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Original

Effects of selenium on the structure of the mandible in experimental diabetics

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Abstract: In the treatment of diabetes-induced pathologies, beneficial results have been obtained with administration of antioxidants. Selenium is an antioxidant and essential trace element in living organisms. The aim of this study was to investigate the possible effects of selenium on the structural alterations of the mandible due to diabetes. In this study thirty-nine Wistar rats were used and a control, a selenium given control, a diabetic and a selenium given diabetes groups were formed. Experimental diabetes was induced by a single i.p. injection (50 mg/kg) of streptozotocin (STZ). The diabetic + selenium and the control + selenium groups were injected with a daily dose of 5 μ mol/kg/day sodium selenite (i.p.) for 4 weeks while the diabetic and the control groups were injected with distilled water. Mandibles of all the animals were excised and examined at the 5th week. High blood glucose level and low body weight in the diabetic group were not significantly affected by selenium administration. Furthermore, a negligible increase in blood glucose level was observed in the selenium given control group. Densitometric analysis revealed a significant reduction in bone density and presence of resorption in the diabetic and the selenium given control groups as compared to the selenium given diabetes and the control groups. In X-ray diffraction analysis, the reduction in peak intensity of the reflected light in both the diabetic and the selenium given control groups indicated a possible alteration in the crystallinity or a poor crystalline substance. Histological investigation showed that there was progressive resorption, trabecular and cortical irregularity and vascular proliferation in the diabetic and the selenium given control groups, whereas a more healthy appearance was detected in the selenium given diabetes group. The results of this study suggest the positive effects of selenium on diabetes-induced structural alterations in the mandible. However, the unexpected results in the selenium given control group necessitate further studies on the mechanism of selenium effects in organisms. (J. Oral Sci. 44, 85-90, 2002)

Key words: bone mineral density; diabetes mellitus; mandible; selenium.

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Introduction

Diabetes mellitus (DM) is a complex syndrome of disordered metabolism and elevated blood glucose. It results from absolute deficiency of insulin secretion (Type 1) or a combination of insulin resistance and inadequate insulin secretion (Type 2) (1,2). Oral and maxillofacial surgery is frequently required to treat diabetic patients (3). The coexistence of DM and altered bone and mineral metabolism has been mentioned in some articles but the pathogenesis of these alterations, their natural evolution and their response to therapy, still remain ill defined (4). Earlier clinical studies implicated poor metabolic control, ketoacidozis and nutritional deprivation (5-7), but recent reports indicate that skeletal derangements in diabetes are not related to the severity of the disease, to hyperglycemia, or to insulin insufficiency, but rather reflect the underlying basic disease (8-10).

Selenium (Se) is recognized as an essential element for mammals, lack of which may result in many diseases such as Keshan disease and Kashin-Beck disease. The exact function of Se is not fully understood; however, it is an integral component of Glutathione peroxidase (GSH-Px), which is an important enzyme of the cellular protecting system against lipid peroxide-initiated damage, so Se also acts as an antioxidant (2,11-13). More recently, *in vitro* studies have demonstrated that Se also has insulin-like actions and in some studies, when it is administrated to experimental diabetics, a significant decrease in blood glucose level and normalization in food intake, weight gain and in some biochemical parameters were noticed (2,11,12,14). Diabetes is well known to increase fatty acids and some oxidative agents that cause many systemic complications (15,16), therefore Se may improve some of the diabetes-induced pathological changes *via* its role in antioxidant system.

The aim of this study was to evaluate the structural alterations in the mandible, which may be caused by diabetes, and to investigate the possible effects of selenium administration.

Materials and Methods

Thirty-nine Wistar rats (aged 3-4 months, weight 200-250 g) were used in this study. The animals were randomly allocated into the control group (Group 1, n = 12), selenium alone group (Group 2, n = 10), untreated diabetic group (Group 3, n = 9) and selenium given diabetes group (Group 4, n = 8). They were weighed and blood glucose levels were determined. All the animals were housed in individual cages at a constant temperature (22°C) with a fixed 12-h light-dark cycle. During the experiment all rats received a standard laboratory chow and water ad libitum. Diabetes was induced in groups 3 and 4 by an i.p. injection of streptozotocin (STZ) (Sigma, 50 mg/kg body weight) dissolved in 0.1 M citrate buffer (pH 4.5). Group 1 and 2 were injected with the buffer only. Hyperglycemia was confirmed by using Medi Sense Card Sensor 2, 3, 7 days after injection. Only rats with two readings of ≥ 200 mg/dl were considered diabetic. The 3 rats that did not demonstrate diabetes in Group 1 were discarded from the study. Then, Group 2 and Group 4 were administrated selenium in sodium selenite form dissolved in distilled water (5 μmol/kg/day, i.p.) for 4 weeks, meanwhile Group 1 and Group 3 were injected with distilled water only. On the 1st, 3rd and 4th weeks blood glucose levels and body weights of the animals were measured again. At the 5th week all animals were sacrificed and mandibles were removed by blunt dissection. Left hemimandibles were prepared for densitometric study and X-ray diffraction analysis, right hemimandibles were prepared for histological examination.

Densitometric analysis

X-rays were taken using Kodak double-packet ultra-speed D group periapical radiographs at a film-focal distance of 20 cm. A Siemens-Heliodent machine was used and the unit was set at 50 Kvp and 7 mA. The periapical radiograph had an aluminum step wedge attached for microdensitometric analysis. The technique was the same as previously described by Kribbs et al. (17). All the radiographs were processed under the standard conditions (21°C, 4.5 min). The Gonion point was determined by orthodontic drawings, so the density of the same point was assessed for each subject. Densitometric readings were done on a Macbeth TD-931 transmission densitometer in terms of diffuse transmission density (DTD).

X-ray diffraction analysis

Just after the periapical graph had been taken and all teeth had been removed, the left hemimandibles were placed in a freezer and then placed in an Edwards vacuum freeze dryer. The samples were powdered in a nitrogen mill to an approximate mesh size of 600 and stored in a dessicator. X-ray diffraction patterns of all powdered samples were recorded on a Rigaku D-Max 2200 model diffractometer using CuKα radiation (40 kV, 30 mA).

Histological examination

A specimen from the molar region was fixed in 10% formalin. Decalcification was carried out in a solution consisting of 8% HCL and 8% HCOOH in 2 weeks. Demineralized samples were dehydrated in ethanols, embedded in paraffin, sectioned serially at 5 Micron, affixed to slides and stained with Hematoksilen Eosin (H.E.).

Statistics

Student's *t*-test was used for statistical analysis and the difference $P < 0.05$ was considered significant.

Results

The untreated diabetic group and the selenium given diabetes group lost weight during the 4-week study period, while both the untreated control group and the selenium given control group gained weight. There was a significant weight loss in the diabetic group in the final measurement ($P = 0.032$) and selenium administration did not improve it significantly ($P = 0.430$). The difference in weight between the untreated control group and the selenium given control group was not significant ($P = 0.442$) (Fig.

1). The final blood glucose level in the diabetic group was significantly higher than that of the initial level (P = 0.014); however, selenium administration did not significantly decrease the blood glucose level either (P = 0.060). We observed a slight increase in the blood glucose level of the selenium given control group compared to the untreated control group (P = 0.136) (Fig. 2).

Densitometric analysis

There was no significant difference in the dry weight of the mandibles among the groups (P = 0.243). The untreated diabetic group had the lowest bone density and pronounced osteopenia, hence the highest DTD value among the investigated groups. Selenium administration to the diabetic group significantly enhanced the bone mineral density (P = 0.011). The selenium given control group had significantly lower bone density than the untreated control group (P = 0.023) (Fig. 3).

X-ray diffraction analysis

Representative XRD patterns of all groups (Group 1, 2, 3 and 4) are shown in figures 4a,b,c,d respectively. As seen in the figures, the intensity of the peak observed at around $2\theta^0$= 31.717 was stronger in the untreated control and the selenium given diabetes groups compared to the diabetic and the selenium given control groups.

Histological examination

In the untreated control group, the teeth and alveolar bone were normal and healthy in appearance (Fig. 5). In the selenium given control group, the findings were similar to those of the diabetic group. We observed destruction of the trabecular structure in resorption areas and osteoclastic activity was present on the trabecular walls (Fig. 6). In the untreated diabetic group, compact structure was lost and replaced by trabecular bone, which may be due to bone resorption. The arrangement of collagenous fibers was irregular and fibroblasts extensively spread (Fig. 7a). The alveolar bone was greatly destroyed and extensive vascular proliferation in connective tissue was obvious (Fig. 7b). The selenium given diabetes group showed sounder bone appearance compared to the diabetic group. Haversian canals and the surrounding circumferential lamellar system were easily identified with the collagenous fibers (Fig. 8).

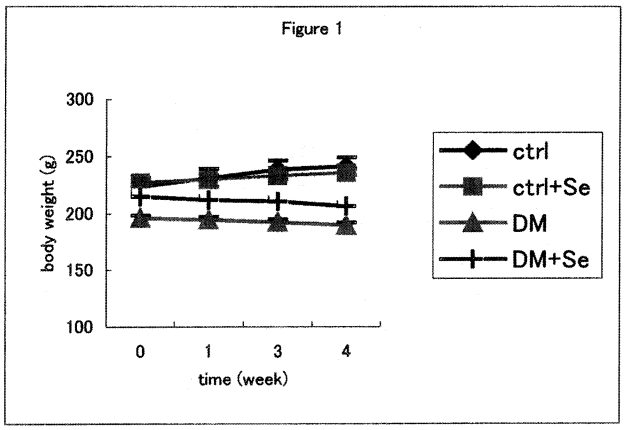


Fig. 1 Body weight differences of the groups during experiment.

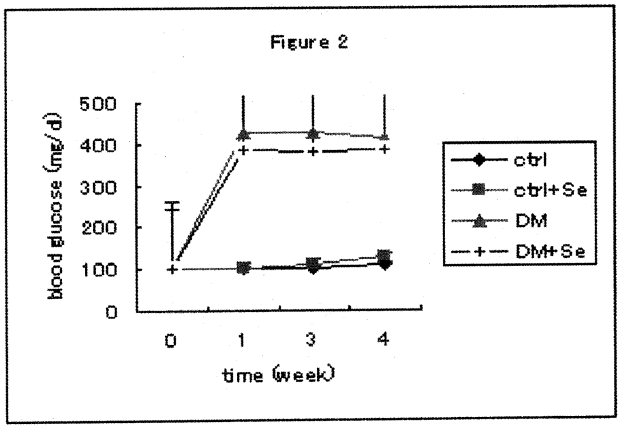


Fig. 2 Blood glucose levels of the groups during experiment.

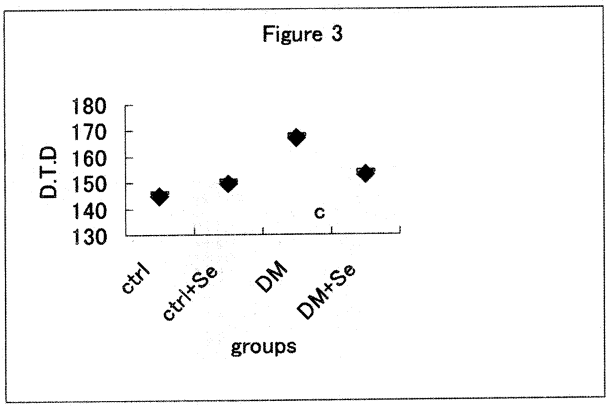


Fig. 3 Bone density values of the groups (DTD).

Discussion

There is some controversy about the effects of diabetes on bone metabolism and bone mineral density (4,8,10,18). This raises the question of whether DM is a risk factor for developing osteopenia and osteoporosis. There is also disagreement as to whether the duration of DM, severity of the disease and/or poor glycaemic control could have adverse consequences on the bone mineral density (BMD). In this study, we found impaired cellular and mineral metabolism in diabetic bone but in selenium administrated diabetics these findings tended to normalize regardless of significant glycaemic and weight control. Some studies

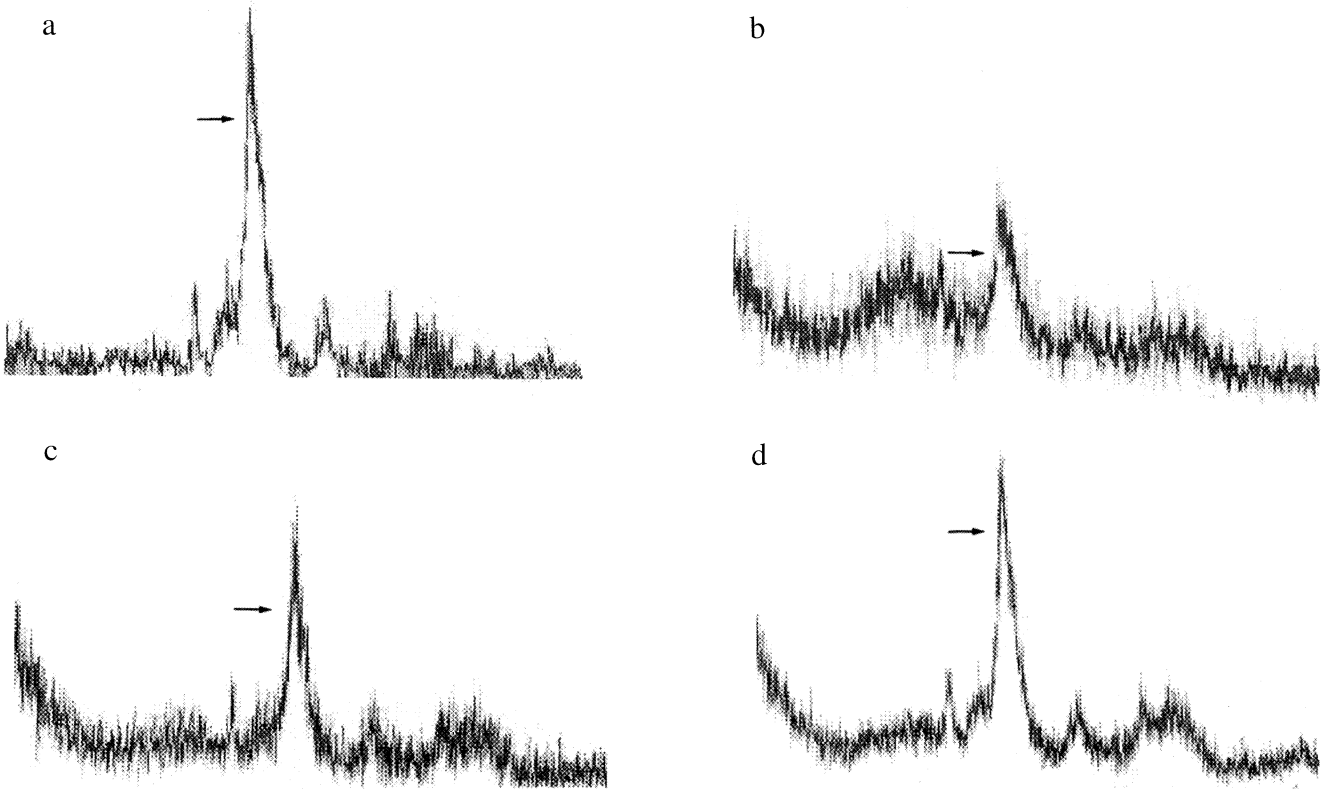


Fig. 4a,b,c,d X-ray diffraction patterns (intensity versus 2 θ) from the control (Group 1), Se given control (Group 2), diabetic (Group 3) and Se given diabetes (Group 4) groups, respectively.

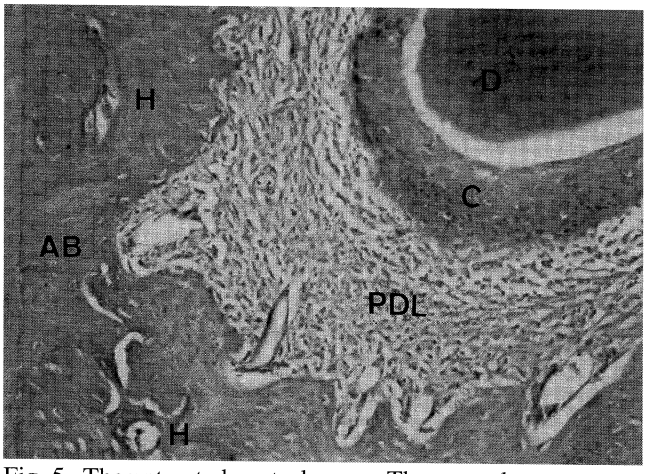


Fig. 5 The untreated control group. The normal appearances of the tooth, periodontal ligament (PDL) and alveolar bone (AB). Haversian canals (H), dentin (D), cementum (C). (HE × 40).

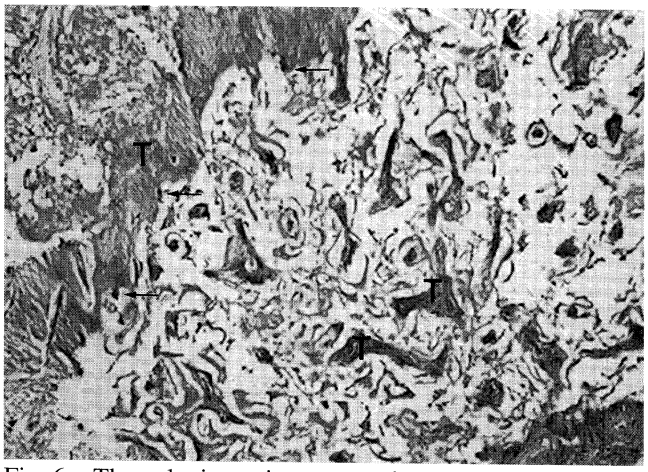


Fig. 6 The selenium given control group. The bone was greatly damaged with a loss of the trabecular structure (T) and osteoclastic activity was present on the trabecular walls (arrows). (HE × 20).

on humans and animals with DM demonstrated a low turnover in bone metabolism, which might be accompanied by a reduced BMD due to a diminished build-up of bone mass. At the present stage, it is not possible to answer the question whether this is of pathological relevance or only the physiological response of the skeleton to a lower weight-bearing load with a reduced mechanical stimulation (10). Reduced bone turnover and impairment in bone formation in experimental diabetes is related to a decrease in the number of osteoblasts that are functioning normally (10,19). Although a complete picture of the pathogenesis of diabetic bone disease is still difficult to draw, it has become clear that no single mechanism can explain all the observed phenomena (5).

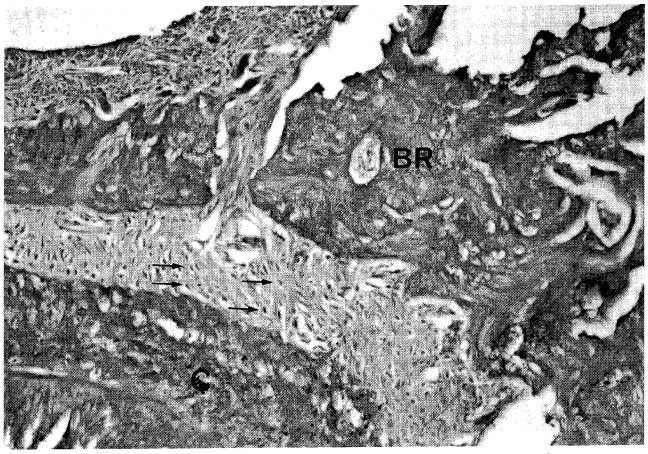


Fig. 7a The untreated diabetic group. Destruction of compact structure due to bone resorption (BR). Periodontal collagenous fibers disorganized and fibroblasts extensively spread (arrows). Cementum (C). (HE × 40)



Fig. 7b The untreated diabetic group. Extensive vascular proliferation (arrow heads). (HE × 40)

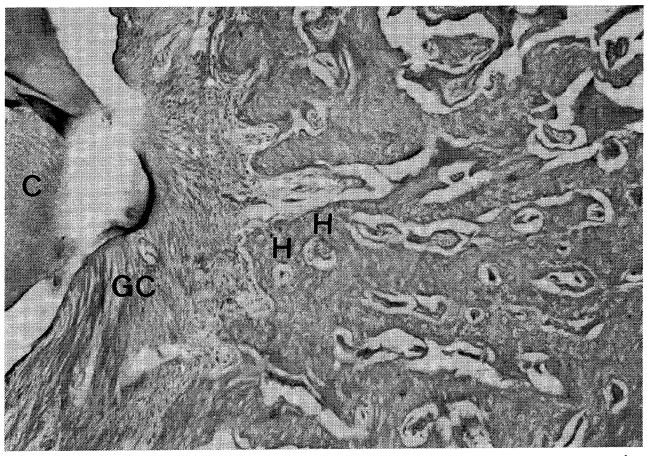


Fig. 8 The selenium given diabetes group. Improvement in bone appearance was noticed compared to the untreated diabetic group. Haversian canals (H), circumferential lamellar system (arrows), cementum (C), gingival collagen (GC). (HE × 20)

In recent years, research has focused on using antioxidant and insulin-mimetic agents to investigate these agents in the prevention and treatment of DM with beneficial results having been reported (20-25). Selenium, an essential mineral for mammals, is important for providing protection against oxidative damage. The action mechanism of Se is not clear, and is under investigation. The effect is not due to the stimulation of the insulin receptor, and does not require the presence of insulin because it can be demonstrated *in vitro* in insulin-free systems. Selenium is unusual among other elements in that it is both toxic and beneficial. The mechanism of Se toxicity as well as deficiency is not clearly established yet. It is thought to be related to its ability to form covalent linkages with intracellular proteins and reduced glutathione (2,12,13). The rats used in this study were treated daily for 4 weeks at the dose of 5 μ mol/kg/day without signs of toxicity. Our results are very encouraging when they are taken into consideration together, because they correlate well with each other.

Many diabetic complications, such as disordered mineral metabolism and osteoporotic bone formation, will adversely affect the outcomes of various interventions in the field of oral and maxillofacial surgery. Thus, the current findings open the possibility that the addition of certain trace elements to the treatment protocols or to preventive measures used for diabetic patients may improve the results of performed surgical procedures. Nevertheless, the unexpected results of selenium given to healthy subjects necessitate further detailed studies evaluating the mechanism of selenium action.

Acknowledgments

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