτων περισσότερες μελέτες είναι απαραίτητες ώστε να διαλευκανθεί αυτή η συσχέτιση.

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