Bioscience Research Communications
Vol. 14, No. 2, April 30, 2002
Printed in Nigeria

0795-8072/2002 $12.00 + 0.00

BRC 2001047/14209

The effect of alloxan on the histochemistry of pancreatic enzymes of albino rats. I. The effect on acid phosphatase activity

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(Received March 30, 2001)

ABSTRACT: A study of the effect of alloxan on the histochemistry of pancreatic acid phosphatase activity was conducted using male albino rats, Wistar strain. The rats were randomly separated into four groups, A, B, C and D of thirty rats each. The first group was used as the primary control group, the second group (B), the third group (C) and the fourth group (D) served as the test groups. The test groups were administered with 200 mg per kg body weight of alloxan in order to induce diabetes. The second group (B) was used to study the direct effect of alloxan on the pancreatic acid phosphatase activity. Group C was used to study the effect of diabetes mellitus on the pancreatic acid phosphatase activity while group D was used to study the possible effect of therapeutic agent insulin on the restoration of pancreatic acid phosphatase activity.

Histochemical examination revealed that the activity of pancreatic acid phosphatase was reduced in diabetic rats. This enzyme, which is necessary for the functioning of the beta islet cells, lost its enzymatic activity as a result of the administration of a diabetogenic dose of alloxan. This suggests that alloxan has a selective degenerative effect on the beta islet cells with consequent loss of acid phosphatase.

Key Words: Acid phosphatase; Pancreatic enzymes; Histochemistry; Alloxan; Diabetes mellitus.

Introduction

The pancreas is composed of two different types of glandular tissues in intimate association with each other. The main mass is exocrine in which are embedded the pancreatic islets of Langerhans. Each islet is a mass of polyhedral cells pervaded by fenestrated capillaries and a rich autonomic innervation (Gerich and Lorenzi, 1978; Williams *et al*., 1995). The major cell types of the islets are distinguished into insulin producing beta (ß) cells, glucagons secreting alpha (α) cells, somatostatin producing delta (δ) cells and PP cells which produce pancreatic polypeptide (Williams *et al*., 1995).

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Environmental agents in the form of harmful substances or drugs may injure the β-cells causing necrosis of these cells. These injuries to the cells may be by direct action on the cell architecture, causing a disarray of organized and oriented macromolecules or by interference with enzymatic actions (Steiner and Freinkel, 1972; Bowman and Rand, 1985; Edwards *et al*., 1995).

Alloxan has been proved to be the most convenient means of producing selective destruction of the β-cells of the Islets of Langerhans in laboratory animals (Barnes and Eltherington, 1964). This results in raised blood glucose level as a result of hepatic overproduction of glucose by glycogenolysis and gluconeogenesis and a decrease in the removal of glucose from the circulation into adipose tissues and muscles (Lazarus and Volk, 1962; Granner, 1995; Haffner *et al*., 1996).

Acid phosphatase has been shown to be one of the three important enzymes participating in the metabolic activity of the Islet cells of the pancreas (Pears, 1972; Murray *et al*., 1996). They hydrolyse organic phosphate esters with optimal activity at pH 5 or less (Bancroft and Stevens, 1982; Onwuka, 1991).

The present study examines the effect of alloxan on the acid phosphatase activity in the pancreas.

Materials and Methods

Alloxan crystals contained in a 250g bottle was used for this work. The alloxan crystals weighing 1.2g were dissolved in 10 ml of injection water, amounting to 1.2 g/10ml. Then 0.3 ml of this solution which is equivalent to 40 mg of alloxan was administered as the diabetogenic dose as reported in the literature (Barnes and Eltherington, 1964; Barbato and Landau, 1977; Bowman and Rand, 1985; Greenspan and Baxter, 1994).

The test animals were 120 male albino rats (Wistar strain) which were randomly separated into four groups, A, B, C and D of thirty rats each. The first group (A) was used as the primary control group. Groups B, C and D were used as the test groups and were administered with 0.3 ml of the 1.2 mg/10ml solution of alloxan, equivalent to 200 mg per kg body weight which contained 40 mg of alloxan. This single diabetogenic dose was administered to the test animals through the intraperitoneal route (ip) while the control group received normal saline through the same route.

Group B animals were the first test group and were used to study the direct effect of alloxan on the pancreatic acid phosphatase activity. The second test group (C) consisted of animals that have developed diabetes mellitus and were used to study the effect of diabetes on acid phosphatase activity. Group C consisted of diabetic animals undergoing insulin therapy and were used to study the effect of the therapeutic agent in restoration of enzymatic activity.

Animals from each group were anaesthetized and their abdomen opened by midline incision and the pancreas from each of the groups were excised. The tissues were placed in ice-cold normal saline until excision of the tissues were completed. Some of the tissues were fixed using cold acetone while others were frozen overnight by the cryostat method. The tissues were sectioned at 20 µm using freezing microtome.

The method used in the demonstration, identification and localization of the pancreatic acid phosphatase activity was the Gomori's lead nitrate method as outlined by Onwuka (1991), Pears (1972) and by Bancroft and Stevens (1982).

Results

The result of the histochemical examination of the pancreas, show isolated pancreatic islets of Langerhans with normal distribution and activity of acid phosphatase enzyme as shown in Plate 1 while Plates 2 and 3 show decreasing activity and finally negative activity of acid phosphatase enzyme after alloxan administration and during insulin therapy.

Discussion

Acid phosptahase has been shown to be one of the three groups of pancreatic enzymes that have high enzymatic activities necessary for the B-cell function within the islets of Langerhans (Lazarus and Volks, 1962, Murray *et al*., 1996). The acids phosphatase show strong enzymatic activities in the intact islets of Langerhans but their activities become reduced and finally disappear in the test groups because of the administration of the diabetogenic dose of alloxan to the animals.

It has been shown that marked acid phosphatase activity is confined to ß-cells in the rat and rabbit which disappeared during islet B-cell degeneration (Duff and Starr, 1974). The significance of the phosphatases in the pancreas is not yet very clear, however, the distribution of acid phosphatase at secretory sites suggested that it plays a role in protein synthesis and that it could function in insulin synthesis as well as synthesis of zymogens (Steiner and Freinkel, 1972; Fitzgerald and Morrison, 1980). This enzyme has been shown to play a part in the formation of S-S containing proteins and peptides of which insulin is one (Pearse, 1972; White and Kahn, 1994). The ß-cells have been shown to contain an additional cytoplasmic SH-dependent adenosine-triphosphatase which could be inhibited by cyanide and which disappear during prolonged steroid administration (Murray *et al*., 1996). The loss of B-cell extremitochondrial ATPase activity after steroid administration suggest a relationship to the B-cell granules and possibly to insulin storage within the cells (Lazarus and Volk, 1962; Pipeleers, 1984).

Acid phosphatase enzyme has been shown to be a major lysosomal hydrolase which occur in many tissues (Onwuka, 1991; Pearse, 1972). de Duve (1970), has shown lysosomes to participate in the production of many pathological disorders. This is because alloxan may have been incorporated in the lysosomal membrane of the ß-cells rendering it more fragile. This may lead to the degeneration of the ß-cells which automatically halts the activities of the enzymes necessary for their action.

Potemkin, (1989), has shown that alloxan is selectively toxic to ß-cells and the destruction of ß-cells in dog associated with prolonged hyperglycemia has been assumed to result from overwork-exhaustion. Goldner and Gomori, (1973) have shown that during the initial hyperglycemia, the pancreas do not show any specific change but changes become apparent during the hypoglycemic stage. This stage revealed an inflamed pancreas with inflammatory reactions on the substance of the tissue. Also at this stage, Govan *et al*., (1992) have reported definite change in the nuclei and cytoplasm of the ß-cells with the diminution of their specific granules early after injection of a diabetogenic dose of alloxan in cats (Lazarus and Volk, 1962).

The acid phosphatase activity in the islets of Langerhans of the pancreas has been shown to be affected by alloxan administration in rats. The effect could be direct as a result of denaturation of the enzymes or indirect due to the consequent effect on the ß-cells of the islets of Langerhans.

ACKNOWLEDGEMENT: The authors are grateful to the Ahmadu Bello University Board of Research for supporting the work and Mr. Mathias Udoh for excellent secretarial services.

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