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Short communication

Effects of benfluorex–vitamin C supplementation on cutaneous capillaries of diabetic rats

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Abstract

The ultrastructural changes on capillaries of the dermis in diabetic and benfluorex–vitamin C treated diabetic rats have been investigated. Three groups of 21 Wistar albino rats were used in the examination: control, diabetes, and benfluorex–vitamin C treated diabetic rats. Diabetes was induced by injection of streptozotocin. The streptozotocin-induced group was treated for 21 days with vitamin C and benfluorex, of which antidiabetic and antihyperlipidemic effects were experimentally proved. Samples taken from the skin of rats' legs were examined under transmission electron microscopy. Swollen endothelial cells, narrowed capillary lumens, a thickened basement membrane, and fusion of mitochondrial cristae in the capillaries of diabetic rat dermis were seen. In the benfluorex–vitamin C treated group, contrary to the diabetic group, neither signs of degeneration in endothelial cells nor a significant difference with the control group with regard to capillary structure were observed. Amelioration in capillaries appears to be due to benfluorex and vitamin C treatment in diabetes.

Keywords: Diabetes; Benfluorex; Vitamin C; Skin; Ultrastructure; Capillary; Rat

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by increased blood glucose levels and abnormalities in the metabolism of carbohydrates, proteins and lipids (Conget, 2002). *Diabetes mellitus*, one of the most common diseases in the world (Wild et al., 2004), may cause morbidity and mortality. *Diabetes mellitus* is not only a serious health problem negatively affecting the daily life of diabetic people, but it also has high economic costs for both governments and the affected patients (Eiselein et al., 2004).

Diabetes mellitus affects various organs and systems of the human body. Complications are especially common in capillaries, skin, eyes, kidneys and the nervous system (Mahajan et al., 2003). Cutaneous manifestations of diabetic complications that may cause various cutaneous lesions (Chishiki et al., 1998) are microangiopathy and macroangiopathy (Romano et al., 1998).

Oxidative damage increases in *Diabetes mellitus* due to the shortages of antioxidative enzymes and vitamins such as vitamin C. Reactive oxygen species produced by the impact of hyperglycemia play an important role in emergence of most complications of diabetes (Ceriello et al., 1998; West, 2000). One way to decrease possible oxidative damage is to increase antioxidant capacity of the human body. Studies show that dietary vitamin C increases global antioxidant capacity of the body (Barja et al., 1994). Therefore, additional dietary vitamin C may prevent development of diabetic complications in patients (McAuliffe et al., 1998).

Antihyperglycemic and hypolipidemic effects of benfluorex (1-(3-trifluoromethylphenyl)-2[*N*(2-benzyloxy-ethyl)amino] propane) which is a hydroxyalkyl β-phenylisopropylamine ester have been explored in diabetic human and various animal models (Ravel and Laudignon, 1996; Kohl et al., 2002). Benfluorex can both diminish hepatic gluconeogenesis

Abbreviations: STZ, streptozotocin; i.g., intragastric.

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and increase glucose utilization in liver and skeletal muscle. Benfluorex inhibits hepatic synthesis of free fatty acids, triglycerides, and sterols (Kohl et al., 2002), and the activity of membrane-bound enzymes involved with lipid metabolism (Ravel and Laudignon, 1996).

2. Material and method

2.1. Ethical approval

Institutional ethical approval for this experiment was granted by the Animal Experimentation Ethics Committee of Gazi University, Ankara.

2.2. Animals and experimental protocol

Twenty-one male adult Wistar albino rats with an averaging 200 ± 20 g were obtained from Refik Saydam Hifzissihha Institute of Ankara. They were housed individually in plastic cages at 24 ± 2 °C with 12 h light–12 h dark cycle, and fed with standard laboratory chow and tap water ad libitum.

Rats were randomly divided into 3 groups each of 7 rats. Control rats received only an injection of citrate buffer (1 ml). Diabetes was induced by intraperitoneal injection of streptozotocin (STZ) (45 mg/kg body weight) in 0.1 M, 1 ml citrate buffer at pH 4.5 after an 18 h fast (Bor et al., 2000; Heidari et al., 2003; Mythili et al., 2004). Rats were considered diabetic if their fasting blood glucose levels exceeded 200 mg/dl at 48 h after STZ injection. Intragastric (i.g.) tap water was given to the rats of the control and diabetic groups for 21 days. The other STZ-induced rat group was treated with benfluorex (50 mg/kg, i.g.) (Brindley et al., 1988; Serradas et al., 1993) and vitamin C (20 mg/kg, i.g.) for 21 days (McLennan et al., 1988).

2.3. Electron microscopy

Animals were anesthetized and the leg skin was rapidly excised. Small pieces of skin tissue were fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH: 7.4) for 2 h, and washed in phosphate buffer. Tissues were post-fixed in 1% osmium tetroxide in phosphate buffer for 2 h, washed in the phosphate buffer, dehydrated in serial concentrations of ethanol and finally, embedded in araldite. The araldite blocks were cut with an ultramicrotome (OM U2, Reichert, Hamburg, Germany) into thin sections. These sections (600 nm) were mounted on copper grids (200 mesh) and stained with uranyl acetate and lead citrate for examination under a transmission electron microscope (JEOL 100CXII).

3. Results

Skin samples of control, diabetes and benfluorex–vitamin C treated diabetes groups were examined by transmission electron microscopy. The results for each are as follows.

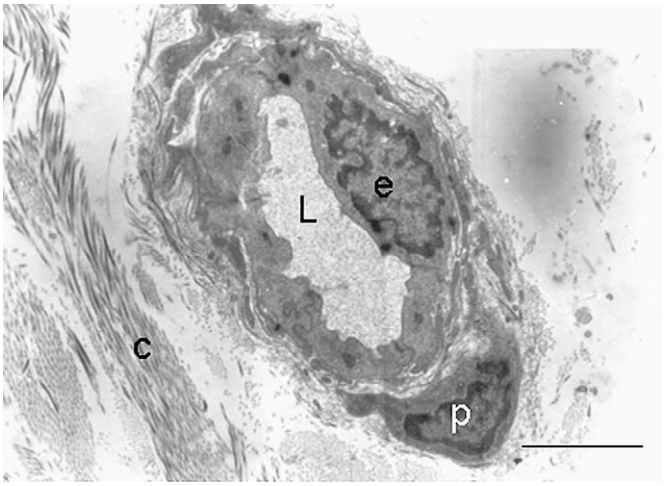

Fig. 1. Pericyte (p), capillary lumen (L), endothelial cell (e), and collagen fibrils (c) in dermis of control group. Bar = 2.8 μm.

3.1. Control group

Capillaries were located between the collagen fibrils in the dermis (Fig. 1). Capillary lumens were clear and surrounded by endothelial cells. The structure of endothelial cells in this group was normal, with flat nuclei and few organelles. Pericytes were located by the endothelial cells. No anomalies were observed in smooth muscle cells or the thin basement membrane (Fig. 2).

3.2. Diabetic group

Various changes in capillaries were detected in the diabetic group compared to the control group. Electron microscopic examinations revealed narrowed capillary lumens due to swollen endothelial cells and (Figs. 3 and 4, see also Fig. 6) a thickened basement membrane resulting from amorphous material deposits (Fig. 5). A reduction in the density of

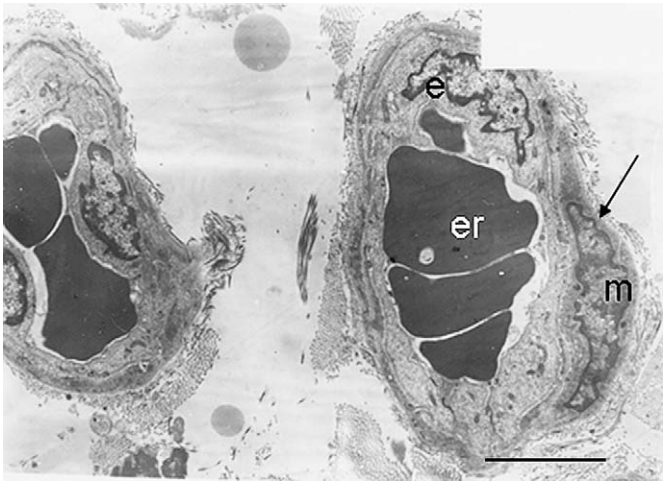

Fig. 2. Erythrocyte (er) within the lumen, endothelial cell (e), basement membrane (arrow), and smooth muscle cell (m) in capillary of control group. Bar = 3.7 μm.