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Morphological and morphometric changes in the small intestine in drug-induced diabetic rats.

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ABSTRACT: The study of Morphological and Morphometric changes in the small intestine in drug-induced rats was undertaken using 120 albino (Wistar) rats. The animals were randomly separated into four groups, A, B, C and D of thirty rats in each group.

The first group (A) was used as the primary control group, the second group (B), the third group (C) and the fourth group (D) was used as test groups. The test groups were administered with 200mg per kg body weight of alloxan, which is the diabetogenic dose through the intraperitoneal route (IP). The second group (B) was used to study the direct effect of alloxan on the small intestine. The third group (C) was used to study the effect of diabetes mellitus on the small intestine. The fourth group (D) was used to study the effect of therapeutic agents on the small intestine.

Histological examination of the intestinal mucosa and villi showed that they were affected in the test groups as compared to the control. The surface amplification factor due to the jejunal villi was 5 to 6 in the Control group and 3 to 4 for the test groups of which the difference was significant between the Control group (A) and the test groups ($P<0.05$). The mucosa and villi of the small intestine are reduced in height and the stroma enlarged in the test groups when compared with the Control. The results revealed that there are morphological and morphometric changes in the small intestine due to the administration of diabetogenic dose of alloxan which consequently lead to diabetes mellitus in the rats.

Key words: Morphological, Morphometric, Diabetic rats, Alloxan, Small Intestine, Amplification factor, Mucosa.

Introduction

Diabetes mellitus is one of the several metabolic disorders resulting from disturbances in islet function and is the most severe and epidemiologically important (Weiss, 1984). Diabetes mellitus is a family of diseases that result from improper uptake of sugar from the blood and its subsequent utilization by the cells of the body. The Islet Beta (B) cells are almost always involved by being functionally deficient, numerically reduced or totally absent in severe cases (Edwards et al., 1995; Weiss, 1984), while the remaining islet endocrine cells proliferate in the diabetic state thus exacerbating the condition (Andrew and Henry, 1989; Lazarus and Volk, 1962).

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There are two types of primary diabetes mellitus which are world-wide in distribution and incidence. These are the Insulin Dependent Diabetes Mellitus (IDDM) sometimes called Type 1 diabetes and Non-Insulin Dependent Diabetes Mellitus (NIDDM) called Type II diabetes. They are classified thus, depending on the variations in onset, severity and complications (Alberti and Krall, 1991; Haffner et al., 1996). The prevalence of diabetes mellitus varies considerably in different parts of the World due to both genetic and environmental factors (Falkner et al., 1970; Alberti and Krall, 1991; Kumar and Clark, 1991; Edwards et al., 1995).

In type I diabetes mellitus, the metabolic changes are the same as those produced by pancreatectomy or after the administration of some drugs, Alloxan and Streptozotocin. These drugs selectively destroy the B-cells of the Islets of Langerhans raising the blood glucose level as a result of hepatic overproduction of glucose by glycogenolysis and gluconeogesis and a decrease in the removal of glucose from the circulation into adipose tissues and muscles(Bowman and Rand, 1985; Murray et al., 1996).

The cause of some forms of diabetes mellitus is known and are classified as secondary diabetes. These include, diabetes caused by the destruction of the pancreas by drugs., diseases or surgery and diabetes due to hormonal imbalance (Bloom and Ireland, 1980; Oakley et al., 1978;Haffner et al., 1996). Diabetes have been shown to be a hereditary disease in that some members of the family of known diabetics have been found to have a number of peculiar diabetic characteristics even though their blood sugar may be normal at the time of examination while a large number of such people develop typical metabolic diabetes some years later (Levine, 1963; Lazarus and Volk, 1962; Granner, 1995).

Since the small intestine is the site of most of the digestion and absorption in the body, any derangement of the small intestine may lead to malabsorption which impair growth and other body functions. The present study examine the significant morphological and morphometric changes in the jejeunum in drug-induced diabetic rats.

Materials and Methods

The drug usedfor the experiment was white Alloxan crystals contained in a 250 gms bottle. The alloxan crystals weighing 1.2 gms were dissolved in 10mls of injection water amounting to 1.2gm/10ml. Then 0.3ml of the 1.2gm/10ml which is equivalent to 40mg of alloxan was administered as the diabetogenic dose according to Barnes and Eltherington, (1964); Barbato and Landau (1977); Bowman and Rand, (1985); Greenspan and Baxter, (1994).

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

The group B animals were the first test group and were used to study the direct effect of alloxan on the small intestine during diabetic induction. The second test group © are animals that have been successfully induced with diabetes mellitus and were used to study the effect of diabetes mellitus on the small intestine. The third test group (D) were diabetic animals undergoing therapy and were used to study the effect of therapeutic agents on the small intestine.

Tissue preparation for microscopy

Animals from each of the groups were anaesthetized and their abdomen opened by a midline incision and part of the small intestine from each of the groups were excised. The tissues were placed in cold normal saline until excision of the tissues were completed. The tissues were fixed using 10% neutral formalin and Bouins fluid. The tissue processing techniques used were both manual and automatic tissue processing techniques using the Histokinette bench model tissue processor obtained from the Department of Human Anatomy, Ahmadu Bello University, Zaria.

The tissues were embedded in paraffin and sections between 5 – 8 microns were made using the Rotary Microtome. The tissues were stained using Haematoxylin and Eosin (H and E) method and periodic Acid

Schiff (PAS) method. These methods were used as outlined by Gurr, (1992); Culling (1993); Drury and Wallington (1973).

Statistical Analysis

Different parameters obtained from the morphometric analysis from the different groups were compared using their mean and standard deviation (SD). The student's t-test was used for testing the level of difference. A, P-values less than 0.05 was considered significant. One-Way Analysis of Variance (ANOVA) was used for comparing the means while the Multiple Range Test was used to find the Least Significant Difference (LSD) between the groups.

Results

Following the administration of 200mg/kg body weight of alloxan to the rats, some observations were made. These observations include, initial increased activity, increased sensitivity to touch and sound. These were gradually replaced by decreased activity and loss of appetite which consequently lead to loss in weight in the test animals.

The result of the microscopic examination of the small intestine, show the jejunal mucosa with normal, tall slender villi and stroma in group A animals as shown in Plate 1. The stroma and the villi were enlarged in the jejunum of group B animals and contain inflammatory cells as shown in Plate 2, while the jejunal mucosa and villi group C and D animals were enlarged and reduced in height with inflammatory cells present as shown in Plates 3 and 4.

The jejunal surface amplification factors due to the jejunal villi estimated by transverse sectioning approach for the different groups of animals as shown in Table 1. The result show the surface amplification factors as 5.61 ± 0.64; 3.82 ± 0.51 and 35 ± 0.40 for groups A, B and C respectively. This means that the jejunal villi amplify the mucosal surface area five to six times, three to four times and three to four times for groups A, B and C animals respectively. The amplification factor differs significantly between the control and the test groups ($P<0.05$) while the difference between the test groups (B and C) was significant ($P<0.05$) and there was no difference between group C and D.

The result on the villous length, crypt height and stromal dimension are shown in Table 1. The villous length are 490.0 ± 37.30µm; 429.0 ± 65.83µm and 381.67 ± 41.03µm for groups A, B and C respectively while the crypt height are 117 ± 17.75µm; 152.0 ± 24.27µm and 135.33 ± 16.13µm for groups A, B and C respectively. The stromal cross-section for groups A, B and C are 78.50 ± 32.80µm; 94.33 ± 35.91µ and 97.67 ± 40.99µm respectively.

The length of the villi differs significantly between the groups ($P<0.05$). The difference between the stromal cross-section of the groups was not significant ($P<0.05$), while the difference in the Crypt height was significant between the Control and the test groups ($P<0.05$).

Discussion

The loss in weight as evidenced from the test groups may be due to reduced gut absorption. This may be attributed to the inability of the small intestine of the test animals for effective nutrient absorption which may be caused by the oxidative toxicity of the mucosa of the small intestine bearing the villi which form the main organ responsible for the absorption of the nutrients (Junqueira et al., 1995; Mcphee et al., 1995). Results obtained from photomicrographs of the small intestinal mucosa and villi of the test and control animals support this. The mucosa, villi and stroma of the test animals showed some changes in comparison to the control animals. The higher body weight of the control showed their effective nutrient absorption are normal (Hills, 1976; Plea, 1975; Abbas et al., 1989; Spencer, 1960).

The inability of the small intestine for effective nutrient absorption can also be due to the direct effect of diabetes in the small intestine which may be regarded as a secondary effect of alloxan administration. This inability of the test animals to effectively absorb more protein from the small intestine to meet

increased demands was established by Lazarus and Volk (1962); Oakley et al (1978) and Podolsky (1980). They showed that the relative ability to absorb protein was defective and concluded that irreparable damage may have been inflicted upon the intestinal morphology of the test animals (Mayhew, 1988; Palay and Karlin, 1957).

Table 1: Small Intestinal Measurements (Mean, S.D. and Range) of Control rats (Group A), rats administered with Alloxan; Group B and Group C (n = 30).

Variable	Group A	Group B	Group C
1. Villous Length (μm)			
Mean	490.00	429.00	381.67
S.D.	± 37.30	± 65.83	± 41.03
Range	(420-570)	(300-540)	300-450)
2. Crypt Length (μm)			
Mean	117.67	152.0	135.33
S.D.	± 17.75	± 24.27	± 16.13
Range	(90-150)	120-180)	
3. Stromal Dimension (μm)			
Mean	78.50	94.33	97.67
S.D.	± 32.80	± 35.91	± 40.99
Range	(30-180)	(30-180)	(60-190)
4. Amplification factor			
Mean	5.61	3.82	3.55
S.D.	± 0.64	± 0.51	± 0.40
Range	(5-6)	(3-4)	(3-4)

The loss in body weight of the test animals may be attributed to the reduction of surface amplifications of the intestinal mucosa due to the reduction in the length of the villi. The study showed decreased length of the villi in the test groups which may lead to decreased rate of absorption because of reduction in surface of the small intestine. (Spencer, 1960; Leblond and Stevens, 1948). This finding is in agreement with Mayhew (1988) who showed that the villi are relatively fixed in number during life but the amplification factor change during experimental fasting and diabetes (Lee and Toner, 1980; Baron, 1980).

These effects may be associated with the influence of fat soluble vitamins which may have been destroyed by alloxan in the test groups of which the deficiency of vitamin A, have been shown to lead to stunted growth, pathological changes in the intestine and necrosis of the tips of the villi (Keele and Neil, 1964; Guyton, 1986; Hills, 1976; Ganong, 1995; Baron, 1980).

Asquith (1979) has shown the small intestinal mucosa with shortened and broaden villi giving the appearance of total or partial villous atrophy. The basic abnormality is a diffuse dense monoculear

infiltrate of the lamina propria causing wide separation of Crypts of Liberkuhn and obliteration of the villous architecture without significant impairment to the integrity of the surface epiethlium (Asquith, 1979). The cellular infiltrate is predominantly plasma cells but the stage of maturation of the cells varies and the cellular infiltration is shown to be confined to the lamina propria (Cheville, 1989; Ritchie, 1990; Lee and Toner, 1980).

In conclusion, the administration of the diabetogenic dose of alloxan to rats may lead to morphological and morphometric changes in the small intestinal mucosa, villi, crypt and villous stroma of the test groups.

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