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CATHEPSIN D INHIBITOR FROM POTATO REVERSES INHIBITION OF COLLAGEN BIOSYNTHESIS IN WOUNDED SKIN OF RATS WITH EXPERIMENTAL DIABETES

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It was found that streptozotocin-induced diabetes is accompanied by increased proteolytic activity and decreased collagen biosynthesis in rat skin wounds. External application of cathepsin D inhibitor from potatoes normalized the proteolytic activity and restored collagen biosynthesis in wounded skin of these animals.

The content of collagen in animal tissues is a resultant of two processes: collagen biosynthesis and its degradation [1]. In rats with streptozotocin-induced diabetes the collagen content in skin is decreased [2]. Also, decreased biosynthesis and accelerated degradation of newly synthesized collagen have been observed [3]. These two effects of insulin deprivation disturb wound healing processes in diabetic subjects.

Previously, we have found that increased proteolytic activity (mainly of cathepsin D) was accompanied by decreased collagen biosynthesis in wounded skin of diabetic rats. The proteolytic activity was increased also in liver and serum of those animals. Furthermore, pepstatin, an inhibitor of cathepsin D, reversed the decline in collagen biosynthesis in the skin of diabetic rats [2].

In the present studies we evaluated *in vivo* the effect of cathepsin D inhibitor from potato on collagen biosynthesis in wounded skin of diabetic rats.

MATERIAL AND METHODS

The experiments were performed on male Wistar rats (body weight 180-200 g). Animals were kept at room temperature, fed with standard diet and water *ad libitum*. Diabetes was induced with streptozotocin according to Phillips & Young [4]. Rats were divided into two groups of 12 rats each. The animals of the first group were injected with 30 mg of streptozotocin dissolved in 0.4 ml of 0.1 mM citrate buffer pH 4.0, into the tail vein under pentobarbital anaesthesia. The control group received 0.4 ml of citrate buffer. Glucose level was determined every day with Haemo-Gluco-test 20-800 (Boehringer

Mannheim, Germany). On the fifth day the rats were shaved under pentobarbital anaesthesia and four 2 cm longitudinal incisions, 0.5 cm apart from each other, were made through the epidermis, dermis and subcutaneous tissue symmetrically on the back of animals in sterile conditions.

Sterile dressings containing the inhibitor tested (2 mg/cm^2) were put on the wounds of six animals from each group and after 72 h the animals were sacrificed, all area of injured skin together with subcutaneous tissue were excised. The tissue was homogenized with the use of Polytron homogenizer and centrifuged ($1000 g$) for 30 min at 4°C . Proteolytic activity was determined in supernatant by the method of Worowski & Roszkowska [5]. Collagen synthesis was evaluated according to Peterkofsky *et al.* [6].

Cathepsin D inhibitor was isolated from potato as described by Worowski & Mariak [7]. DNA was determined by the method of Burton [8].

RESULTS AND DISCUSSION

The specific cathepsin D inhibitor — pepstatin, at a concentration of $8 \mu\text{g}\cdot\text{ml}^{-1}$, totally inhibited proteolytic activity in homogenate of the skin of diabetic rats. The cathepsin D inhibitor from potato at the same concentration evoked a half of the pepstatin's potency (Fig. 1).

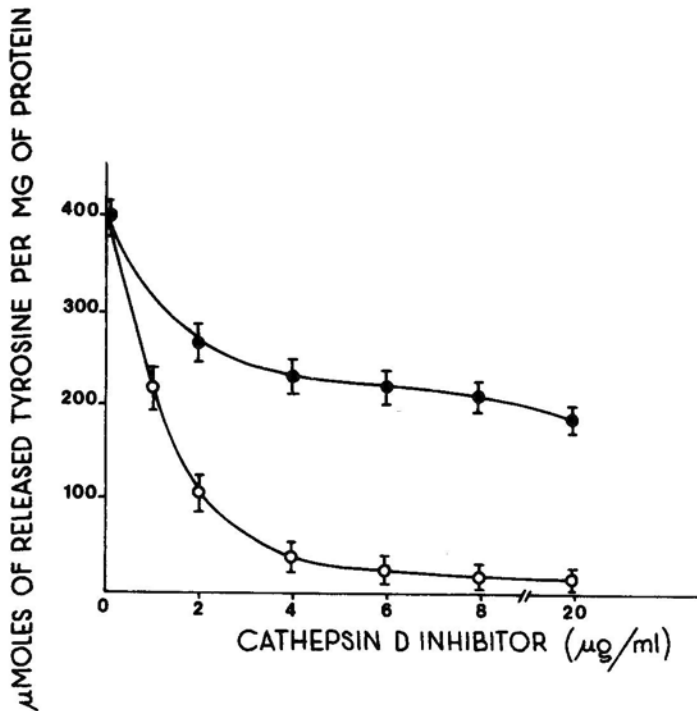


Fig. 1. The effect of cathepsin D inhibitors: pepstatin (○) and the inhibitor isolated from potato (●) on proteolytic activity of diabetic rat skin homogenate measured at pH 4.5. Mean values from 6 experiments \pm SD are presented

In the wounded skin of diabetic rats the proteolytic activity (mainly that of cathepsin D) was 2-3 times higher and collagen synthesis less than half that, in controls. On addition of the cathepsin D inhibitor the proteolytic activity in controls remained unchanged, whereas collagen synthesis was slightly decreased. On the other hand, in diabetic rats the inhibitor caused a decrease, almost by half, of the proteolytic activity with a simultaneous increase in collagen synthesis, almost reaching the control values (Fig. 2).

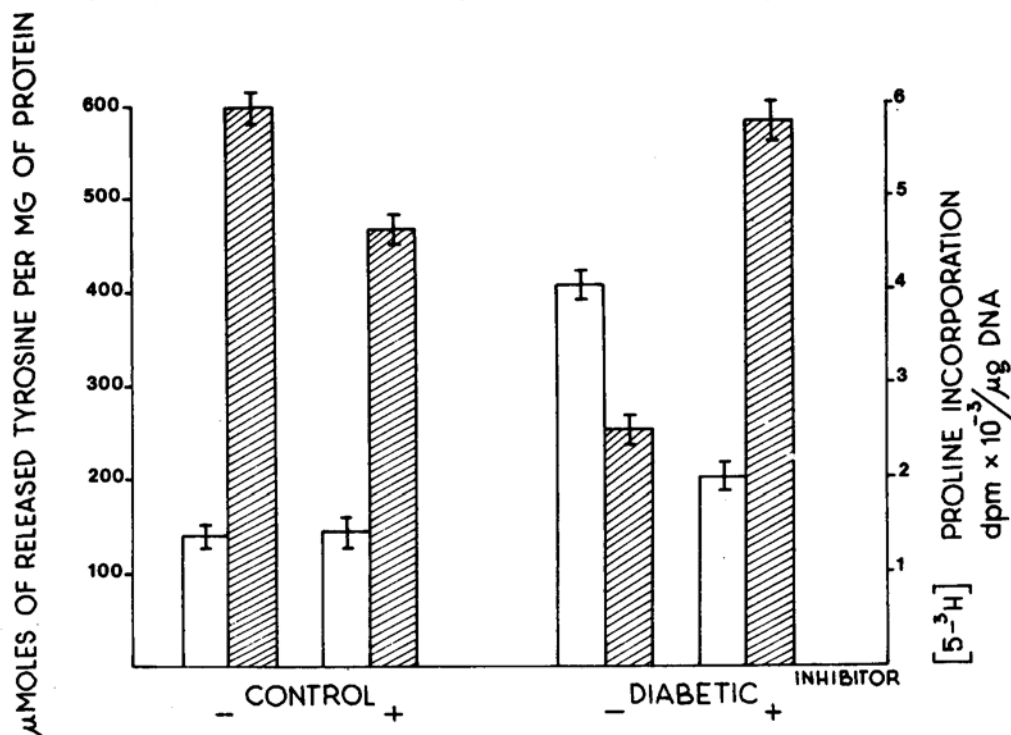


Fig. 2. The effect of cathepsin D inhibitor isolated from potato on proteolytic activity (open bars) and collagen synthesis (hatched bars) in the wounded skin of control and diabetic rats. Mean values from 6 experiments \pm SD are presented

As can be seen from the results, cathepsin D inhibitor from potato applied on the skin wounds of diabetic rats evoked a decrease of proteolytic activity accompanied by an increase of collagen biosynthesis in the injured tissue.

Insulin and insulin-like growth factor-I (IGF-I) stimulate collagen biosynthesis in fibroblasts [9, 10]. The plasma levels of both hormones are decreased in diabetic subjects [4, 11]. The mechanism of insulin deficiency in streptozotocin treated rats is commonly known and the reduced level of IGF-I is probably due to the increase in the content of plasma low molecular mass IGF-I binding proteins (LMMBPs) which inhibit the biological activity of this hormone [12]. As suggested previously [13, 14], it is possible that the increased proteolytic activity in tissue induces inhibitors of IGF-I (LMMBPs) as a result

of degradation of high molecular mass IGF-I binding proteins which are carriers of active IGF-I in normal plasma.

In addition to decreased collagen biosynthesis in diabetic animals, an inhibition of fibroblast's growth has been observed [4, 10]. The growth of many cells is promoted by insulin, IGF-I and IGF-II [15]. Their physiological action is mediated by specific interaction with cell surface receptors [16]. It was discovered recently that the IGF-II receptor binds both lysosomal enzymes and IGF-II [17, 18]. It seems possible that in this way the lysosomal enzymes may modulate the biological response of collagen producing cells to IGFs. Thus, the increased proteolytic activity of cathepsin D in the skin of diabetic rats may disturb both the growth of collagen producing cells and biosynthesis of this protein.

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