MYOCARDIAL CAPILLARY NET AND BLOOD CONSTITUENTS IN STREPTOZOTOCIN (STZ)-INDUCED DIABETIC RATS

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Abstract: Type 1 diabetes was induced in Wistar rats by injection of streptozotocin (STZ). Changes in the myocardial capillary network were examined using the double-staining enzymatic method for alkaline phosphatase (AP) and dipeptidylpeptidase IV (DPPIV). This method allows the identification of the arteriolar (AP-containing) and the venular (DPPIV-containing) portions of the capillary network. In addition, blood plasma was analysed. The AP- and AP/DPPIV-containing capillary portions increased significantly, accompanied by a decrease in the DPPIV-containing portions in 60 days. A significant increase in AP was observed in the plasma. The capillary domain areas of each capillary portion were larger in the STZ-injected group than in the controls. It appears that oxygen transport to the subendocardial myocardial tissues may be decreased in the STZ group. In rats fed with Saji-supplemented chow there was a decrease in plasma AP, with increases in hemoglobin, hematocrit and vitamin C, suggesting a partial improvement of metabolic function and oxygen supply in these diabetic Wistar rats.

1. INTRODUCTION

Cardiac insufficiency is one of the main complications in diabetes. Several causes for the development of insufficiency have been postulated in relation to microvessels. These include excessive deposition of collagen surrounding capillaries, increased activity of the plasminogen activator inhibitor, PAI-1 (reported in the OLETF rat model of non-insulin dependent diabetes mellitus) and a decrease in angiogenic factors as found in diabetic patients. It also seems probable that hyperglycemia may affect the expression of several enzymes.

A significant increase in alkaline phosphatase (AP)-containing capillaries has been observed in the study on OLETF rats. However, the converse result, that is a decrease in AP despite an overall increase in capillaries, has been reported in the hearts of STZ-diabetic rats. Different types of diabetes in different strains of rat may cause different...
modifications to the capillary networks and enzyme expression. In the present experiments we have studied changes in myocardial capillaries in the STZ-induced diabetic Wistar rats.

It has been established by a number of different techniques that the capillaries in the subendocardium that are stained blue, red, and violet by the double-staining method represent, respectively, alkaline phosphatase-containing arteriolar portions, venular portions containing dipeptidylpeptidase IV (DPPIV) and intermediate portions containing both enzymes. In the present studies, using this method, particular attention has been paid to the capillaries expressing AP. It is known that expression of proliferating cell nuclear antibody is low in AP-sensitive capillary portions, suggesting AP acts to suppress endothelial cell proliferation, resulting in the arterialization and calcification of blood vessels.

In addition AP in the plasma was studied since it is multi-functional and widely distributed in the whole body. Blood plasma was also analysed for some other factors. Further, in an attempt to find a herbal medicine that might be active against diabetes, the effects of supplementing rat chow with Saji (Hippophae rhamnoides) were studied. Preliminary results will be presented.

2. METHODS

The present study conformed with the guidelines of Hokkaido University for the care and use of laboratory animals.

STZ was dissolved in a 0.1M citrate buffer solution (pH 4.3) (100mg/ml). Six 7-week-old male Wistar rats were injected with STZ (50mg/kg) in the tail vein; five controls received 0.5 ml citrate buffer. Sixty days after the injections, rats were anesthetized with i.p. Nembutal (50mg/kg) and blood samples from the abdominal vein collected in heparinized cylinders for analysis (see below).

Hearts were removed, divided horizontally, placed in O.C.T. compound® and quickly frozen in liquid nitrogen. Frozen cross-sections of the left ventricle were sectioned at 16 µm with a cryotome and stained for AP and DPPIV using the double-staining method with a slight change in timing. In STZ-diabetic tissues the normal 25 min reaction time produced intense blue coloration throughout the whole section; the staining time was therefore reduced to 5 minutes, after which sections were rinsed several times in water for visualization and counting of AP- or DPPIV-containing capillaries. The number of each type of capillary portion was counted in four visual fields in each of four sections of the subendocardium from each heart. The polygonal capillary domain area (CDA) which represents the tissue area perfused by each capillary was calculated by the method proposed by Hoofd et al. About 100 to 150 capillaries were present in a visual field viewed at x400, hence the four visual fields counted in each of the four sections from each of the five rats in a group yielded 3000 to 4000 values for statistical analysis of CDAs. The proportion of the three of capillary types, and the CDAs for each, were statistically analysed by the Mann-Whitney test (means ±S.D. p<0.05)

The blood plasma was analysed by routine methods: AP by the JSCC standard method, glucose, total cholesterol (TC) and triglyceride (TG) by enzymatic methods; vitamin C (VC) by HPLC, and hemoglobin (Hb) and hematocrit (HCT) by an automated hemoanalyzer. The values for the control and treated groups were compared by Student’s unpaired t-test (means ±S.D. p<0.05).

In a preliminary study on the effects of the traditional herbal medicine, powdered Saji fruit was obtained from North Eastern China. It was added to dry rat chow at a ratio