Effects of Insulin and 1 α -Hydroxy Vitamin D₃ on Streptozotocin-Induced Diabetic Rats Fed a Low Zinc Diet

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Streptozotocin-induced diabetic rats (LZ-DM rats) developed nephrocalcinosis after being fed a low zinc diet for 4 weeks. However, administration of 1 α -hydroxy vitamin D₃ (1-OHD₃) or insulin inhibited the formation of nephrocalcinosis.

The urinary calcium level increased when 1-OHD₃ was administered to these rats, but decreased in the group receiving insulin. It was also observed that the femur recovered its hardness when insulin was administered, although the hardness in the LZ-DM rats remained lower than that of the control rats. Rat femur hardness, however, did not completely recover with 1-OHD₃ treatment.

These results indicate that the effects of insulin and 1-OHD₃ differ, even though nephrocalcinosis was reduced by administration of either insulin or 1-OHD₃. It appears that insulin assists bone formation, and the 1-OHD₃ hastens calcium excretion.

Keywords nephrocalcinosis; insulin; 1 α -hydroxy vitamin D₃; diabetes; low zinc diet; femur

Introduction

It is well known that diabetes mellitus (DM) induces various metabolic disorders in humans; one such disorder associated with renal dysfunction, hypercalciuria, can be induced in humans and in experimental animals.^{1,2)} However, disorders occur less frequently in animals with DM than in man. One reason is that experimental animals are given a regular balanced diet and there is little chance that disorders due to unbalanced nutrition will occur spontaneously. To induce hypercalciuria, we fed a zinc-deficient diet to experimental rats that were later treated with streptozotocin (STZ).

In this manner, as has been described in our previous report, zinc deficiency induced nephrocalcinosis in the kidneys of diabetic rats.³⁾ Moreover, we also found that hydroxyapatite was a component of the kidney stones that developed and the hardness of the femur decreased.

However, since the mechanisms leading to the formation of hydroxyapatite remain unclear, we administered insulin or 1 α -hydroxy vitamin D₃ (1-OHD₃) to diabetic rats fed a low zinc diet, and studied the effects of these drugs on nephrocalcinosis.

Materials and Methods

Male Wistar rats, weighing 170 to 190 g, were purchased from Japan SLC Co., Ltd. (Shizuoka, Japan).

These animals were given either a modified B diet (SC) with 6.9 mg Zn/100 g or a modified B diet lacking zinc (LZ) with only 0.5 mg Zn/100 g. Both groups of rats were given distilled water *ad libitum*.

SC and LZ diets were supplied by Oriental Yeast Co. (Tokyo, Japan). The SC diet contained protein (22.6%), lipids (6.2%), crude fiber (4.9%), ash (3.9%), and a nitrogen-free extract (53.4%), and provided a metabolized energy of 3600 kcal per kg. The diet was enriched with vitamins and trace elements. The LZ diet was the same as the SC diet, but with the zinc compounds removed.

The insulin, (Insulin Insulatard Nordisk), was purchased from Yamanouchi Pharmaceutical Co., Ltd. (Tokyo), and 1-OHD₃, (Alpharol), was purchased from Chugai Pharmaceutical Co., Ltd. (Tokyo).

After one week, 50 mg/kg of STZ was intravenously administered to the SC and LZ groups (SC-diabetic group and LZ-diabetic group). 0.05 μ g/d of 1-OHD₃ was administered orally to STZ-treated LZ rats daily following the STZ treatment until the end of the experiment (LZ-AC group), and

5 U/d of insulin was injected subcutaneously in STZ-treated LZ rats daily until the end of the experiment (LZ-Ins group). In place of STZ, a 0.05 M-citrate buffer (pH 4.5) was administered to the two control groups (SC-control group and LZ-control group).

Four weeks after STZ treatment, urine was collected over a 24-h period from each group of rats. Following this, heparinized blood samples were collected from the aorta, and after sacrifice, the kidneys and femurs of each animal were removed.

After wet combustion with nitric acid, the calcium content of all tissue samples was measured by atomic absorption spectrophotometry (170-70, Hitachi Co., Tokyo). The urea nitrogen (BUN) and creatinine levels of the plasma were measured using "NESCAUTO" kits (Nippon Shoji Co., Osaka).

The hardness of each femur was measured by a Monsanto durometer (2030-2, Kayagaki Co., Tokyo).

All results are given as a means $\pm$ S.E. Differences between groups were determined by a one-way analysis of variance, and the comparison of means between groups was evaluated using the Scheffe method.

Results

Figure 1 shows the renal calcium levels of the various groups of rats. When STZ was administered to the rats fed the SC diet, renal calcium increased. Renal calcium also increased in the rats fed the LZ diet. When compared with the LZ-diabetic rats, the renal calcium levels in LZ-Ins and LZ-AC rats were significantly lower.

Figure 2 indicates the plasma levels of the different parameters. Plasma calcium increased when 1-OHD₃ was administered to LZ-diabetic rats, but the plasma BUN and creatinine increased in both the 1-OHD₃- and insulin-treated groups compared with the control groups.

Figure 3 shows the urinary calcium and the hardness of the femur in all rat groups. Urinary calcium increased in the rats receiving STZ. Also, urinary calcium increased when 1-OHD₃ was administered to the LZ-diabetic rats. However, urinary calcium in LZ-Ins rats decreased, when compared with LZ-diabetic rats.

Femur hardness did not differ between the SC-control group and the LZ-control group. However, when STZ was administered to the rats, fed either the SC or LZ diet, the femur hardness decreased, compared with each control group. The femur hardness of the LZ-Ins group was

significantly higher than that of the LZ-diabetic group. However, no difference was noted in femur hardness between the LZ-diabetic group and the LZ-AC group.

Discussion

Decreased bone mineral content has been noted in diabetic humans⁴⁾ and in diabetic rats.⁵⁾ Hypercalciuria has also been found in diabetic humans and rats.^{1,2)} In this regard, Garland *et al.*⁶⁾ reported that calcium absorption by the loop of Henle is significantly lower in diabetic animals. Mars *et al.*⁷⁾ also reported that extreme kidney calcification was seen when a 0.8% phosphorus diet was

fed to young female rats. In this present report, nephrocalcinosis was induced in diabetic rats fed a low zinc diet. Moreover, our results indicate that the renal calcium content in LZ-Ins and LZ-AC rats decreased, compared with the calcium content level in LZ-diabetic rats (Fig. 1). However, there were differences between the effects of insulin and those of 1-OHD₃ on diabetic rats fed a low zinc diet (Fig. 3). Although neither drug completely prevented renal injury, plasma BUN and creatinine levels were increased (Fig. 2).

These results indicate that the effect of insulin on nephrocalcinosis differed from that of 1-OHD₃. 1-OHD₃ is known to have the same activity as 1,25-dihydroxy vitamin D₃,⁸⁾ and that in the liver, vitamin D₃ is converted to 25-hydroxy vitamin D₃, and then to 1,25-dihydroxy vitamin D₃ in the kidney. This 1,25-dihydroxy vitamin D₃ reacts to an increase in the intestinal absorption of calcium and renal calcium reabsorption.⁹⁾

In addition, it has also been reported that there is a decrease in the hydroxylation enzyme activity in the kidney and in the concentration of 1,25-dihydroxy vitamin D₃ when renal dysfunction occurs.¹⁰⁾ In this regard, Fukushima *et al.*¹¹⁾ reported that plasma calcium increased when 1-OHD₃ was administered to nephritic rats. However, no difference was noted in urinary calcium levels of 1-OHD₃-administered rats and nephritic rats, although the urinary calcium in the nephritic rats was higher than in the controls. Similarly, plasma calcium increased in the LZ-AC group (Fig. 2) and it could be that the increase in calcium absorption from the intestine induced the increase in plasma calcium. As shown in Fig. 3a, calcium excretion in the urine was accelerated by administering 1-OHD₃. Vitamin D increased calcium uptake and reabsorption.⁹⁾ However, renal dysfunction occurred in the diabetic rats and calcium reabsorption declined. Moreover, calcium excretion was accelerated when plasma calcium increased. Therefore, the urinary calcium increased when 1-OHD₃ was administered to the LZ-diabetic rats.

Taking these findings into consideration, it appears that an imbalance of calcium and phosphate induces nephrocalcinosis, but 1-OHD₃ inhibits the formation of neph-

Fig. 1. Effects of Insulin and 1-OHD₃ on Renal Calcium

SC-C, SC-control group; SC-DM, STZ-treated rats fed SC diet; LZ-C, LZ-control group; LZ-DM, STZ-treated rats fed LZ diet; LZ-AC, 0.05 µg/d of 1-OHD₃ was administered daily to LZ-DM rats until the end of the experiment; LZ-Ins, 5 U/d of insulin was administered daily to LZ-DM rats until the end of the experiment. Results are expressed as means ± S.E. a) $p < 0.01$ vs. LZ-control group, b) $p < 0.01$ vs. LZ-DM group.

Fig. 2. Effects of Insulin and 1-OHD₃ on Plasma Parameters

SC-C, SC-control group; SC-DM, STZ-treated rats fed SC diet; LZ-C, LZ-control group; LZ-DM, STZ-treated rats fed LZ diet; LZ-AC, 0.05 µg/d of 1-OHD₃ was administered daily to LZ-DM rats until the end of the experiment; LZ-Ins, 5 U/d of insulin was administered daily to LZ-DM rats until the end of the experiment. Results are expressed as means ± S.E. a) $p < 0.05$, b) $p < 0.01$ vs. LZ-C group, c) $p < 0.05$ vs. LZ-DM group. ■, calcium; ▨, BUN; ▩, creatinine.

Fig. 3. Effects of Insulin and 1-OHD₃ on Urinary Calcium and Femur Hardness

a) Urinary calcium in each group. Urine collected over 24 h was analyzed. b) The hardness in the femur in each group.

SC-C, SC-control group; SC-DM, STZ-treated rats fed SC diet; LZ-C, LZ-control group; LZ-DM, STZ-treated rats fed LZ diet; LZ-AC, 0.05 µg/d of 1-OHD₃ was administered daily to LZ-DM rats until the end of the experiment; LZ-Ins, 5 U/d of insulin was administered daily to LZ-DM rats until the end of the experiment. Results are expressed as means ± S.E. a) $p < 0.05$, b) $p < 0.01$ vs. LZ-C group, c) $p < 0.05$, d) $p < 0.01$ vs. LZ-DM group.

rocalcinosis by increasing calcium excretion.

As shown in this study, calcium excretion from the bone occurred in the LZ-diabetic rats (Fig. 3b), and it is well established that insulin is essential for bone growth.¹²⁾ Furthermore, as seen in this study, in the rats that received insulin, calcium excretion from the bone was reduced, and nephrocalcinosis was inhibited.

Therefore, nephrocalcinosis occurred in STZ-induced diabetic rats fed a low zinc diet, but was reduced by the administration of either insulin or 1-OHD₃. However, the effects of insulin and 1-OHD₃ on nephrocalcinosis differ.

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