ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LXII, N 1, 60 SECTIO D 2007

Human Anatomy Department, Clinical Pathomorphology Department
Medical University of Lublin

ZBIGNIEW WÓJTOWICZ, JUSTYNA SZUMIŁO, AGNIESZKA FRONCZEK, PIOTR PRZYBYLSKI, TERESA HERMANOWICZ-DRYKA, JOANNA KIŚ, FRANCISZEK BURDAN

Morphology of the aorta in experimentally-induced diabetes in rabbits

Diabetes mellitus is a chronic disease that is complicated by various pathological changes, i.e., nephropathies, neuropathies and vasculopathies (5, 10). Diabetic angiopathy is the most frequent complication and is also the main cause of mortality in those diseases (10).

Pathophysiological mechanisms of micro- and macroangiopathy may be considered as manifestation of endothelial dysfunction at distinct vascular sites (9). It is well known that hyperglycemia is identified as indirect factor of the diabetic vascular pathology. However, a direct pathway of vessels destruction is still not fully established. Combination of hyperglycemia, dislipidaemia, hyperhomocysteinemia, and other factors may accelerate the process and cause an early vascular wall dysfunction, as well as influence normal hemostasis. The initial functional disorders are later substituted by morphological lesions of the blood capillaries (microangiopathy) or arteries (macroangiopathy) (11).

The aim of this study was to evaluate the aortic morphology in the alloxan-induced diabetes in rabbits.

MATERIAL AND METHODS

The study was performed on mature New Zealand rabbits in accordance with international animal principles of conduct. The examined protocol was fully approved by the Local Bioethical Committee. The animals of 2.6–3.2 kg weight (mean 2.88 kg) were selected for the study. The regular diet and water were provided *ad libitum*. Diabetes was induced by intravenous injection of 10% alloxan solution (100 g/kg; Sigma Chemical Co., USA). In the control group the animals were injected with physiological solution of sodium chloride. Seven days after the injection the glucose level was measured. The level over 11.1 mmol/l (200 mg/dl) was considered as primary biochemical criterion for selection to the experimental group.

The animals were sacrificed at the end of week 6 (group A6) and 12 (group A12), and the necropsy was performed. The aorta was dissected, separated and taken for morphological studies. After longitudinal opening and gross inspection, three segments of the vessel, i.e. arch, thoracic and abdominal vessels, were fixed in 10% buffered formalin. After routine processing to the paraffin blocks cross sections of the aorta were stained with hematoxilin and eosin (H+E), resorcin-fuchsin, alcian blue/periodic acid-Schiff and Masson's trichrome. Formalin-fixed samples of the aorta were

also taken for frozen sections and Sudan IV stain to visualize lipids. Histological evaluation was performed in six animals for each experimental group. The slides were microscopically examined (Olympus BX45) without knowledge of animal groups.

RESULTS

The structure of the aorta was normal in all but one of the animals from the control group (Fig. 1A, 1B). Four of the six rabbits from group A6, as well as in all the animals sacrificed in week 12 (group A12) developed lesions in the abdominal aorta. The lesions were located mostly in the arch, however single ones were noted in the descending aorta as well. All of them were macroscopically visible as small (<1 mm in diameter), slightly elevated yellowish spots. They corresponded to mild thickness of *tunica intima* due to focal subendothelial accumulation of foam cells accompanied by the increase of the extracellular matrix (Fig. 2A, 2B). The foam cells were laden by lipids, which was confirmed by special staining (Fig. 2C). Most lesions were covered by intact endothelium, however shedding of overlying endothelial cells was also observed. In a few cases increased amount of the ground substance between sheets of elastic fibers as well as irregular arrangement of these sheets within *tunica media* underneath intimal lesions were noted (Fig. 2A). Nevertheless, *tunica media* like adventitia were usually unaffected. There were no differences in the severity and composition of lesions in aorta between both groups exposed to alloxan. Similar lesions were also found in one animal from control group. No advanced atherosclerotic lesions were revealed in any rabbits' aorta.

Unexpectedly, in one rabbit exposed to alloxan and sacrificed in week 6 a true succular aneurysm located in the abdominal part of the aorta was also found (Fig. 3). *Tunica intima* of the aneurysmatic part of the aorta was unaffected, however the media was markedly reduced and composed of 3–4 sheets of the elastic fibers. The wall of the aorta on the level of the aneurismal neck was thickened due to local calcium salts depositions in the media. It seems that the aneurysm represents rather congenital than acquired anomaly of the aortic wall, especially for the fact that intimal lesions in this particular case were minimal.

DISCUSSION

In the present study it was shown that intravenous injection of alloxan increased the risk of morphological changes in the aortic wall. All of the revealed lesions were time-dependent and localized mainly in the aortic arch, less frequent in the descending part of the vessel. Our results confirm previous reports on the similar type of histological changes and the same localization of atherosclerotic loci in gerbils (6, 7), hamster (2, 3, 12), mice (4) and rats (1).

The main cause of death and great percentage of morbidity in patients with diabetes mellitus is atherosclerosis. However, the pathogenesis of cardiovascular complications in those patients is multifactorial and can be affected not only by metabolic but also other factors like the socioeconomic status (10). A hypothesis for the initial event of atherosclerosis is endothelial dysfunction associated with changes in the concentration of the chemical messengers produced by the endothelial cells and/or by blunting of the nitric oxide-dependent vasodilatory response to acetylcholine. The endothelial abnormalities observed in patients with diabetes are poorly understood, but the loss of normal endothelial function could be involved in pathogenesis of diabetic long-term complications as angiopathy, as endothelial dysfunction associated with diabetic micro- and macroangiopathy. Some authors reported activation of protein kinase C, overexpression of growth factors, cytokines and oxidative stress in endothelial cells of arteries in patients with diabetes (10).

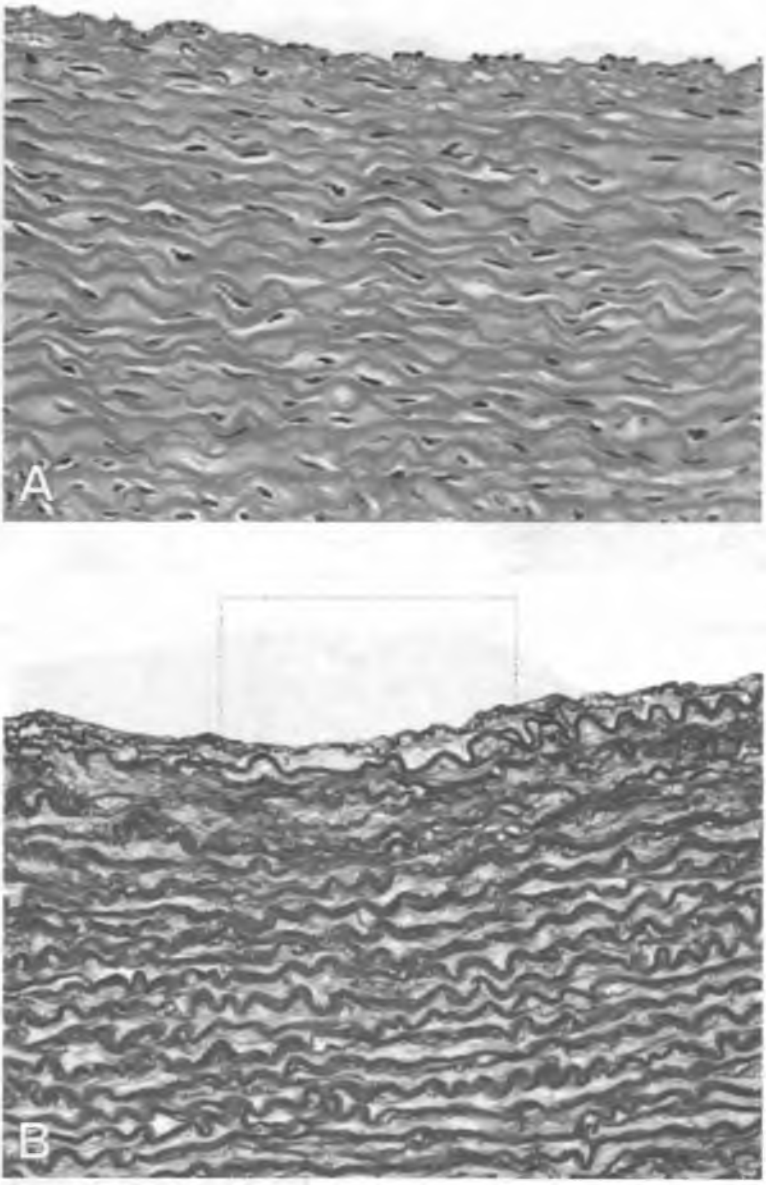

Fig. 1 A, B. Normal structure of aortic arch in rabbit from the control group (A: H&E, B resorcin-fuchsin stain; lens magn. x10)

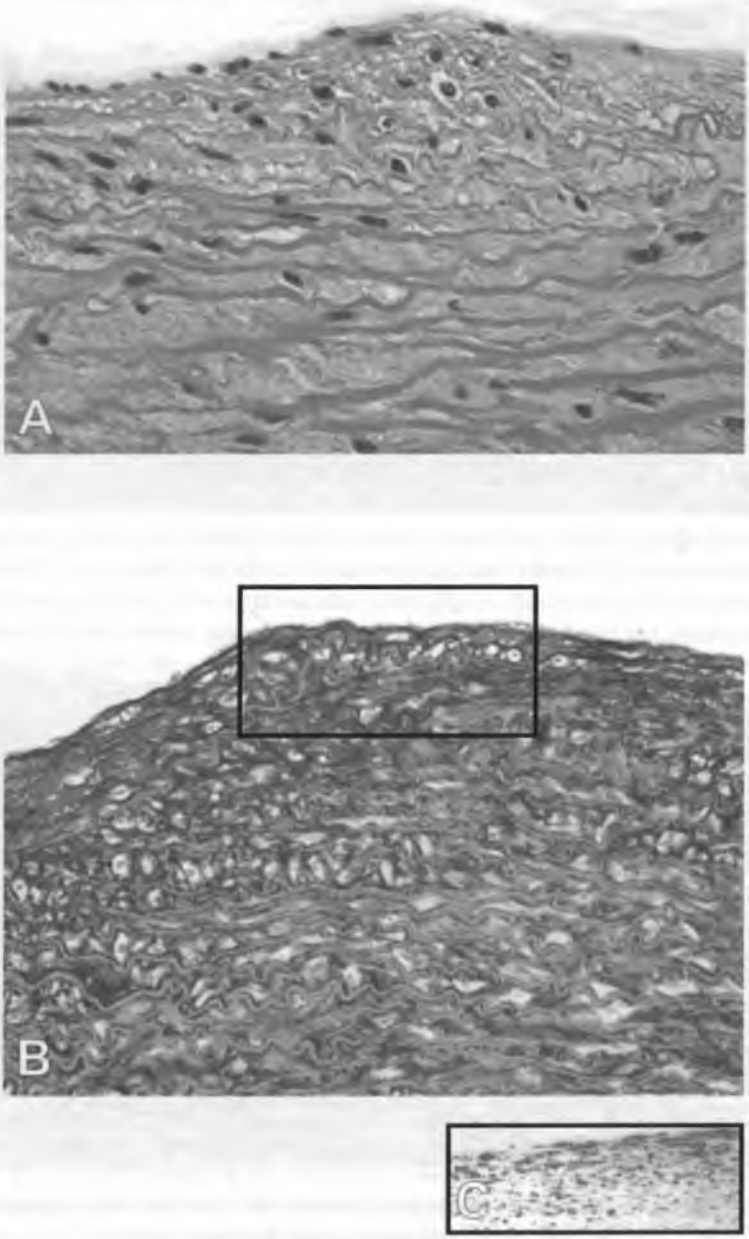

Fig. 2 A. Fatty streaks in the abdominal aorta in the rabbit exposed to alloxan and sacrificed on week 6 (H&E; lens magn. x20); B,C: Increased amount of ground substance (C, alcian blue/PAS; lens magn. x10) and accumulation of lipids (D: Sudan IV; lens magn. x10) in *tunica intima* of the aorta arch in the rabbit exposed to alloxan and sacrificed in week 12

Fig. 3. Succular aneurysm of the abdominal aorta in the rabbit exposed to alloxan and sacrificed in week 6 (H&E; lens magn. x5)

Primary changes in plasma and endothelial parameters go over to latter alteration represented by advanced atherosclerosis when typical plaques are formed. Failure of protective scavenger mechanisms is one possible explanation of vessel wall pathology in diabetes (11). Endothelial dysfunction represents a very early step in the development of atherosclerosis. As a result of hyperglycemia and hyperlipidemia during the process of atherosclerosis in large vessels of diabetic patients, fatty streaks characterized by a subendothelial accumulation of foam cells, fibrous plaques and more complex plaques containing calcium salts or cholesterol crystals were observed. Moreover, deposition of apolipoprotein E and advanced glycation end-products were also present in those lesions (12).

Hyperglycemia as a cause of accelerated atherosclerosis influenced the process of glycoxidation and lipid oxidation, causing an early impairment of the vessel wall (11). Diabetic macroangiopathy is also characterized by a series of diffuse, non-atherosclerotic alterations that hypothetically increase the vulnerability of the vessel wall to atherogenic process. One prominent feature of the macroangiopathy are linear media calcifications, which have been found to impose a strong risk for prospective cardiovascular events. Osteoprotegerin (OPG), also present in the vascular system, is increased in the *tunica media* of arteries from the diabetic patients. This bone-related protein has been linked to vascular calcifications in immunohistochemical analysis of atherosclerotic lesions. It is possible that increased arterial OPG concentrations reflect an osteogenic transformation of the vessels in diabetes as an aspect of diabetic macroangiopathy (8).

Smooth muscle cells proliferation and extracellular matrix (ECM) protein deposition are key features of diabetic macroangiopathy. The crucial role in diabetes-induced vascular hypertrophy and

remodelling is played by endothelial A (ETA) receptor. Plasma molecules as fibronecti, plasminogen activator inhibitor-1 (PAI-1) and their receptors as indicators of increased EMC protein synthesis in vessels' wall are up-regulated in diabetes. These changes in the molecules level are associated with increased medial thickness and diabetes-induced vascular hypertrophy and remodelling. It was also found that they are endothelium-dependent and may be mediated via TGF-β1 and angiotensin (1).

It was also established that polymorphonuclear leukocytes (PMN) play the role in the development of diabetic vascular complications (5). Activation of PMN is associated with increased expression of some adhesion molecules on the surface of those cells, e.g., β2-integrin and selectin L. These molecules play the role in leucocytes diapedesis into the vascular wall and progression of the inflammatory process (5).

In conclusion, it could be stressed that intravenous injection of alloxan increased the risk of time-dependent morphological changes in the aortic wall.

REFERENCES

1. Fukuda G. et al.: Endothelin-mediated remodeling in aortas of diabetic rats. Diabetes Metab. Res. Rev., 21, 367, 2005.
2. Horiuchi K. et al.: Histopathological studies of aortic dissection in streptozotocin-induced diabetic APA hamsters. Exp. Anim., 54, 363, 2005.
3. Horiuchi K. et al.: The effect of probucol on atherosclerosis in streptozotocin-induced diabetic-hyperlipidemic APA hamsters in different stages of atherosclerosis. Exp. Anim., 51, 457, 2002.
4. Lassila M. et al.: Imatinib attenuates diabetes-associated atherosclerosis. Arterioscler. Thromb. Vasc. Biol., 24, 935, 2004.
5. Mastej K., Adamiec R.: Role of polymorphonuclear leukocytes in development of vascular complications in diabetes. Pol. Merkuriusz Lek., 20, 36, 2006.
6. Naito Z. et al.: Localization of extracellular matrix and mitogen-activated protein kinase (MAPK) in aorta of streptozotocin treated Mongolian gerbils. J. Nippon Med. Sch., 68, 37, 2001.
7. Nishigaki R. et al.: Ultrastructural changes and immunohistochemical localization of nitric oxide synthase, advanced glycation end products and NF-kappa B in aorta of streptozotocin-treated Mongolian gerbils. Nippon Ika. Daigaku. Zasshi., 66, 166, 1999.
8. Rasmussen L. M., Ledet T.: Osteoprotegerin and diabetic macroangiopathy. Horm. Metab. Res., 37, 90, 2005.
9. Santilli F. et al.: The role of nitric oxide in the development of diabetic angiopathy. Horm. Metab. Res., 36, 319, 2004.
10. Shahab A.: Why does diabetes mellitus increase the risk of cardiovascular disease? Acta Med. Indones., 38, 33, 2006.
11. Skrha J.: Pathogenesis of angiopathy in diabetes. Acta Diabetol., 40, 324, 2003.
12. Yamanouchi J. et al.: Aortic atheromatous lesions development in APA hamsters with streptozotocin induced diabetes: a new animal model for diabetic atherosclerosis. 1. Histopathological studies. Exp. Anim., 49, 259, 2000.

SUMMARY

Vasculopaties are very common complications of diabetes. The aim of this study was to evaluate the morphology of the aorta in alloxan-induced diabetes in rabbits. The animals were sacrificed at

the end of week 6 and 12 of the experiment. Gross and microscopic evaluation of different segments of aorta was performed. Fatty streaks located mostly in the aortic arch were found in the majority of animals from experimental groups. In one rabbit true succular aneurysm in the abdominal part of the aorta was also found.

Morfologia aorty u królików z cukrzycą doświadczalną

Zmiany naczyniowe są bardzo częstymi powikłaniami cukrzycy. Celem pracy była ocena morfologii aorty u królików z cukrzycą wywołaną przez podanie alloksanu. Zwierzęta uśmiercano w szóstym i dwunastym tygodniu doświadczenia. U większości zwierząt z cukrzycą obserwowano ogniska stłuszczenia w błonie wewnętrznej, zlokalizowane głównie w łuku aorty. U jednego królika w aorcie brzusznej obecny był także tętniak workowaty.