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Ultrastructure of atheromatous lesions experimentally induced in Syrian hamsters of the APA strain

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Summary. In order to examine whether diabetes enhances primary aortic lesions up to atherosclerotic ones, mild primary lesions were induced in aorta of APA hamsters by an administration of vitamin D_2 (VD) and/or stop-and-reflow (SR)-operation, a modification of renal artery clamping. At 2 months after the treatment with the combination of VD-administration and SR-operation, atheromatous lesions, characterized by an appearance of many foam cells in the intima, were observed in the abdominal aorta, the site of SR-operation, in streptozotocin (SZ)-induced diabetic APA hamsters. Foam cells in the atheromatous lesions were originated from smooth muscle cells and monocyte/macrophages. On the other hand, neither VD-administration alone nor SR-operation alone developed atheromatous lesions in SZ-induced diabetic APA hamsters. In conclusion, we succeeded in a rapid induction of atherosclerotic lesions in abdominal aorta of SZ-induced diabetic APA hamsters by the combination of VD-administration and SR-operation.

Key words: APA hamster, Atheromatous lesion, Diabetes, Primary lesion

Introduction

Diabetes mellitus has been considered to be one of the major risk factors for atherosclerosis (WHO, 1985; Krolewski et al., 1987). One speculation is that diabetes alone may directly induce atherosclerotic lesions in the aorta, and another is that diabetes may enhance some pre-existing mild lesions in the aorta. Recently, the latter has been more supported than the former (Ledet et al., 1992; Heickendorff et al., 1994). In our preliminary study using Syrian hamsters of the APA strain (APA hamsters), neither arterio- nor athero-sclerotic lesions developed in the aorta at 6 months after the induction of diabetes by streptozotocin (SZ), as reported by Reinilä (1981) and Harano et al. (1992).

In this study, we experimentally induced mild aortic lesions in SZ-induced diabetic APA hamsters and examined whether diabetes may enhance such primary lesions in the aorta or not. Primary lesions were induced by an administration of vitamin D_2 (VD) (Yasoshima et al., 1982) and/or by stop-and-reflow (SR)-operation, a modification of renal artery clamping (Fried et al., 1984; Lennon et al., 1991).

An administration of VD is considered to accelerate the permeability of arterial endothelium to serum molecules and to induce degenerative changes in medial smooth muscle cells (SMCs) due to calcium-overloading (Yasoshima et al., 1982). In our preliminary study on the aorta of APA hamsters treated with VD, mild intimal edema and medial calcium deposition were observed in the acute phase. On the other hand, SR-operation is expected to induce transient hypoxia in the aortic wall, bringing about mild injury in endothelial cells (ECs). Actually, in our preliminary study on the aorta of APA hamsters following SR-operation, focal degeneration and peeling-off of ECs were observed in the acute phase. Moreover, in our preliminary study on the aorta of APA hamsters treated with the combination of VD-administration and SR-operation, no atherosclerotic lesions were observed at 2 months after the treatment.

Materials and methods

Twenty-one male APA hamsters obtained from our breeding colony were used. They were maintained under controlled conditions (temperature, 24±2 ºC; humidity, 55±5%) in plastic cages with sterilized wood shavings for bedding, and fed a commercial diet, CMF (Oriental Yeast Co. Ltd., Tokyo) with tap water *ad libitum*.

Eighteen 2-month-old animals were injected intraperitoneally with 40 mg/kg body weight (b.w.) of SZ (Sigma Chemical Co., Lot 43H0208) dissolved in 0.1M citrate buffer (pH 4.5), and then they were divided into 4 groups: 1) 3 animals without any additional treatments (SZ-group); 2) 6 animals orally administered with 8 mg/kg b.w. of VD for 4 days at 2 weeks after SZ-injection (SZ+VD-group); 3) 4 animals treated with the

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SR-operation as mentioned below at 4 weeks after SZ-injection (SZ+SR-group); 4) 5 animals administered with 8 mg/kg b.w. of VD for 4 days at 2 weeks and then treated with the SR-operation at 4 weeks after SZ-injection (SZ+VD+SR-group). The remaining 3 animals served as non-treated controls (C-group).

As to SR-operation, after laparotomy under anesthesia, abdominal aorta just below the bifurcation of the left renal artery was clamped atraumatically using a stainless clip, «Serufin type A» (Natsume Seisakusyo Co. Ltd., Tokyo) and blood flow was stopped for 1 minute. Immediately after that, the blood was reflowed by removal of the clip. This procedure was successively repeated 3 times before suture.

At 5 months of age (about 2 months after each treatment), each animal was weighed and killed by exanguination from vena cava under ether anaesthesia. Immediately, proximal thoracic aorta (TA) and abdominal aorta (AA) just below the bifurcation of the left renal artery (just below the clamping portion) were taken from each animal and fixed in 10% neutral-buffered formalin. For electron microscopic examination, small pieces of TA and AA were fixed in 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1M phosphate buffer (pH 7.4), postfixed in 1.0% osmium tetroxide in the same buffer, and embedded in epoxy resin, Quetol 812 (Nissin EM Co. Ltd., Tokyo). Ultrathin sections were double-stained with uranyl acetate and lead citrate, and observed by electron microscopy, 1200 EX (JEOL Co. Ltd., Tokyo).

Blood samples were collected after overnight fasting from the orbital sinus of each animal once a month through the experimental period, and measured colometrically for serum glucose levels using a commercial test kit, Glucose CII Test-Wako (Wako Pure Chemical Industries Inc., Osaka). In addition, blood samples collected at the end of the experiment were also subjected for the measurement of the contents of serum total cholesterol and triglyceride in the same way.

Results

Body weights and serum glucose levels

Body weights decreased at 1 month after SZ-injection and thereafter increased again (Fig. 1). Serum glucose levels were significantly higher in all SZ-injected groups than in the C-group, and there was no significant difference among 4 groups injected with SZ throughout the experimental period (Fig. 2). As to the content of serum total cholesterol and triglyceride, they showed significantly higher levels in the 4 groups treated with SZ than in the C-group, though there was no significant difference between these 4 groups because of their large deviations (Fig. 3).

Electron microscopic findings

In the SZ-group, degenerative changes in ECs and an accumulation of swollen mitochondria in irregular-shaped medial SMCs were simultaneously observed in both TA and AA, and their severity was similar to that found in the C-group. However, an invasion of medial SMCs into the discontinued internal elastic lamina (IEL), which showed triple or more branching, was more prominent in the SZ-group (Fig. 4).

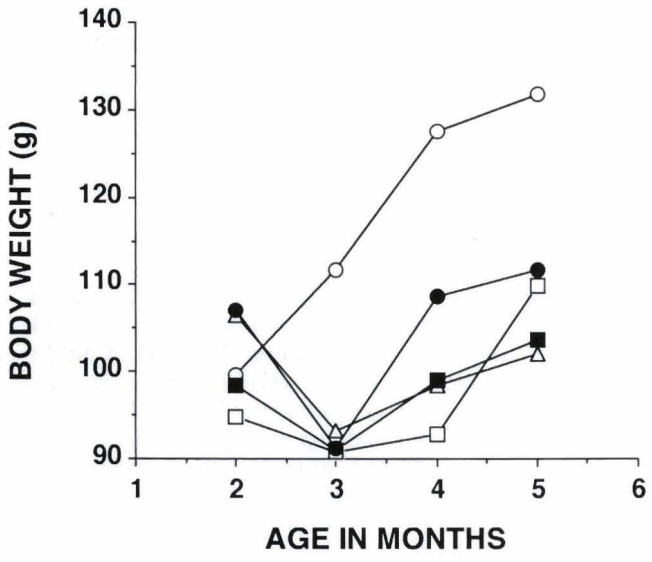


Fig. 1. Changes in body weights of APA hamsters. white circle: C-group; triangle: SZ-group; black square: SZ+VD-group; white square: SZ+SR-group; black circle: SZ+VD+SR-group. The data are expressed as the mean of the animals of each group.

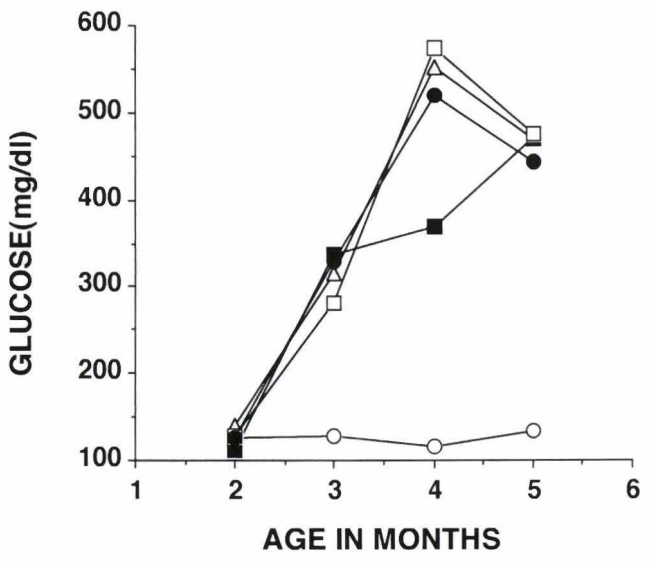


Fig. 2. Changes in serum glucose level of APA hamsters: white circle: C-group; triangle: SZ-group; black square: SZ+VD-group; white square: SZ+SR-group; black circle: SZ+VD+SR-gorup. The data are expressed as the mean of the animals of each group.

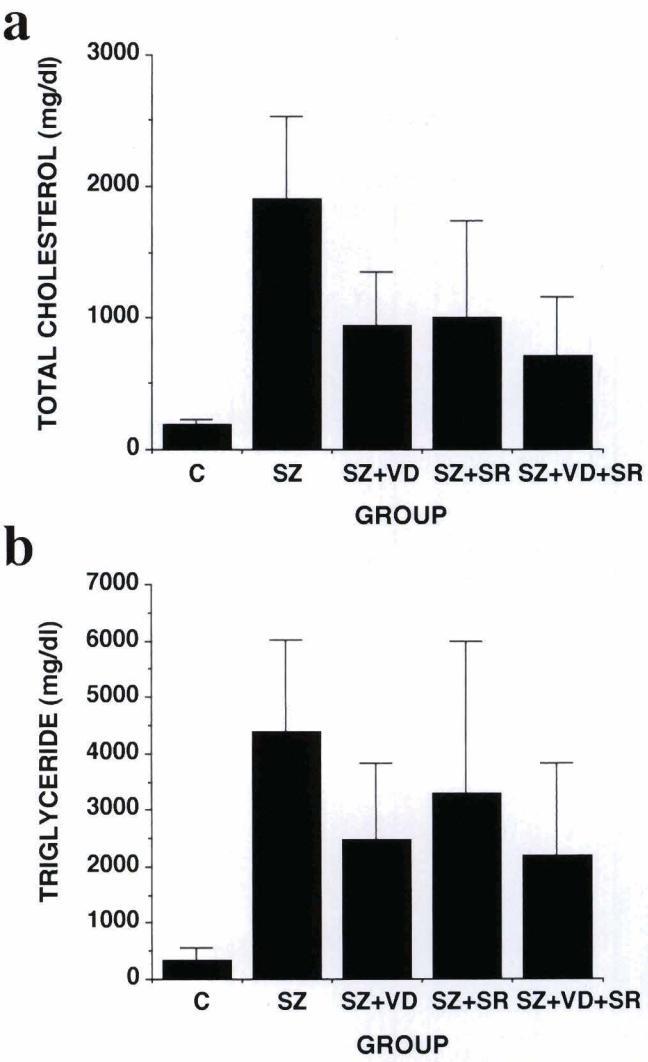
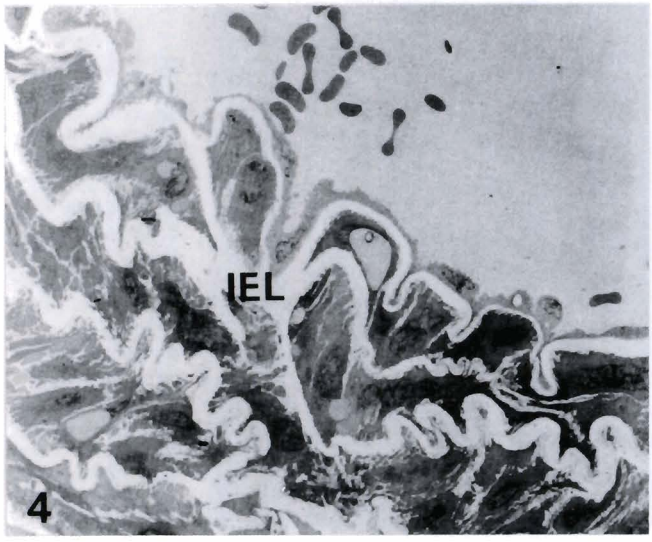


Fig. 3. Serum levels of total cholesterol **(a)** and triglyceride **(b)** of APA hamsters at the end of the experiment (5 months of age). The data are expressed as the mean and the standard deviation of each group.



In the SZ+VD-group, in addition to the similar changes to those in the SZ-group, calcification in the medial interstice and a small aggregation of cell debris were sometimes found in both TA and AA. Some parts of the intima included a small number of SMCs with increased extracellular matrix materials, resulting in

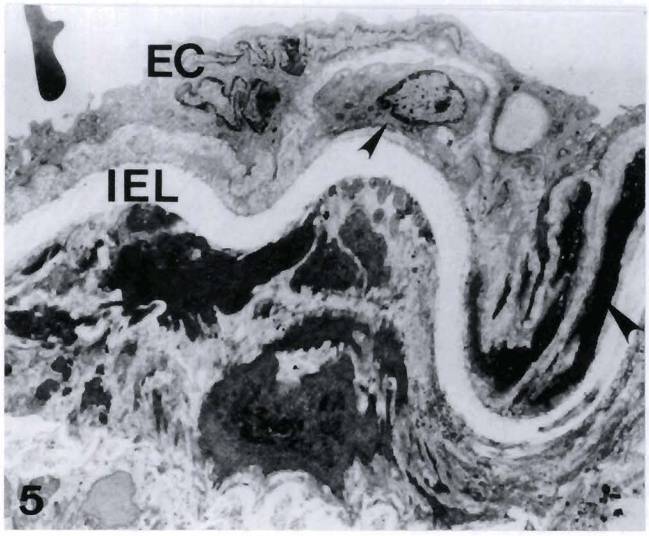


Fig. 5. Abdominal aorta of an APA hamster in the SZ+VD-group. The thickened intima includes some smooth muscle cells (arrowheads) with increased extracellular matrix materials. Ec: endothelial cell; IEL: internal elastic lamina. x 2,400

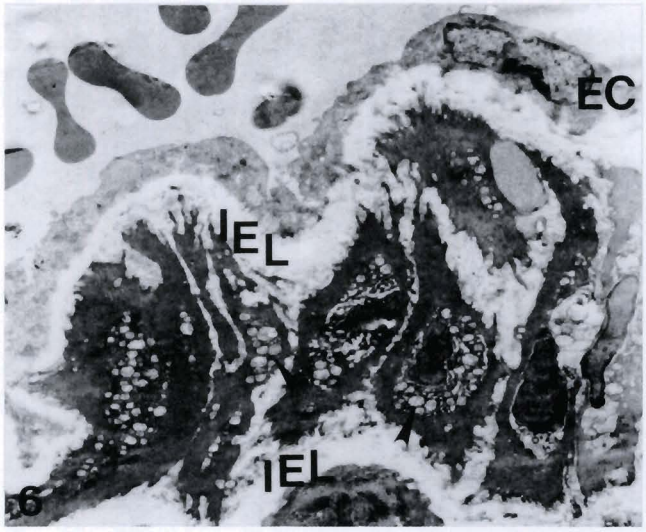


Fig. 6. Abdominal aorta of an APA hamster in the SZ+SR-group. Smooth muscle cells between the layers of the discontinued internal elastic lamina (IEL) show degenerative changes, such as an accumulation of swollen mitochondria (arrowheads). EC: endothelial cell. x 2,700

Fig. 4. Abdominal aorta of an APA hamster in the SZ-group. The discontinued internal elastic lamina (IEL) shows triple or more branching. x 1,000

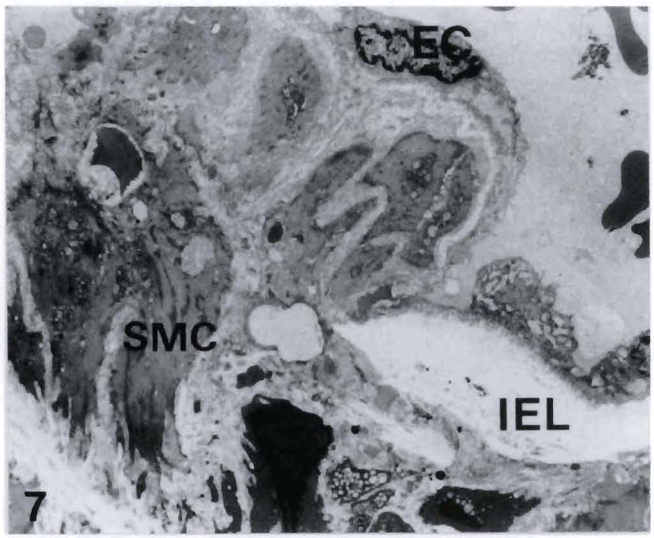


Fig. 7. Abdominal aorta of an APA hamster in the SZ+VD+SR-group. The thickened intima accompanies disintegration of the internal elastic lamina (IEL) and an invasion of medial smooth muscle cells (SMCs) with a large amount of extracellular matrix materials. Ec: endothelial cell. x 2,500

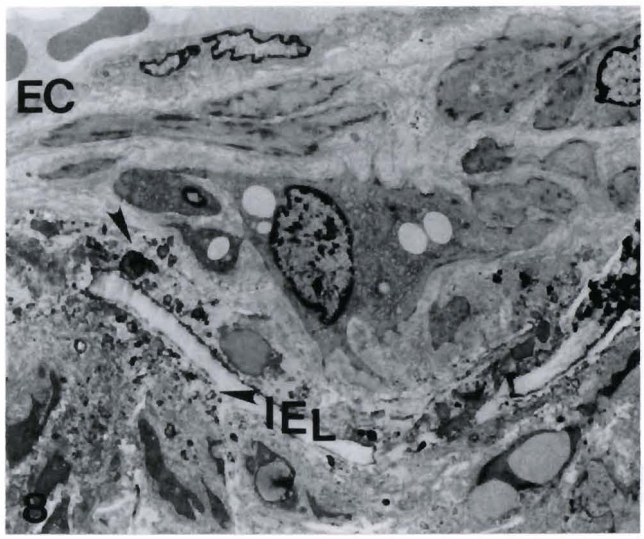


Fig. 8. Abdominal aorta of an APA hamster in the SZ+VD+SR=group. The thickened intima includes a lipid-laden smooth muscle cell. Severe disintegration of the internal elastic lamina (IEL) and a deposition of cellular debris and calcium salts (arrowheads) are observed. EC: endothelial cell x 2,400

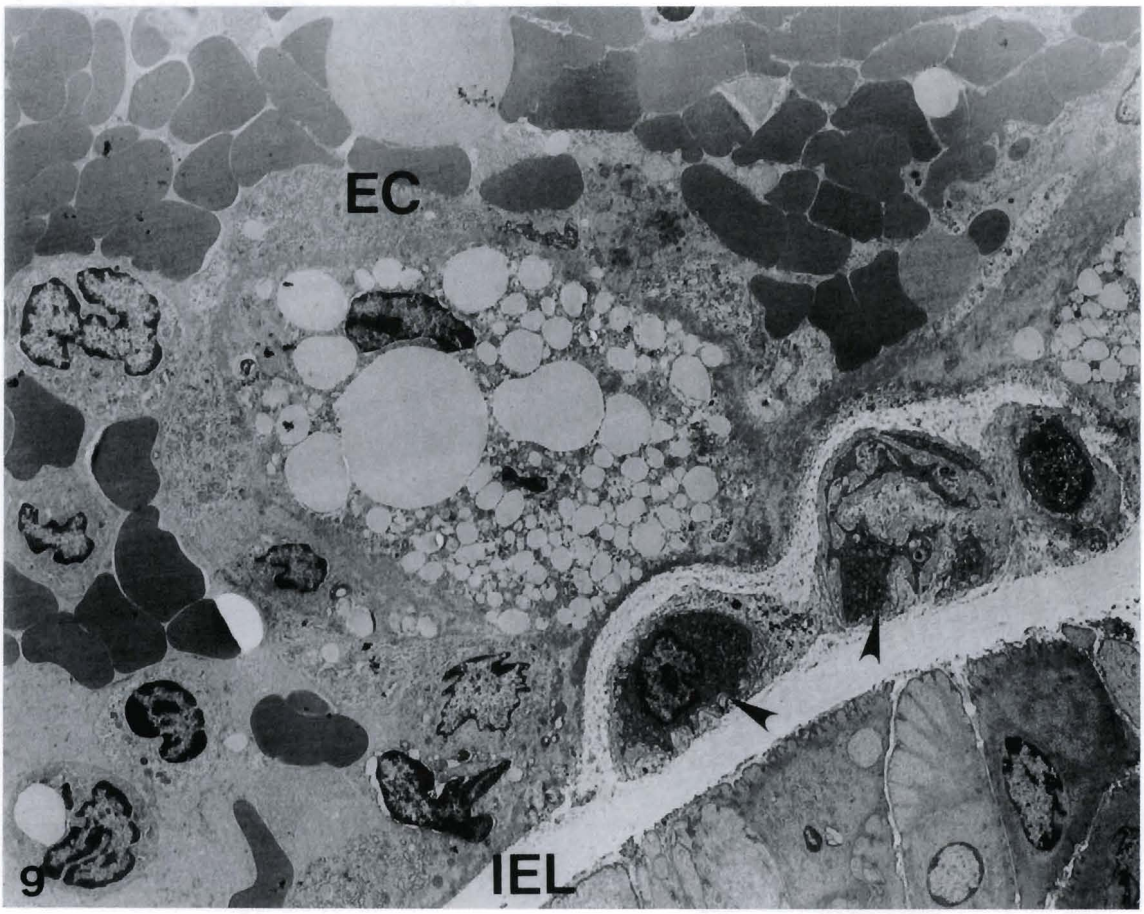


Fig. 9. Abdominal aorta of an APA hamster in the SZ+VD+SR-group. The thickened intima includes a few foam cells and accompanies an invasion of some smooth muscle cells (arrowheads) with increased extracellular matrix materials. The endothelial cells (ECs) covering the foamy portion show degeneration. IEL: internal elastic lamina. x 2,800

intimal thickening (Fig. 5).

In the SZ+SR-group, the similar changes of TA to those in the SZ-group were found. On the other hand, degenerative changes of SMCs which were situated between the layers of the discontinued IEL and showed an accumulation of swollen mitochondria in the cytoplasm were more prominent in AA as compared with those in the SZ-group (Fig. 6).

In the SZ+VD+SR-group, similar changes of TA to those in the SZ+VD-group were observed. On the other hand, as compared with other groups, a more prominent increase in extracellular matrix materials and aggregation of cellular debris in AA resulted in a widening of medial interstice. SMCs often invaded the subendothelium through the disintegrated IEL and produced a large amount of extracellular matrix materials, inducing a prominent thickening of the intima (Fig. 7). Some of the SMCs in the thickened intima contained lipid droplets (Fig. 8). A few foam cells were also observed in the subendothelium (Fig. 9) in some portions of AA, sometimes making up atheromatous lesions (Fig. 10). Some foamy cells contained myofilaments and others did not. Many of the ECs covering atheromatous lesions showed severe degenerative changes (Figs. 9, 10).

Discussion

In this study, we examined the effects of diabetes on different types of primary lesions in the aorta of APA hamsters from the viewpoint of their capacity for the formation of atheromatous lesions, i.e. diabetic macroangiopathy. As a result, atheromatous lesions characterized by an appearance of many foam or foamy cells were observed only in the SZ+VD+SR-group at the site of AA where the SR-operation was done. On the other hand, no atheromatous lesions were detected in the SZ+VD- and SZ+SR- groups. But the intimal thickening in the SZ+VD-group might progress to atheromatous lesion for a longer period of diabetes.

As mentioned in the Introduction, in our preliminary study, aortic lesions induced by the combination of VD-administration and SR-operation were almost repaired and no atherosclerotic lesions were detected at 2 months after the treatment. And also, no atherosclerotic lesions developed in the aorta under diabetic condition alone with duration of less than 6 months. Therefore, our present results and those of preliminary studies support the hypothesis that diabetic atherosclerotic lesions, i.e. diabetic macroangiopathy, may be induced by

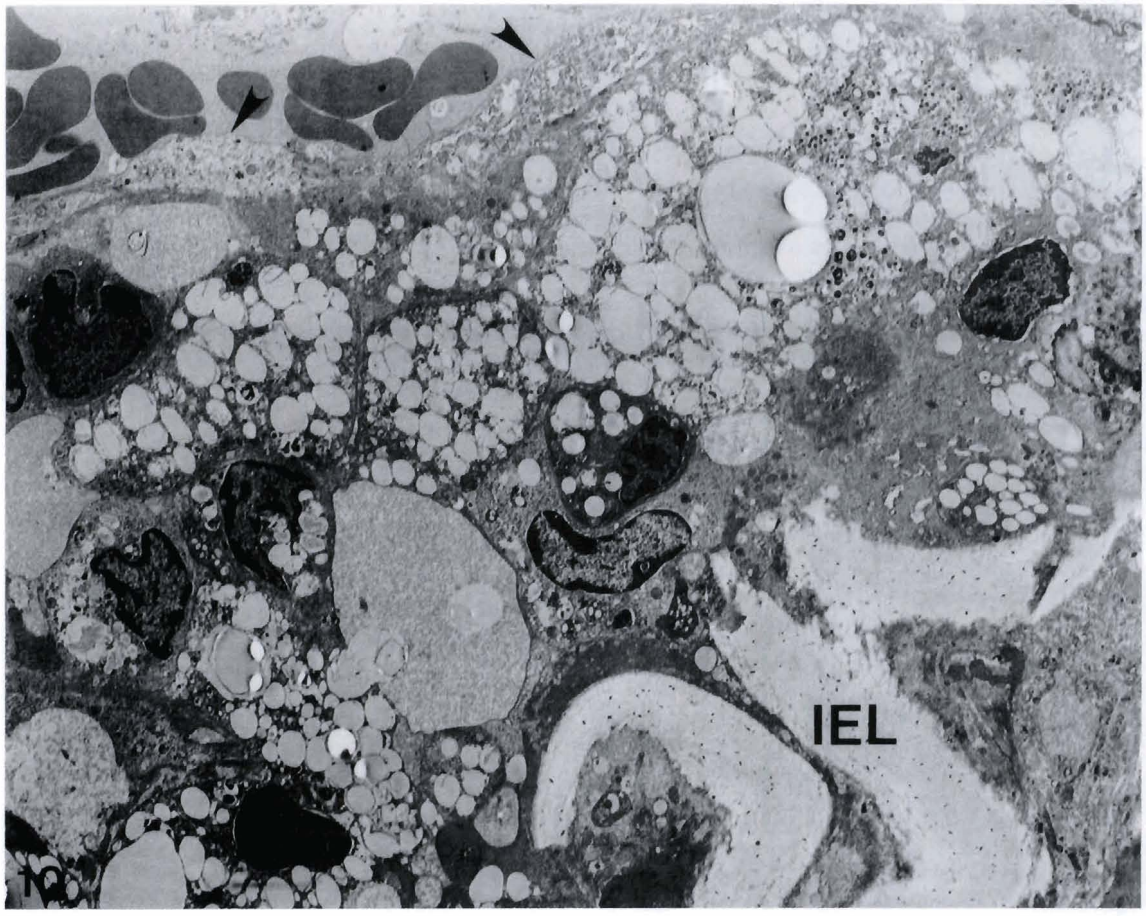


Fig. 10. Abdominal aorta of an APA hamster in the SZ+VD+SR group. Atheromatous lesion includes many foam cells. The endothelial cells (arrowheads) covering the lesion show severe degeneration. IEL: internal elastic lamina. x 2,800

enhancement of some primary lesions in the aortic wall under diabetic condition (Ledet et al., 1992; Heickendorff et al., 1994).

Atheromatous lesions contained lipid-laden SMCs, which invaded from the media into the intima through the disrupted IEL, and lipid-laden mononuclear cells without myofilaments perhaps from the vascular lumen. From electron microscopic findings in the present and previous studies (Gerrity, 1981a,b), those mononuclear cells were considered to be monocytes/macrophages. Therefore, foam cells found in the aortic intima in the present study seem to be both SMC- and monocyte/macrophage-origin, as proposed previously (Joris et al., 1983; Faggiotto and Ross, 1984; Faggiotto et al., 1984; Nistor et al., 1987).

Excluding that the discontinued IEL showed triple or more branching, ultrastructural changes observed in the SZ-group were similar to that found in the C-group, that is, the age-related changes detailed in our previous report (Yamanouchi et al., 1995). So, increased potential of medial SMC invasion into the intima is suggested to be a part of diabetic effects on the primary lesions in the formation of atheromatous lesions.

In conclusion, we succeeded in a rapid induction of atherosclerotic lesions in AA of SZ-induced diabetic APA hamsters by the combination of VD-administration and SR-operation, and this model is considered to be useful for the investigation of the development of diabetic macroangiopathy.

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