Effects of vitamin E supplementation on bone metabolism in nicotine-treated rats

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ABSTRACT

Introduction: Nicotine has been shown to exert negative effects on bone. This study determined whether vitamin E supplementation is able to repair the nicotine-induced adverse effects in bone.

Methods: 24 male rats were divided into three groups. The first group was the baseline control and killed untreated at the beginning of the study. Groups 2 and 3 received nicotine at 7 mg per kg for three months but during the second and third months, group 2 was supplemented with alpha-tocopherol (N+ATF) while group 3 was given palm tocotrienol mixture (N+TT). Serum interleukin-1 (IL-1), serum interleukin-6 (IL-6), serum osteocalcin, urine deoxypyridinoline (DPD) and bone calcium content were measured.

Results: Palm tocotrienol mixture was able to prevent the increment of IL-1 and IL-6 due to nicotine treatment. No changes were seen in the osteocalcin levels, but the N+ATF group had lower urine DPD levels after treatment. However, bone-remodelling index revealed no significant changes. No significant differences were seen in the femoral bone calcium content results, although the fourth lumbar bone calcium content was reduced in both groups with 66.5 percent reduction in the N+ATF group and 59.6 percent reduction in the N+TT group.

Conclusion: Palm tocotrienol mixture was better than alpha-tocopherol in reversing the effects of nicotine on IL-1 and IL-6. Both forms of vitamin E were not able to restore the nicotine-induced bone calcium loss, but the N+ATF group suffered a greater loss. Tocotrienol seemed to be superior to alpha-tocopherol in combating against the adverse effect of nicotine.

Keywords: alpha-tocopherol, bone remodelling, nicotine, tocotrienols, vitamin E

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INTRODUCTION

Bone is a unique connective tissue which constantly undergoes remodelling. Bone remodelling involves bone formation by osteoblasts and bone resorption by osteoclasts. Serum osteocalcin and urine deoxypyridinoline (DPD) are among the specific biochemical markers used to measure bone formation and bone resorption activities, respectively.[1,2] Cytokines such as interleukin-1 (IL-1) and interleukin-6 (IL-6) are called bone-resorbing cytokines and play a major role in bone resorption.[3] The bone matrix is composed of organic and inorganic components. The organic components include collagen and glycoprotein while the inorganic components include minerals such as calcium and phosphorus. Both the organic and inorganic components provide rigidity and strength to the bone.[4]

Nicotine is the active alkaloid component found in tobacco. Nicotine has been associated with many diseases. It increases oxidative stress[5] and the risk of coronary artery disease,[6] and promotes tumour growth as well as atherosclerosis formation.[7] Studies have found that nicotine reduces bone mineral density,[8] inhibits osteoblasts[9] and delay bone healing.[10] Vitamin E, a lipid-soluble vitamin, is available in two biologically active forms: tocopherol and tocotrienol.[11] Vitamin E is known to have antioxidant properties and has been proven beneficial in some disease processes. It protects the body's biological systems[12] by preventing lipid peroxidation.[13] Tocotrienols are effective in preventing breast cancer growth[14] and reducing blood cholesterol levels.[15] Vitamin E also affects bone by increasing bone trabecular formation,[16] preventing bone calcium loss

due to an oxidising agent, ferric nitrilotriacetate,[17] and preventing bone calcium loss in ovariectomised rats.[18] This study was carried out to determine whether vitamin E supplementation is able to reverse the detrimental effects of nicotine on bone biochemical markers, bone density and bone calcium content.

METHODS

24 three-month-old male Sprague-Dawley rats obtained from the Laboratory Animals Resource Unit, Universiti Kebangsaan Malaysia, were randomly assigned to three groups of eight rats each. The first group acted as the baseline control (BC) for determination of bone calcium content. This group was not treated and was sacrificed at the beginning of the study. Group 2 (N + ATF) was given nicotine at the dose of 7 mg/kg for three months and alpha-tocopherol (60 mg/kg) was added to the treatment regime in the last two months. Group 3 (N + TT) received treatment similar to group 2 but alpha-tocopherol was replaced by palm tocotrienol mixture (60 mg/kg).

The rats were kept four per cage under 12-hour natural light/dark cycles and given deionised water ad libitum. All rats received normal rat chow obtained from Gold Coin (Port Klang, Selangor, Malaysia). The nicotine used was nicotine hydrogen tartrate salt, which was purchased from Sigma Chemical Co. (St Louis, MO, USA). Alpha-tocopherol acetate was purchased from Sigma Chemical Co. (St Louis, MO, USA) while the Malaysia Palm Oil Board (Bangi, Selangor, Malaysia) supplied the tocotrienol mixture. Blood and urine samples were taken before the start of treatment and at the end of the treatment period.

Nicotine 7 mg/kg was prepared by mixing 0.07 g of nicotine in 10 ml normal saline. The vitamin E solutions were prepared by mixing 3 g of the respective vitamin Es in 50 ml olive oil (Bertolli, Secaucus, NJ, USA). A total of 0.1 ml/100 g rat weight of the nicotine and vitamin E preparations were respectively given intraperitoneally and orally, six days a week.

Serum IL-1 was measured by means of Rat interleukin-1 alpha ELISA kit (Biosource International Inc, Camarillo, CA, USA) (Cat no MG45292). Serum IL-6 was measured by using Rat interleukin-6 ELISA kit (Bender Medsystem, Burlingham, CA, USA (Cat no BMS625). Osteocalcin ELISA kit (Nordic Biosciences Diagnostics, Hovedgade, Hevlev, Denmark) (Cat no 70SC4000) was used to measure serum osteocalcin, and Metra DPD EIA kit (Quidel Corporation, San Diego, CA, USA) (Cat no 8007) was used to measure urine DPD. Urine DPD values obtained were corrected with urine creatinine levels. This was done to eliminate errors due to the difference in renal function of individual rats. Creatinine levels were measured using kits (Cat no 1489291) obtained from Roche Diagnostics, Indianapolis, IN, USA.

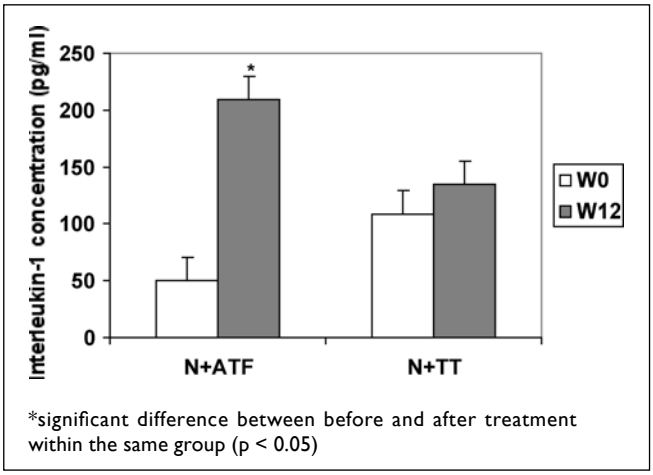

Fig. 1 Bar chart shows serum interleukin-1 concentration in rats treated with nicotine and supplemented with vitamin E.

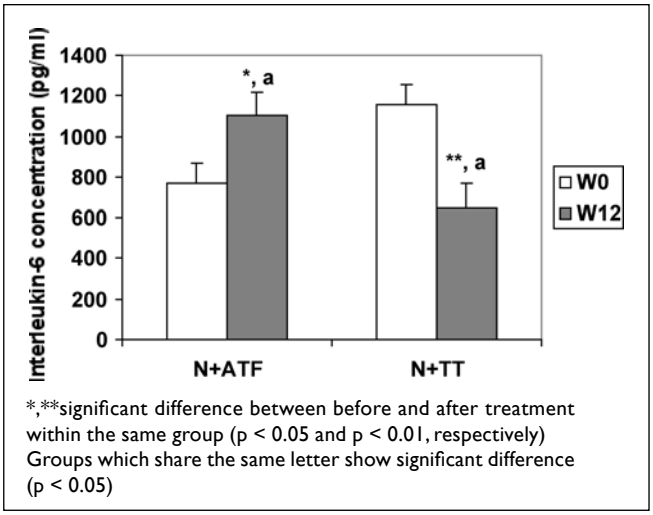

Fig. 2 Bar chart shows serum interleukin-6 concentration in rats treated with nicotine and supplemented with vitamin E.

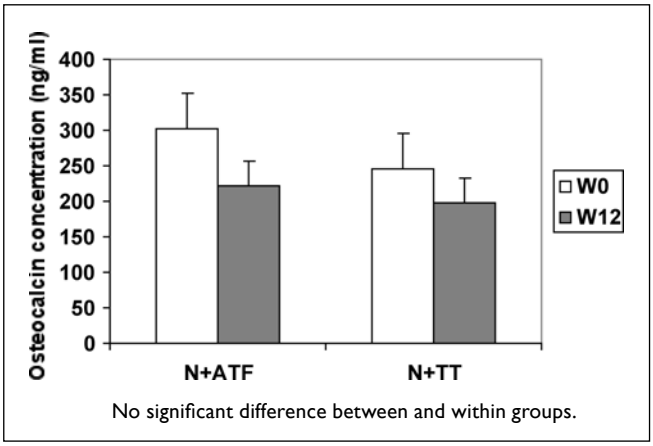

Fig. 3 Bar chart shows serum osteocalcin concentration in rats treated with nicotine and supplemented with vitamin E.

Upon sacrifice, the left femur and the fourth lumbar bones were dissected and cleaned of all soft tissues. The bones were left at room temperature for 24 hours, dried in an oven at 100°C for 24 hours and then ashed in a furnace at 800°C for 12 hours. The ash was dissolved in 3 ml nitric acid and then diluted in lanthanum chloride. Calcium content was measured with a flame atomic absorption spectrophotometer (Analyst 100, Perkin Elmer™ Instruments, Wellesley, MA, USA) at 422.7 nm.

Data were analysed using the one-way analysis of variance (ANOVA) and Tukey's honestly significant difference test was selected as the post-hoc test. To compare data between before and after treatment, paired t-test was used. All the analysis was carried out using the Statistical Package for Social Sciences version 12.0 (SPSS Inc, Chicago, IL, USA) software. This study was approved by the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC) with the approval number FAR/2002/IMA/23-JULY/076.

RESULTS

Serum IL-1 levels were increased in the N+ATF group after the treatment as compared to the beginning of the study ($p < 0.05$). However, no significant changes were observed within the N+TT group and also between the two different supplemented groups (Fig. 1). Serum IL-6 levels were also increased in the N+ATF group after the treatment as compared to the beginning of the study ($p < 0.05$). Meanwhile, the N+TT group had lower serum IL-6 levels than before treatment ($p < 0.01$). In addition, serum IL-6 levels of the N+TT group were significantly lower than that of the N+ATF group ($p < 0.05$) (Fig. 2).

No significant changes were seen in the serum osteocalcin levels in both groups (Fig. 3). DPD levels were reduced in the N+ATF group at month 3 compared to month 0 ($p < 0.01$). No other significant differences were seen (Fig. 4). No significant changes were observed in the bone remodelling index, which is the ratio of osteocalcin levels to DPD levels (Fig. 5). No significant findings were discovered in the femoral bone calcium content results (Fig. 6). However, the fourth lumbar vertebra bone calcium content of both N+ATF and N+TT groups were lower than the BC group ($p < 0.01$ and $p < 0.05$, respectively).

DISCUSSION

IL-1[19] and IL-6[20] are cytokines responsible for the bone resorption process. IL-1 induced vitamin D_3-induced bone resorption[21] while IL-6 was involved in osteoclastogenesis.[22] IL-6 levels were high in ovariectomised rats showing high bone resorption rate.[23] Increment of these cytokines levels implied that the bone resorption rate was increased. Our previous study showed that nicotine (7 mg/kg) significantly increased the IL-1 level.[24] In the present study, we observed that IL-1

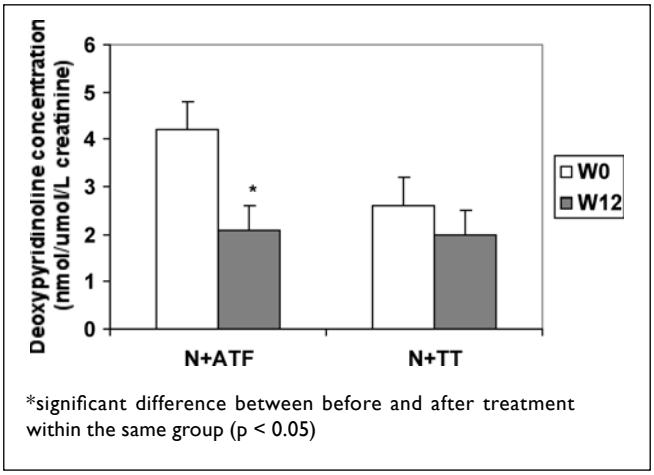

Fig. 4 Bar chart shows urine deoxypyridinoline concentration in rats treated with nicotine and supplemented with vitamin E.

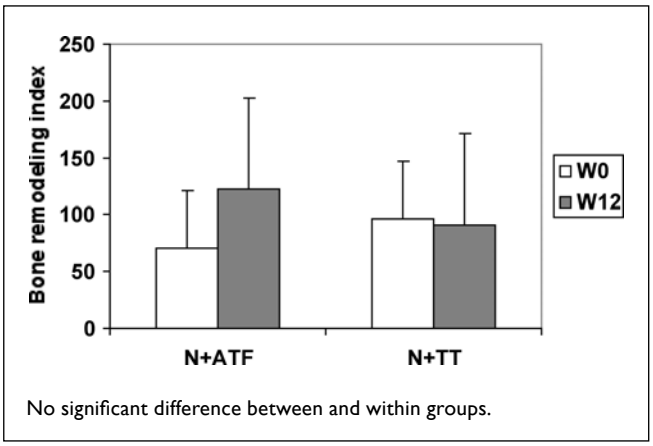

Fig. 5 Bar chart shows bone remodelling index in rats treated with nicotine and supplemented with vitamin E.

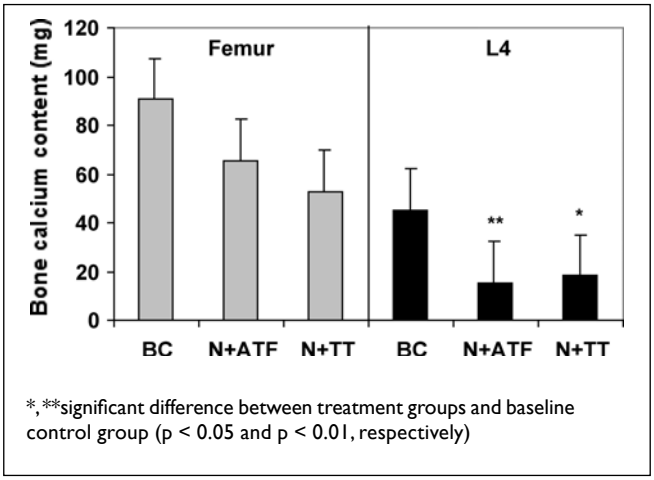

Fig. 6 Bar chart shows bone calcium content of left femur and fourth lumbar vertebra in rats treated with nicotine and supplemented with vitamin E.

levels were elevated in the N+ATF group but not in the N+TT group. This suggested that alpha-tocopherol was not effective in maintaining the level of IL-1 in nicotine-treated rats, unlike tocotrienol. A similar trend was observed in the serum IL-6 results. The N+ATF group showed a higher serum IL-6 level after three months compared to month 0, and this effect was not seen in the N+TT group. In fact, the serum IL-6 levels of the N+TT group reduced significantly compared to the beginning of the study. Besides preventing the nicotine-induced increment of IL-6, tocotrienol further reduced the level of IL-6, indicating suppression of bone resorption process. Once again, tocotrienol was more effective compared to alpha-tocopherol.

Previous studies have shown the advantages of tocotrienol over tocopherol. In our own study, vitamin E-deficient rats given palm vitamin E showed an improvement in bone calcium content whereas pure alpha-tocopherol failed to do the same.[25] Palm vitamin E has a high content of tocotrienols (60%–70%). Tocotrienols have also been shown to be superior to tocopherol in preventing hydrogen peroxide-induced neurotoxicity.[26] In another study, alpha-tocotrienol was shown to be 40 times more effective than alpha-tocopherol in reducing lipid peroxidation.[27]

Osteocalcin is the bone formation marker while urine DPD indicates bone resorption rate. In this study, no significant differences were observed in both groups which suggest either nicotine 7 mg/kg had no effect on osteocalcin level or both of the vitamins E were able to overcome the effects of nicotine on osteocalcin. Our previous study showed that serum osteocalcin levels were not different in rats treated with nicotine 7 mg/kg for one month, compared to control group.[24] However, there was one study which showed that nicotine affected osteocalcin levels. Osteocalcin levels were found to be lower in smokers.[28] The failure to see any differences in the present study might be due to the short treatment period.

Urine DPD levels were lower in the N+ATF group as compared to the beginning of the study but no differences were seen in the N+TT group. Since urine DPD indicates bone resorption activity, our results implied that bone resorption activity was reduced in the N+ATF group which contradicted our cytokine results, in which both cytokines, IL-1 and IL-6, were increased. To resolve the issue, we calculated the bone remodelling index and it was found that there was no significant difference. This showed that there were no changes in bone metabolism rate in both groups.

Even though significant results were seen in the cytokine levels, the changes were not translated into the bone remodelling parameters. IL-1 and IL-6 are not specific markers for bone. Many other cells can release these cytokines. Nicotine has been found to increase IL-1 levels in culture of oral keratinocyte cells[29] and IL-6 levels in the liver and spleen.[30] However, other studies pointed out that serum IL-6 is a predictor of postmenopausal bone loss[31] and serum cytokines may give a picture of the mechanisms regulating bone ageing.[32] The short treatment period may be the causative factor on our failure to detect differences in bone remodelling parameters.

Calcium is the most abundant mineral which makes up the bone and contributes to the bone's strength. Our previous study showed that at eight weeks, femoral bone calcium content was increased in the control group as compared to the BC group. Administration of nicotine at a dose of 7 mg/kg reduced the femoral bone calcium content compared to the control group but comparable to the BC group.[33] In the present study, femoral bone calcium content of both vitamin E supplemented groups did not differ from the BC group. However, the lumbar bone calcium content was lower in both the vitamin E supplemented groups compared to the BC group. These results suggested that both alpha-tocopherol and palm tocotrienol were able to prevent the nicotine-induced loss of bone calcium from the femoral bones but not from the lumbar bones.

Nicotine is found to inhibit the absorption of calcium and vitamin D.[34] This may contribute to the bone calcium lowering effect of nicotine. Our results also showed obvious changes in the lumbar vertebrae compared to the femoral bones. Lumbar bones, being cancellous in nature, has a much larger surface area per unit volume and is more active metabolically, compared to cortical bones (such as the femur).[35] Hence, the lumbar bones in this study were more affected than the femoral bones. A similar trend was observed in our previous studies which applied different stress conditions. Ovariectomised rats tend to lose cancellous bone faster than cortical bone,[36] and vitamin E deficiency decreased the fourth lumbar bone calcium content but not the femoral bone calcium content.[37] A longer treatment period may give more consistent findings.

In addition, the reduction in the fourth lumbar bone calcium content is greater in the N+ATF group (66.5%) compared to the N+TT group (59.6%). This observation further implied that tocotrienol is better than alpha-tocopherol even though tocotrienol failed to completely repair the adverse effects induced by nicotine. Greater bone calcium loss in the N+ATF group may be due to the increase in bone resorption activity as shown by elevated levels of the bone resorbing cytokines, IL-1 and IL-6. The N+TT group did not display any increment in the cytokine levels, implying reduction in bone resorption, thereby arresting the bone calcium loss. In conclusion, palm tocotrienol mixture is more effective than alpha-tocopherol in preventing the deleterious effects of nicotine on bone metabolism.

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