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The effect of impaired lipid metabolism on the smooth muscle cells of rabbits

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Summary. Our clinical data enabled us to demonstrate a correlation between impaired lipid metabolism and vasculogenic impotent men. Our aim was to evaluate the effect of an impaired lipid metabolism on the smooth muscle of the corpus cavernosum. A total of 16 rabbits were given a cholesterol-enriched diet for 3 months, and 8 of these received additional thromboxane A_2 receptor antagonist; 10 other rabbits (control) were fed a normal diet. Subsequently, cavernous tissue biopsies were taken, and tissue lipid extractions and electron microscopic evaluation were made from 3 rabbits in each group. In the untreated high-cholesterol diet group, cholesterol levels reached approx. 2.1 µg/mg body weight compared with 1.07 µg/mg b.wt. in the thromboxane A_2 receptor antagonist-treated group and elevated levels compared with control group. Similar results were found for the triglyceride and free fatty acid levels. Lecithin tissue levels in treated rabbits were distinctly elevated against those of other 2 groups. Ultramorphological examination of the control group disclosed normal smooth muscle cell (SMC) architecture with numerous sites of intercellular contacts. These findings contrasted with those of the high-cholesterol diet groups which showed significant SMC degeneration with loss of intercellular contacts. Our data imply that impaired lipid metabolism causes cavernous SMC degeneration which plays a major role in the pathogenesis of erectile dysfunction. The thromboxane A_2 receptor antagonist seems to produce a protective metabolic effect on the erectile tissue which may have some consequences future treatment strategies.

Key words: Impotence – Cholesterol – Cavernous smooth muscle cells – Thromboxane A_2 receptor antagonist

The incidence of organic impotent men has been determined to be between 50% and 80% [10, 15, 16]. However, very little is known about the pathogenesis which leads to organic failure. In 1988, Persson-Jünemann and colleagues [12] reported on ultrastructural morphological alterations of the cavernous smooth muscle in vasculogenic impotent men. Smooth muscle cell (SMC) degeneration with a consecutive increase of fibroconnective tissue as well as the loss of cell membrane contacts was the most pronounced finding in human penile biopsies. These results led us to investigate the pathogenetic factors favoring the development of penile ultrastructural alteration. For this reason, we examined the risk factors of erectile dysfunction [8]. Four major erectile risk factors have to be taken into account: smoking, diabetes, lipid disorders, and hypertension. It has already been shown that smoking inhibits the erectile mechanism [7], and hypertension is a well-known general risk factor of arterial circulary disturbances. Diabetes, as well as hyperlipemia, must be considered in the detection of any metabolic disturbances which may lead to penile ultrastructural changes. Erectile failure in diabetics is age-dependent and can be diagnosed as 25% in younger men (30–34 years) and up to 75% in older men (60–64 years) [14]. Furthermore, as reported by Persson and colleagues [11], diabetics displayed the most severe intracavernous SMC alterations.

There is very little knowledge on the incidence of impaired lipid metabolism in impotent men and its possible pathogenic role. The incidence of hyperlipidemia and erectile dysfunction varies between 40% and 50% [3, 6]. Recently, we reported on prospective data from 65 patients undergoing our routine impotence work-up, including an additional analysis of the lipid metabolism. Cholesterol levels in patients with or without vascular erectile disturbance were similar and within the normal range. Further analysis of the low-density lipoprotein (LDL) and high-density lipoprotein (HDL) fractions revealed, however, that patients with a vascular pathology showed distinct pathological changes in the LDL and HDL fraction, whereas in the nonvascular group, these

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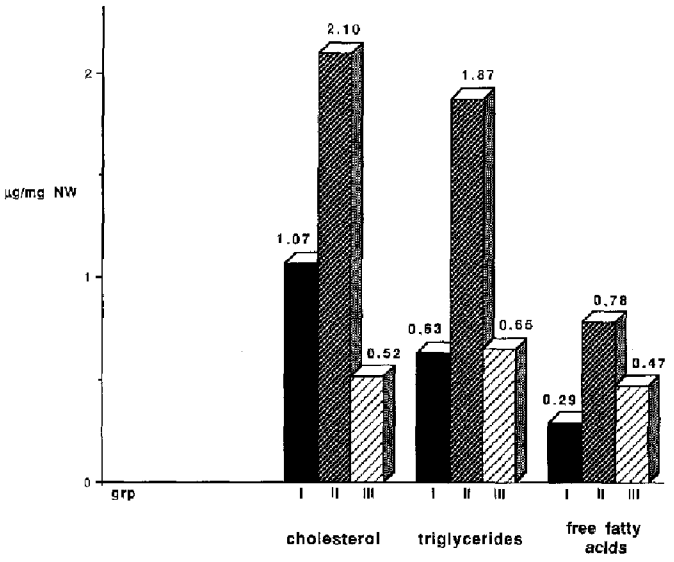

Fig. 1. Cavernous tissue lipid extractions in rabbits. Group I = cholesterol-enriched diet plus thromboxane A_2 receptor antagonist treatment; group II = high-cholesterol diet plus drinking water only; group III = control group. Preliminary results

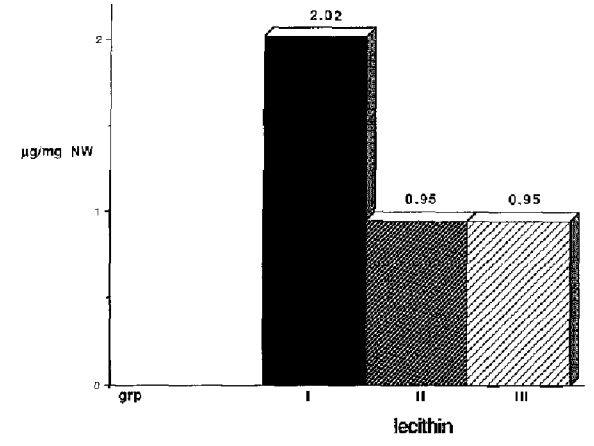

Fig. 2. Cavernous tissue lecithin levels in all three groups (vide supra). Preliminary results

values were normal [8]. These data implied a strong correlation between impaired lipid metabolism and vasculogenic erectile dysfunction. Our clinical results led to the investigation of the effect of a diet-induced hypercholesterolemia on the SMC of the corpus cavernosum in rabbits.

Materials and methods

Sixteen male white New Zealand rabbits (WNZ), weighing 1.5–2 kg, were placed on a standardized cholesterol-enriched diet for 96 days. Two groups were formed: Group 1 ($n = 8$) was additionally treated with a special thromboxane A_2 receptor antagonist at a dosage of 25 mg/kg body weight; group 2 ($n = 8$) received drinking water only. A third group ($n = 10$), serving as a control, was fed a standard diet (low cholesterol) without additives. The animals were sacrificed on completion of the diet, the cavernous tissue of the penis dissected, and tissue lipid extractions and electronmicroscopic evaluation made.

Lipid extractions

Small amounts (approx. 100 mg) of corpora cavernosa tissue were homogenized and extracted three times with chloroform/methanol in accordance with Bligh and Dyer [13]. The lipids (cholesterol, triglycerides, free fatty acids, and lecithin) were measured in an isopropanolic phase by commercially available enzymic methods.

Electron microscopy

Preparation of the biopsies from the corpora cavernosa was similar to previous reports described elsewhere [11]. The ultrastructural examination was performed with a transmission electron microscope, and the ultramorphological findings were correlated with the tissue lipid extraction data.

Results

Up to now, 3 animals in each group have been assessed, and we consider these initial results a pilot study providing a basis for future research.

Lipid extraction studies

Four different lipid extraction parameters of the corporeal tissue were analyzed: cholesterol, triglycerides, free fatty acids, and lecithin. Tissue extracts of the three groups displayed marked differences in the various lipid parameters. In the non-treated cholesterol-enriched diet group (2), tissue cholesterol levels of approx. 2.1 µg/mg b.wt. were measured (Fig. 1). In group 1, rabbits additionally treated with thromboxane A_2 receptor antagonist showed clearly 50% lower cholesterol levels in the cavernous SMC tissue compared with the untreated group but elevated levels in comparison with the control group (3). With respect to the triglyceride levels, a marked increase was found in the cholesterol diet group in comparison with the other two, although no marked deviation was noted between normal controls (0.65) and drug-treated animals (0.63 µg/mg b.wt.). In the free fatty acid analysis, the tissue level deviations of each group were comparable with those of the triglycerides one. On examination of the lecithin levels, the thromboxane antagonist-treated rabbits showed a distinct elevation of tissue levels in comparison with the untreated and control group (2.02 vs. 0.95 µg/mg b.wt.); the levels of the latter two groups were assessed with the same values (Fig. 2).

Electron microscopy studies

Similar to microscopy, semithick cross sections of the rabbits penile segments revealed numerous fat bodies located next to or within the cavernous SMCs (Fig. 3a) in the two groups fed on the cholesterol-enriched diet. However, in the drug-treated animal group, the amount of fat incorporation was less, and the smooth muscle architecture appeared more compact (Fig. 3b). The control group displayed normal tissue configuration with no sign

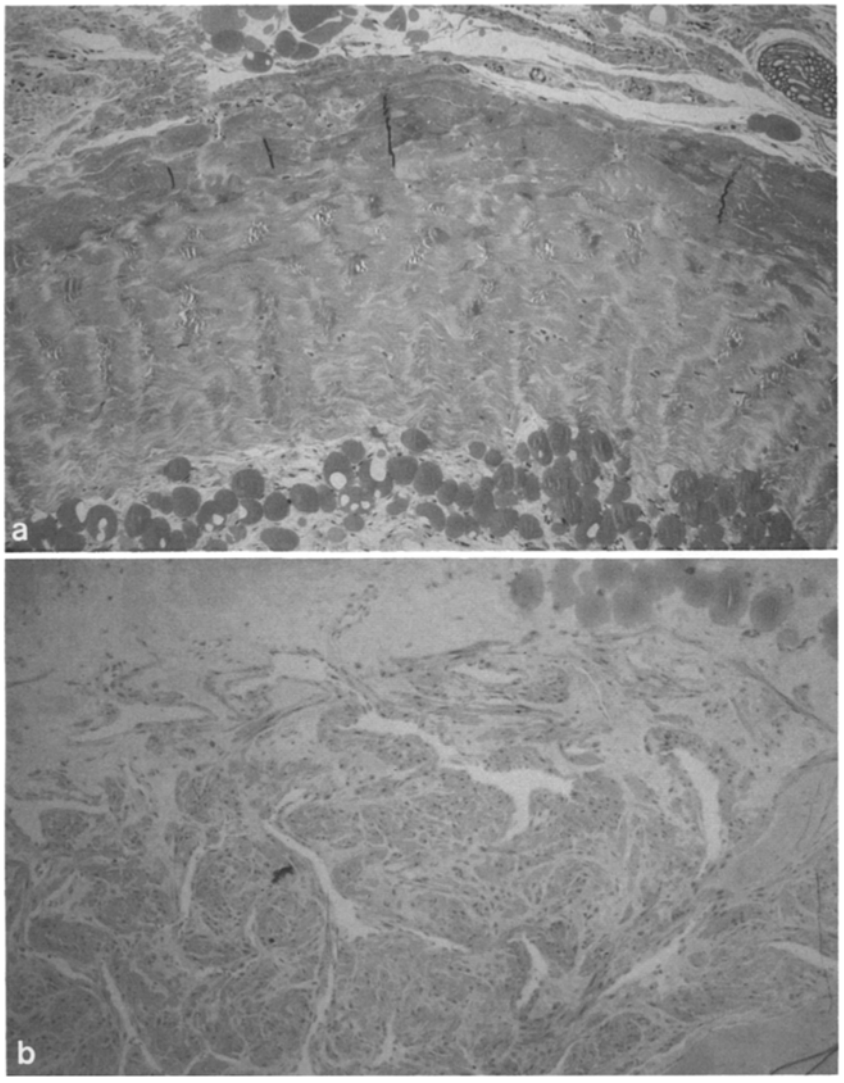

Fig. 3a, b. Semi-thick cross-sections of rabbit's penile segments in both cholesterol-enriched diet groups. a In the drinking-water group, numerous fat were found next to or within the corporeal smooth muscle cell. Cross-section shows area immediately beneath the tunica albuginea. b Animals additionally treated with thromboxane A_2 receptor antagonist showed less frequent fat incorporation, and the smooth muscle architecture appeared more compact

of fat incorporation. The electron microscopy (TEM) studies disclosed more distinct alterations at the ultrastructural level. In the control group, a normal trabecular ultrastructure of the cavernous tissue was found in all specimens investigated. Bundles of intimately related SMCs with densely packed myofilaments were embedded in strands of fibroconnective tissue (Fig. 4a). The round-shaped cell nucleus with a homogenous chromatin network was surrounded by a double membrane. A distinct base membrane, surrounding the SMC exhibited numerous sites of intracellular contacts of adjacent cells. No fat cells or cell debris were found inbetween the SMCs. The ultrastructural findings were similar in both hypercholesterol diet groups. The SMC replacement by dense connective tissue separated individual cells, and an irregular SMC contour with fragmentation and loss of basal lamina resulted in a consequent decrease of cell membrane contacts (Fig. 4b). Intercellular alteration of the SMC included displacement and reduction of the contractile myofilaments to the periphery and pleiomorphic nuclei with unevenly distributed chromatin. Fat bodies were encountered lying within the SMC in close proximity to the nucleus (Fig. 4c).

Discussion

The effect of hypercholesterol or hyperlipemia on the SMC of the cavernous tissue has not yet been investigated. Therefore, we acquired an appropriate animal model: diet-induced hypercholesterolemia in rabbits showing atherosclerotic plaque formation, similar to that in humans [1]. Surprisingly, the ultramorphological changes observed in the cholesterol-enriched diet groups in rabbits were similar to previous results of Persson and colleagues [11] reporting on vasculogenic impotent men. SMCs had been replaced by dense connective tissue separating individual cells. Furthermore, the SMC contour had become irregular with fragmentation or loss of basal lamina. Both structural transformations resulted in a loss of intracellular contacts, thus interfering with the intracellular transmission of excitation [4, 2]. As former studies have shown, the SMC represents a structural basis for sinusoidal relaxation, a key factor in penile erection [5, 7, 9]. Loss or degeneration of cavernous SMCs results in an impaired sinusoidal relaxation with consequent erectile failure. Although no marked morphological deviations were observed between the treated and untreated hyper-

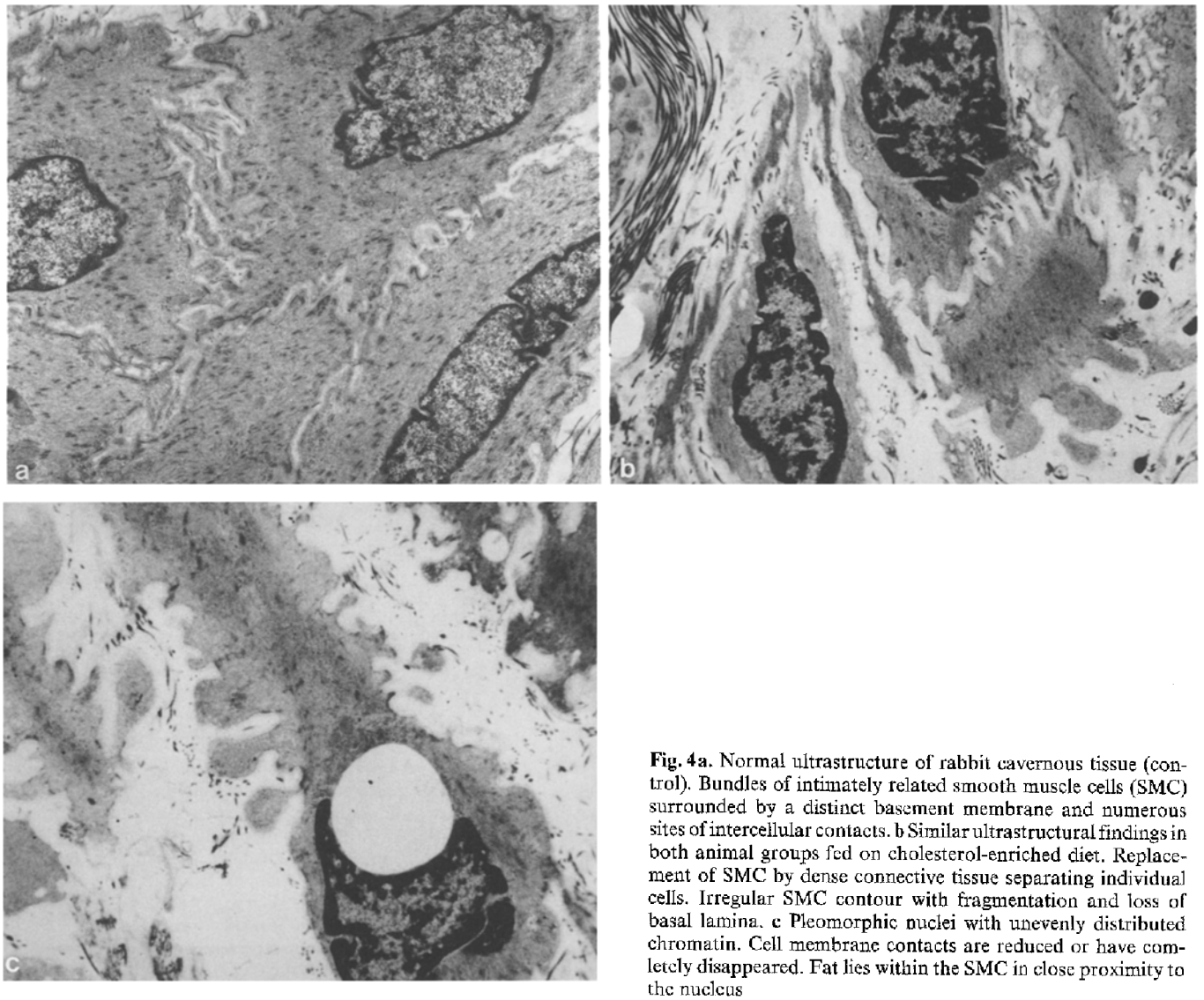

Fig. 4a. Normal ultrastructure of rabbit cavernous tissue (control). Bundles of intimately related smooth muscle cells (SMC) surrounded by a distinct basement membrane and numerous sites of intercellular contacts. b Similar ultrastructural findings in both animal groups fed on cholesterol-enriched diet. Replacement of SMC by dense connective tissue separating individual cells. Irregular SMC contour with fragmentation and loss of basal lamina. c Pleomorphic nuclei with unevenly distributed chromatin. Cell membrane contacts are reduced or have completely disappeared. Fat lies within the SMC in close proximity to the nucleus

cholesterol groups under TEM, the semithick cross-sections revealed a much lower incorporation of fat into the SMCs of the cavernous smooth muscle in the thromboxane antagonist-treated animals.

The lipid tissue extraction studies were of major interest. As far as the lipid metabolism in the erectile tissue was concerned, cholesterol, triglycerides, and free fatty acid levels were distinctly elevated in the cholestrol-enriched diet group in comparison with the control and thromboxane antagonist-treated groups. The thromboxane A_2 antagonist imparts a protective effect on the metabolism of SMCs in the corpora cavernosa. A thromboxane antagonist-related lecithin increase in tissue could provide an explanation for the protective mechanism of the drug. However, this process remains unknown. The lecithin levels of the control and high-cholesterol groups were half that of the treated group. These data imply an active protection mechanism of the antagonist. Whether this effect is due to an altered cellular cholesterol metabolism or inhibition of the platelet function remains open to discussion. Although the case numbers are very small at the moment, we conclude from this pilot study that hyperchlesterolemia in rabbits induces ultramorphological alterations similar to those found in vasculogenic impotent men and results in pathologically elevated cholesterol and triglyceride levels in rabbit cavernous tissue. The thromboxane receptor antagonist treatment prevents rises in tissue cholesterol and triglycerides but elevates lecithin tissue levels with marked reduction of fat incorporation in the erectile tissue. This phenomenon may have some consequences on future treatment strategies in impotent men. It may be assumed that impaired lipid metabolism plays a major role in vascular/cavernogenic erectile dysfunction.

References

1. Anitschkow N, Chalatow S (1913) On experimental cholesteatosis and its influence on the formation of some pathologic processes (in German). Zentralbl Allg Pathol 24:1
2. Bergmann RA (1956) Proceedings of First European Regional Conference on Electron Microscopy, Stockholm

3. Bouilly P, Virag R (1984) Results of a comparative study of the impotence incidence on the occasion of a medical examination and the incidence of arterial risk factors in a series of impotent subjects. In: Virag R, Virag-Lappas H (eds) Les Editions du CERI: Proceedings of the First World Meeting on Impotence, Paris, June 1984, p 23
4. Caesar R, Edwards GA, Ruska H (1957) Architecture and nerve supply of mammalian smooth muscle tissue. J Biophys Biochem Cytol 3:867
5. Fournier GR Jr, Jünemann KP, Lue TF, Tanagho EA (1987) Mechanisms of venous occlusion during canine penile erection: an anatomic demonstration. J Urol 137:163
6. Hebrang A, Vrhovski D, Vidakovic Z, Subic N (1986) The correlation of angiographic findings and atherosclerotic risk factors in vascular impotence. Second World Meeting on Impotence, Prague, June 17–20
7. Jünemann KP, Luo JA, Lue TF, Tanagho EA (1986) Further evidence of venous outflow restriction during erection. Bri J Urol 58:320
8. Jünemann KP, Persson-Jünemann C, Alken P (1990) Pathophysiology of erectile dysfunction. Semin Urol 8:80
9. Lue TF, Takamura T, Schmidt RA, Palubinskas AJ, Tanagho EA (1983) Hemodynamics of erection in the monkey. J Urol 130:1237
10. Montague DK, James RE Jr, de Wofe V (1979) Diagnostic evaluation, classification and treatment of men with sexual dysfunction. Urology 14:545
11. Persson C, Diederichs W, Lue TF, Yen TS, Fishman IJ, McLin PH, Tanagho EA (1989) Correlation of altered penile ultrastructure with clinical arterial evaluation. J Urol 142:1462
12. Persson-Jünemann C, Diedrichs W, Lue TF (1988) Correlation of impaired penile ultrastructure to quantified clinical impotence. In: Goldstein I, Krane RJ, Padma-Nathan (eds) Proceedings of the Sixth Biennial International Symposium for Corpus Cavernosum Revascularization – Third Biennial World Meeting on Impotence, Boston, MA, Oct. 6–9
13. Bligh EG, Dyer WF (1959) Con J Biochem 37:911
14. Rubin A, Babbott D (1958) Impotence and diabetes mellitus. JAMA 168:498
15. Sarramon JP, Rischmann P, Elman B (1986) Within the last five years our experience in impotence vascular surgery. Second World Meeting on Impotence, Prague, June 17–20
16. Virag R (1986) The screening of impotence by the use of visual sexual stimulation after intracavernous injection of a small (8 mg) dose of papaverine. Second World Meeting on Impotence, Prague, June 17–20

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