Intercalated Duct Cells in the Chicken Pancreatic Islet with Special Reference to the Alloxan Administration

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ABSTRACT. The intercalated duct cells were observed in the A and B islets of the chicken pancreas. These cells adhered with each other by intercellular junctional complexes at the apical side. They had many microvilli projecting into the lumen. Abluminally, they displayed extended slender cytoplasmic processes between islet endocrine cells. Administration of alloxan resulted to denser cytoplasm and a more prominent thickening of cytoplasmic processes of the intercalated duct cells, although the blood glucose levels did not show appreciable changes by the treatment. The intercalated duct epithelial cells appeared clearly as stellate cells. The lysosomes increased in size and number with passage of time after alloxan administration. The present findings may suggest that intercalated ducts are not only anatomically important as a structure passing through the islet but also play physiologically by protecting the islet endocrine cells.—KEYWORDS: alloxan, chicken, intercalated duct, pancreatic islet, stellate cell.


RESULTS

After alloxan administration, the A cells did not show any remarkable ultrastructural changes (Figs. 1, 3), whereas the B cells showed degranulation (Figs. 2, 4). Any degenerated endocrine cells, however, was not observed (Figs. 3–5). Other remarkable morphological changes were also noted in the intercalated ducts. In the control, intercalated ducts were localized in the periphery and/or near the center of the pancreatic islets. These ducts were removed immediately after sacrifice, cut at 1 mm thickness. The thin tissue slices were fixed successively into two solutions of 0.1 M phosphate buffer, pH 7.4: first in the solution containing 1.5% glutaraldehyde and 0.5% paraformaldehyde, and then in a solution containing 1% osmium tetroxide. The tissues were dehydrated in a graded series of alcohol, and embedded in Epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and then examined under electron microscope (H-7000, Hitachi, Tokyo, Japan).

MATERIALS AND METHODS

A total of 11 white Leghorn chicken, 1–2 years old, was used in this study. They were grouped as alloxan-treated (8 birds) and untreated/control (3 birds). For the alloxan-treated group, the birds were injected intravenously with a single dose of alloxan monohydrate (Wako Pure Chemical Industries, Tokyo, Japan) at 400 mg/kg body weight. Two birds were sacrificed under barbital anesthesia at an interval of 24 hours after alloxan administration.

Plasma glucose concentration of the control and alloxanized chicken was determined just before sacrifice. Analysis of blood glucose levels was done by easy paper method using glycostick or dextrostick (Bayer-Sankyo, Tokyo, Japan).

In the morphological study, the pancreas of the chicken was removed immediately after sacrifice, cut at 1 mm thickness. The thin tissue slices were fixed successively into two solutions of 0.1 M phosphate buffer, pH 7.4: first in the solution containing 1.5% glutaraldehyde and 0.5% paraformaldehyde, and then in a solution containing 1% osmium tetroxide. The tissues were dehydrated in a graded series of alcohol, and embedded in Epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and then examined under electron microscope (H-7000, Hitachi, Tokyo, Japan).

It is well-known that alloxan provides a quick and convenient method to produce experimental diabetes in a variety of vertebrates, but not birds [1, 7]. The direct and specific action of alloxan on the B cells has singled out B-cell as the focus of alloxanized pancreas [1]. In spite of the numerous cytological studies on the avian pancreatic islets [3, 5, 8, 9], morphological interrelationship between the exocrine and endocrine pancreatic has not yet been clarified. Our preliminary study in avian pancreas, however, revealed that the intercalated ducts which are part of exocrine pancreatic duct have been observed in the pancreatic islets of the avian [4].

The present study, therefore, aimed primarily to investigate the ultrastructural characteristics of avian pancreas with special reference to intercalated duct epithelial cells under normal and alloxanized conditions. The plasma glucose level of the birds subjected to the aforementioned conditions was also monitored.

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Plasma glucose concentration of the control and alloxanized chicken was determined just before alloxan administration and shortly prior to sacrifice. Analysis of blood glucose levels was done by easy paper method using glycostick or dextrostick (Bayer-Sankyo, Tokyo, Japan).

In the morphological study, the pancreas of the chicken was removed immediately after sacrifice, cut at 1 mm thickness. The thin tissue slices were fixed successively into two solutions of 0.1 M phosphate buffer, pH 7.4: first in the solution containing 1.5% glutaraldehyde and 0.5% paraformaldehyde, and then in a solution containing 1% osmium tetroxide. The tissues were dehydrated in a graded series of alcohol, and embedded in Epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and then examined under electron microscope (H-7000, Hitachi, Tokyo, Japan).
microtubules, and microfibrils, but no endocrine granules (Figs. 1, 2).

In the alloxanized pancreas, the microfibrils and lysosomes of the intercalated duct epithelial cells increased resulting to a denser cytoplasm (Figs. 3–6). Accompanying the increase in size and number of lysosomes with the passage of time after alloxan administration is the further thickening of the cytoplasmic processes which led the cells to assume a more prominent stellate in form. The cytoplasmic projections surrounded the endocrine cells, reaching the basement membrane of the endocrine islet cells. The lumen of the ducts was generally empty although sometimes dark granular materials were observed (Fig. 6).

The plasma glucose level did not show appreciable change after alloxan administration. The blood glucose concentration of alloxanized-chicken ranged from 165–180 mg/dl whereas in the control it ranged from 160–175 mg/dl.

DISCUSSION

Our present study supports the previous claim that alloxan administration in the avian does not cause diabetes. The alloxanized-chicken and the control did not show considerable variation in their plasma glucose level. The degranulation of the B-cells in the pancreatic islet, however, can not be disregarded. The degranulation may be speculated as an initial response of B-cells to the possible action of alloxan on insulin secretion. Alloxan has been known to induce insulin release causing hypoglycemia before finally destroying the B-cells which consequently cause hyperglycemia, an indication of diabetes. In our study, we only employed a single dose of alloxan. This dose may not be enough to provoke diabetes. Further study using a wide range of alloxan dosage level may clarify the potential effect of alloxan on the B-cells of the avian pancreas.

Of particular interest are the morphological changes in the intercalated duct epithelial cells brought about by alloxan administration. Certain increase in the size and number of lysosomes was observed as time elapsed after alloxan administration. Since lysosomes contain hydrolytic enzymes, it might be possible that they hydrolyze alloxan. At this standpoint, it may be suggested that intercalated duct epithelial cells may not only serve as a mere anatomical structure but play an important role in protecting the islet endocrine cells. Nonetheless, the potential functional significance associated with the morphological changes in the intercalated duct epithelial cells remains to be clarified. The possible function of intercalated epithelial cells may also be related to that of the folliculo-stellate cells of the pituitary gland. These folliculo-stellate cells which demarcate the margin of follicles have demonstrated slender cytoplasmic projections extending between granulated endocrine cells [6]. Several reports on the folliculo-stellate

![Fig. 1](image1.png)  ![Fig. 2](image2.png)

Fig. 1. Electron micrograph of a portion of an A islet from the normal chicken pancreas showing the organization of the intercalated duct (ID) and endocrine cells. A: A cell, D: D cell, ID: intercalated duct cell, arrowheads: intercellular junctional complex, arrows: lumen of the ID. × 4,000.

Fig. 2. Electron micrograph of a portion of a B islet from the normal chicken pancreas showing the organization of the ID cells and endocrine cells. A lumen of the ID (arrow) is clearly found among the three ID cells. B: B cell, D: D cell, C: capillary, ID: intercalated duct cell, arrowheads: intercellular junctional complex. × 4,000.
Fig. 3. Darkened ID cells in the A islet at 24 hr after alloxan administration. Note the unchanged organelles and endocrine granules in the A cells. A: A cell, ID: intercalated duct cell, arrowheads: intercellular junctional complex, arrows: lumen of the ID. ×5,000.

Fig. 4. Darkened ID cells in the B islet at 24 hr after alloxan administration. Note the unchanged organelles and endocrine granules in the A cells. B: B cell, ID: intercalated duct cell, arrowheads: intercellular junctional complex, large arrows: lumen of the ID, small arrows: basement membrane of endocrine islet cells and ID cell. ×5,000.

Fig. 5. Darkened ID cells in the B islet at 48 hr after alloxan administration. Note the decreased endocrine granules and increased rough surfaced endoplasmic reticulum. B: B cell, ID: intercalated duct cell, arrowheads: intercellular junctional complex, asterisk: lumen of the ID. ×5,000.

Fig. 6. High magnification of a part of the darkened ID cells at 72 hr after alloxan administration. Ly: lysosome, N: nucleus of intercalated duct cell, arrowheads: intercellular junctional complex, arrow: lumen of the ID. ×40,000.
cells have suggested that these cells might be involved in phagocytosis, support, contraction, stem cell function, and paracrine regulation of hormone secretion [2]. In our present study, similar morphological feature, the presence of slender cytoplasmic projections, was observed in the intercalated duct epithelial cells. This finding may imply that intercalated duct epithelial cells may exert similar function as folliculo-stellate cells.

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