Coronary Arterial Lesions in Piglets from Sows Fed Moderate Excesses of Vitamin D

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Toda, T., Toda, Y. and Kummerow, F.A. Coronary Arterial Lesions in Piglets from Sows Fed Moderate Excesses of Vitamin D. Tohoku J. exp. Med., 1985, 145(3), 303-310 — Ultrastructural studies were conducted on the coronary arteries of 6 week-old piglets from sows which received diets containing either 25 μg or 3.7 μg of vitamin D₃ per pound of basal ration. The subendothelial space which was rich in mucopolysaccharide and newly formed tiny elastic fibers, contained numerous macrophages, some of which possessed lipid droplets. Degenerated smooth muscle cells with pyknotic nuclei or vesiculated organelles, and mitotic smooth muscle cells were occasionally seen in the coronary artery. Piglets from sows which were fed high doses of vitamin D had more degenerated smooth muscle cells without stainable lipid and lipid containing cells in their coronary arteries than piglets from sows which were fed low doses of vitamin D. These results suggested that an excess dietary intake of vitamin D by pregnant animals may have potential angiototoxic effects on the coronary arteries of their offsprings. — excess vitamin D; coronary artery; piglet

Extensive calcification has been documented in the cardiovascular system of various animal species that were fed excessive vitamin D (Hass et al. 1958; Kent et al. 1958). Seeling (1969) has indicated a parallel increase in the incidence of vitamin D toxicosis in humans, including idiopathic hypercalcemia, the supravalvular aortic stenosis syndrome, and renal tubular acidosis with increased levels of vitamin D in fortified foods. The British Pediatric Association (1964) reported that the incidence of these diseases decreased several years after vitamin D fortification of foods was reduced.

Taura et al. (1978) and Kamio et al. (1977) indicated that an excess intake of vitamin D induced coronary and aortic arterial lesions in swine; however, the

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minimum level of vitamin D required to induce arterial damage is still not known. Coronary arterial damage can be accelerated in the fetus before birth by the excessive presence of vitamin D in the diet.

From these considerations, we compared the coronary arterial lesions of piglets from sows that had been fed a diet containing a high level of vitamin D₃ with those of piglets from sows that had been fed a low level of dietary vitamin D₃.

**MATERIALS AND METHOD**

A total of 11 six week-old Yorkshire piglets were used in this study; 5 piglets from a sow which had been fed a diet containing 25 μg vitamin D per pound of basal ration (high D-group) were compared to 6 piglets from a sow which had been fed a diet containing 3.7 μg vitamin D per pound of basal ration (low D-group). The basal diet contained 3% fat and 14.3% protein as furnished by 1,745 pounds of ground yellow corn, 200 pounds of pressure cooked and solvent extracted soybean meal and 55 pounds of a premix of multiple vitamins and minerals per ton of basal ration.

The animals were tranquilized with ketamine hydrochloride before the hearts were removed and dissected. The proximal 3 cm of the left anterior interventricular coronary arteries were collected and divided into five specimens. These specimens were fixed in a 3% phosphate-buffered glutaraldehyde solution (pH 7.4), post-fixed in 1% phosphate-buffered osmium tetroxide (pH 7.4), dehydrated in increasing concentrations of ethanol, and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate, and examined with a Hitachi HU-12 electron microscope. Counts and computations of degenerated smooth muscle cells without stainable lipid and lipid containing cells were carried out at a magnification of 5,000 (Toda et al. 1980).

As previously reported by Toda et al. (1983), the levels of 25-hydroxy-vitamin D₃ were determined by high pressure liquid chromatography (Waters Associates Chromatography pump and model 440 absorbance detector) on a μ-porosil column with 2.5% propanol in hexane.

**RESULTS AND DISCUSSION**

Ultrastructural features of the intima and inner media of the coronary artery of a high D piglet are shown in Fig. 1. A single layer of endothelial cells lies on the luminal surface of the coronary artery. Most of the endothelial cells contained abundant dilated rough endoplasmic reticulum. Endothelial cells with pyknotic nuclei and vesiculated organelles were occasionally seen. The subendothelial space contained abundant amorphous material, probably of mucopolysaccharides, and tiny rod-shaped masses of elastic fibers.

The majority of the cellular components in the subendothelial space were macrophages with kidney-shaped nuclei, abundant lysosomal dense bodies and cytoplasmic processes. Some of these macrophages contained lipid droplets of various sizes. The internal elastic lamina was discontinuous. The rough endoplasmic reticulum-rich smooth muscle cells (Stary and Malinow 1982) were occasionally observed around the internal elastic lamina and in the tunica media (Fig. 2). Most of the medial smooth muscle cells displayed plentiful
Fig. 1. Coronary arterial intima from a high D group piglet. Several lipid containing cells (LC) and rough endoplasmic reticulum-rich smooth muscle cells (star) are shown in this photograph (×3,200). E, endothelial cell.
myofilaments and scanty rough endoplasmic reticulum, and were surrounded by a basement membrane. Occasional cell debris (Fig. 3) and mitotic smooth muscle cells (Fig. 4), indicating active cell turnover, were also noted. Some smooth muscle cells in the innermost layer of the intima and in the inner media displayed degeneration without stainable lipid (Fig. 5), a feature frequently found in the coronary arteries of adult swine fed excess vitamin D (Kamio et al. 1977). Such degenerated cells without stainable lipid have also been reported in the arteries which had been subjected to hypoxia (Toda et al. 1981) and the administration of dietary fat and cholesterol (Imai et al. 1970).

The counts and computations of degenerated cells without stainable lipid and lipid containing cells demonstrated that the high D group piglets had more degenerated cells without stainable lipid and lipid containing cells than the low D group piglets. The plasma concentration of 25-hydroxy-vitamin D₃ was two times higher in the high D group than in the low D group (Table 1).

We previously indicated that 25-hydroxy-vitamin D₃, an active metabolite of vitamin D₃, enhances membrane permeability (Toda et al. 1983), which may have been responsible for the presence of degenerated smooth muscle cells without stainable lipid observed in this study. Certain oxigenative sterols have been reported to stimulate cholesterol ester formation in cultured fibroblasts (Brown et al. 1975). Whether excess dietary intake of vitamin D by pregnant women may

Fig. 2. Rough endoplasmic reticulum-rich smooth muscle cell (RE) in the tunica media of the coronary artery from a low D group piglet (×3,300).
Fig. 3. Cell debris (arrow) segregating from a medial smooth muscle cell in the coronary artery from a high D group piglet (×8,300).
B, basement membrane; P, pinocytic vesicle.
Fig. 4. Mitotic medial smooth muscle cell (M) with paired cisternae (arrow) in the coronary artery from a low D group piglet (×11,000).

Table 1. Frequency of degenerated smooth muscle cells without stainable lipid and lipid containing cells, and plasma concentration of 25-hydroxy vitamin D in experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Low D group</th>
<th>High D group</th>
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<tbody>
<tr>
<td>Number of animals</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Total cells counted</td>
<td>1624</td>
<td>1319</td>
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<tr>
<td>Degenerated smooth</td>
<td></td>
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<tr>
<td>muscle cells without</td>
<td>20 (1.2%)</td>
<td>34 (2.6%)</td>
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<tr>
<td>stainable lipid</td>
<td></td>
<td></td>
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<tr>
<td>Lipid containing cells</td>
<td>31 (1.9%)</td>
<td>52 (3.9%)</td>
</tr>
<tr>
<td>Serum level of</td>
<td>15</td>
<td>30</td>
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<td>25-hydroxy vitamin D (ng/ml)</td>
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be responsible for the fatty streaks that have sometimes been observed in the coronary arteries of newborn infants (Moon 1957) is still unknown. Finally, it is very important to note that the 25-hydroxy-vitamin D₃ plasma levels of piglets from the high D group in the present study were similar to those of the American population (Holmes and Kummerow 1983).
Acknowledgments

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References