Lipofundin Arteriosclerosis and Iloprost Treatment

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Introduction

It has been known since Anitschkow and Chalatow's [1] publication in 1913 that cholesterol feeding is an atherogenic factor [2, 13–15, 19, 22–24]. This observation was followed by the discovery of a number of factors similarly causing arteriosclerotic changes in the vessel wall. These factors are collectively designated as risk factors [3–16, 18, 20].

The feeding of a cholesterol-rich diet normally takes a longer period of time, i.e., up to 2 months. In our laboratory a method was developed to produce within 8 days a cellular, fiber-rich plaque observable by light and, particularly, by electron microscopy.

Methods

The experiments were performed on rats by giving them 2 doses per day of 1 ml/100 g body weight Lipofundin-S 20% (Braun, Melsungen). This was continued for 8 days. The animals received their normal feed and water ad libitum. Lipofundin is a stabilized soy bean oil extract used for parenteral feeding [17].

The animals were killed on the 8th day for light and electron microscopy studies. The method of perfusion fixation for electron microscopy has been published elsewhere [17]. In the light microscope small intimal cushions covered by endothelium and consisting of cells of different types were observed. In the intimal plaque above the internal elastic membrane light microscopy revealed the formation of new elastic fibers under the endothelium.

Electron microscopy studies of these cellular plaques showed the appearance of single lipid droplets in the endothelium and/or the smooth muscle cells in the early phase of treatment. Increased transmural permeability was demonstrable by colloidal iron tracer (Nattermann, Köln). In animals killed on the 8th day practically no liped droplets were observed; instead we observed mostly cellular-fibrous plaques, which were covered by intact endothelium. In the initial phase, accumulation of basal membrane-like material was observed under the endothelium, with occasional cross sections of smooth muscle cell processes passing through the elastic fibers. Smooth muscle cells became mobile and, after diluting the elastic fibers, they migrated into the subendothelial space. Here they produced several layers of new basal membrane, thus enlarging the subendothelial space. Later a fibrillar structure appeared in the basal membrane-like matter, which after further organization and elastin-deposition developed into elastic granules and ultimately into elastic fibers, forming a new internal elastic lamina. The formation of collagen fibers was observed simultaneously [17].
This procedure was also used to study the effects of Iloprost (Schering AG, Berlin) treatment. Iloprost (ZK 36374) was administered to rats by means of an Alzet minipump (type 2002) implanted subcutaneously. The dosage rate was 0.5 μg kg⁻¹ min⁻¹ for 8 days. The experimental design included four groups of eight animals each.

Group I received the solvent Iloprost (0.9% saline + 15.8 mg·ml⁻¹ Tris buffer). Group II was given Iloprost. Group III received two daily doses each of 1 ml/100 g body weight Lipofundin. Group IV was given both Iloprost and Lipofundin simultaneously.

Results and Discussion

After 8 days of treatment the animals were killed by overdosage of a narcotic and their tissues were fixed with perfusion [17]. From the specimens removed semithin and ultrathin sections were prepared after embedding.

Fig. 1. Control rat. An endothelial cell (E) is shown facing the lumen (L) and containing a nucleus (N), pinocytic vacuoles (P) and mitochondria (M). The cell is in direct contact with the internal elastic lamina (IEL). Below IEL medial smooth muscle cells (SMC) and collagen fibers (Co) are observed.