

Comparative evaluation of pancreatic histopathology of rats treated with olanzapine, risperidone and streptozocin

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Olanzapine and risperidone are widely prescribed atypical antipsychotics used in the treatment of schizophrenia and various other psychiatric disorders. Both of these drugs have been extensively reported to cause Type 2 diabetes mellitus and pancreatitis, however, the mechanism of olanzapine and risperidone-induced toxicity has not been so far unveiled. We, therefore, compared the streptozocin-induced pancreatic damage with that of pancreas isolated from olanzapine and risperidone treated rats. It was noticed that fibrotic growth, necrosis and derangement of the pancreatic islet cells caused by streptozocin were more pronounced than olanzapine and risperidone.

Keywords: Histopathology/pancreas. Antipsychotic drugs/evaluation. Olanzapine. Risperidone.

INTRODUCTION

Olanzapine and risperidone are atypical antipsychotic drugs which are widely prescribed to treat schizophrenia and other psychotic illnesses. These drugs have also been reported to induce diabetes both in rats and humans (Shah *et al.*, 2015; Weston-Green, Huang, Deng, 2013). Several mechanisms of olanzapine- and risperidone-induced hyperglycemia and diabetes have been reported so far including their antagonistic effect on α_1 , α_2 , H_1 and M_3 receptors (Shah *et al.*, 2015; Weston-Green, Huang, Deng, 2013). It has been reported that antagonism of H_1 receptors is associated with weight gain while M_3 receptors' antagonism with pancreatic damage which leads to insulin insufficiency. Weston-Green and co-workers have shown in their review article that acetylcholine muscarinic receptors (M_3) on the pancreas are one of the reasons that lead to insulin dysregulation (Weston-Green, Huang, Deng, 2013). Studies have also shown that M_3 receptors antagonism is a risk for diabetes (Duttaroy *et al.*, 2004; Weston-Green *et al.*, 2013). The M_3 receptors

located on pancreas are involved in regulating the glucose-stimulated cholinergic pathway of insulin release from the beta cells of the islet of Langerhans. These receptors, when stimulated, results in the release of insulin (Duttaroy *et al.*, 2004; Weston-Green, Huang, Deng, 2013). Olanzapine and risperidone have been shown to block the M_3 receptors on pancreas that decreases the insulin release (Weston-Green, Huang, Deng, 2013). However, such studies are still lacking that report the direct effect of olanzapine and risperidone on the architecture of pancreas of rats. We, therefore, designed this study to explore whether these drugs have any impact on the pancreatic histology by comparing it with the streptozocin-induced pancreatic damage.

METHODS AND MATERIAL

Sprague-Dawley rats were bred in the Animal House and Bioassay Laboratory of the Department of Pharmacy, University of Peshawar. During experimental period, the rats were constantly observed and handled carefully in accordance with the rules specified under the Animals (Scientific Procedure) Act, 1986, UK and as approved by the Departmental Ethical Committee.

The rats had a free access to water whereas they were provided hygienic, palatable, dried and nutritional

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food in restricted amount (20 g/12 h). Fresh water was frequently filled in bottles during day-time, and before the start of dark-cycle. The flow of water to the feeding tube was regularly checked.

The sawdust was used as a bedding material in sufficient amount to avoid infection and to maintain the softness, dryness and hygiene of the surface. The bedding material was changed on daily basis or when required.

Procedures

Drug treatment

The animals were kept fasted for 24 hours before first dose was administered. Olanzapine was formulated by dissolving it in small quantity of 0.1 N acetic acid followed by volume make-up with normal saline in a 1:100 (Pouzet *et al.*, 2003). Olanzapine was used once in daily doses of 5 mg/kg/d (Pouzet *et al.*, 2003) for first 8 weeks, 10 mg/kg/d for next 4 weeks (Andersen, Pouzet, 2001; Pouzet *et al.*, 2003) and 15 mg/kg/d for last 2 weeks (Angelucci *et al.*, 2005) during the fourteen weeks experiment. Controls were administered with normal saline in a volume based on body weight (not greater than 5 ml/kg/d) (Pouzet *et al.*, 2003). Both olanzapine and saline freshly prepared were administered on daily basis by oral gavage method during the same period (Pouzet *et al.*, 2003; Shah *et al.*, 2015; Terry *et al.*, 2003). The experiment continued for fourteen weeks.

Risperidone was used orally in a dose of 2.5 mg/kg/d for three weeks (Terry *et al.*, 2003). It was formulated by dissolving in small quantity of 0.1 N acetic acid followed by volume make-up with normal saline in a ratio of 1 to 100. Normal saline as a control was administered in a volume based on body weight (not greater than 5ml/kg/d). Drug containing solution and normal saline were administered by oral gavage method. Fresh solution of drug was prepared each day and the experiment continued for three weeks.

Streptozocin was administered at a dose of 50 mg/kg/ip for diabetes induction. The samples of pancreas of rats treated with streptozocin were obtained from rats with confirmed diabetes (Ali *et al.*, 2015).

Histological study

The animals were sacrificed by cervical dislocation, selected tissues were isolated and washed with phosphate buffered saline (PBS) and fixed in neutral buffered formalin (NBF) for not less than 6 hours. The fixed samples of tissues were sliced into small pieces of 3–5 mm and embedded separately in paraffin blocks. These blocks were sectioned using microtome (SLEE MAINZ, CUT

5062). Sections of 5 μ size were taken and stained using H & E staining technique (Ali *et al.*, 2015; Shah *et al.*, 2015).

Microscopy

The stained slides were analyzed under a light microscope (LABOMED LX400, USA) equipped with camera (iVu 3100). The images obtained were labeled, saved and interpreted for any drug induced changes.

RESULTS

Normal saline

The pancreas of saline treated rats showed normal architecture of the Islet of Langerhans with compact arrangement of beta and non beta cells. The islet appeared lightly stained than the surrounding acinar cells, and consisted of slightly stained polygonal cells arranged in cords separated by a network of blood capillaries. The acinar cells were characterized by its basal basophilia and apical acidophilia, as depicted in Figures 1A and 1B.

Olanzapine

As shown in Figure 1C and 1D, olanzapine was associated with reduction in the number of beta cells. The islets were irregularly shaped having a lobular appearance along with increase in fibrotic growth. The beta cells showed extensive necrosis, degeneration and vacuolization with eosinophilic cytoplasm. Most of the nuclei exhibited karyolysis and pyknosis while others were darkly stained with chromatin condensation along the periphery. The blood vessels of the islets showed dilatation and were heavily congested with red blood cells.

Risperidone

The islets of Langerhans in the pancreas of risperidone treated rats seemed scattered showing derangement of pancreatic islets cells. The cells lost their arrangement in cords and compaction contrary to the architecture observed in normal saline treated pancreas, showing lobular appearance and fibrotic growth in the similar fashion as seen in olanzapine treated rats, depicted in Figures 1E and 1F.

Streptozocin

As depicted in Figure 1G and 1H, streptozocin seemed extremely toxic to the pancreatic cells thereby rendering severe damage to the beta cells. The islet cells

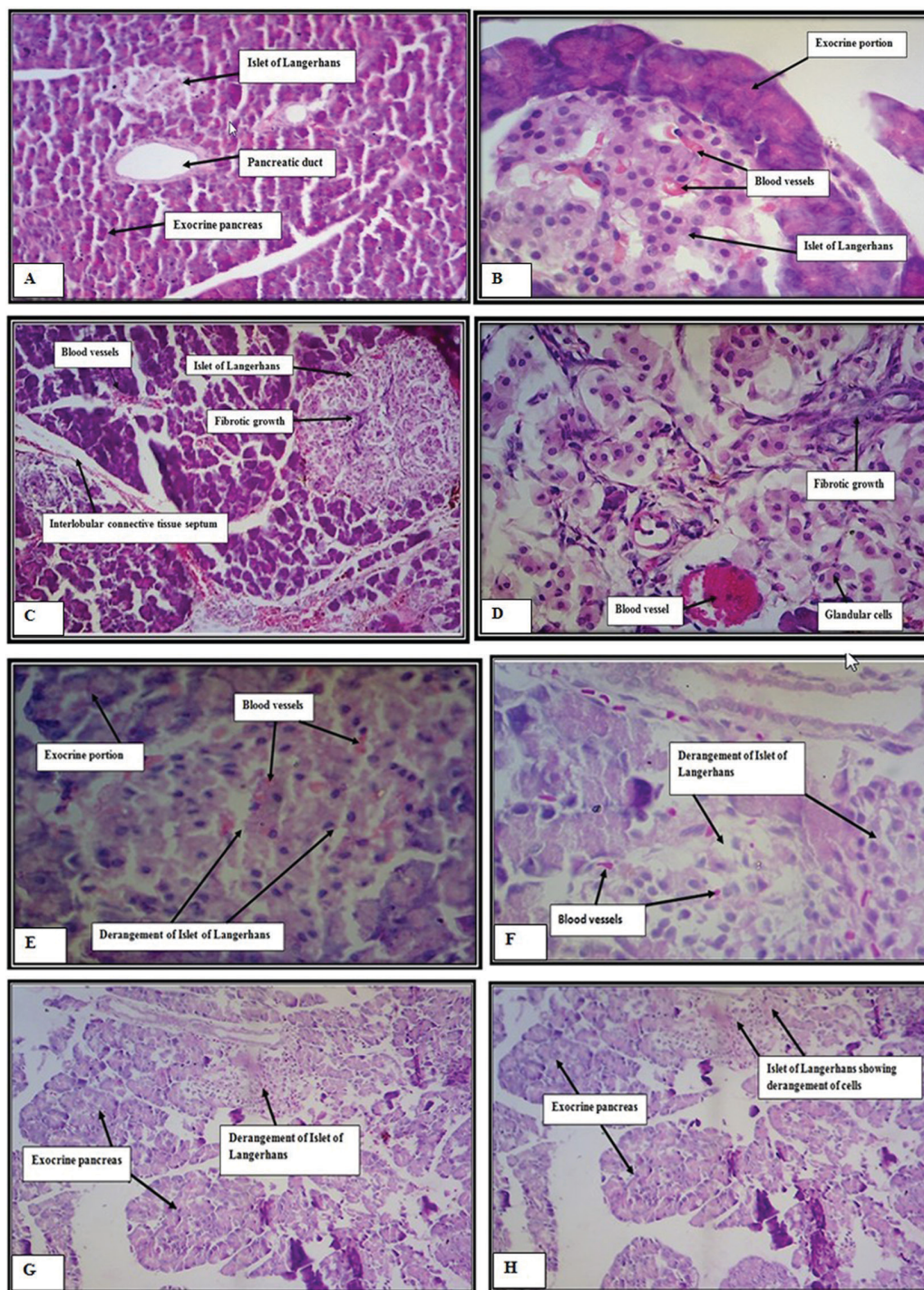


FIGURE 1 - (A-D): Effect of normal saline and olanzapine on the pancreas of rats (H & E, 100x, 400x, 5 μ): Representative image of pancreas of rats treated with normal saline (A & B) orally for three weeks showing islets of Langerhans, packed as compact and intact glandular mass. Photomicrographs of a section (5 μ m) of pancreas from a rat treated with olanzapine (C & D) (5 mg/kg/d for the first eight weeks, 10 mg/kg/d in next four weeks and 15 mg/kg/d in the last two weeks), showing nodular appearance of islet of Langerhans due to fibrotic growth with derangement of beta cells. Effect of risperidone on the pancreas of rats (H & E, 100x, 400x, 5 μ) (E-F): Representative images of pancreas of rats treated with risperidone (2.5 mg/kg/d) orally for three weeks showing derangement of islets of Langerhans, lacking compaction of glandular cells as compared to rats treated with normal saline (A & B). Effect of streptozocin on the pancreas of rats (H & E, 100x, 400x, 5 μ): Representative images of pancreas of rats treated with streptozocin (G -H) (50 mg/kg/d/ip) showing derangement of islets of Langerhans, lacking compaction of glandular cells as compared to rats treated with normal saline.

were shown dispersed, irregular and necrosed. The beta cells exhibited extensive necrosis, degeneration, fibrosis and vacuolization. Paucity of islet cells was observed in streptozocin treated pancreas.

DISCUSSION

We compared the histopathology of streptozocin treated pancreas of rats with normal saline, olanzapine and risperidone treated pancreas. We noticed derangement of islet cells in olanzapine and risperidone treated rats, while fibrotic growth in olanzapine and streptozocin treated rats, however the streptozocin-induced pancreatic damage was more pronounced (Shah *et al.*, 2015; Ali *et al.*, 2015).

It is believed that the diabetogenic effect of streptozocin, is due to oxidative stress, since increased reactive oxygen species have been observed in pancreatic tissues (Coskun *et al.*, 2005; Ihara *et al.*, 1999). It has been shown that streptozocin depletes the intracellular nicotinamide dinucleotide (NAD) in islet cells (Coskun *et al.*, 2005; Schein *et al.*, 1973). Furthermore, it has also been proved that streptozocin causes the breakdown of DNA strands that results in methylation within the pancreatic islet cells, which in turn, leads to pancreatic damage (Coskun *et al.*, 2005; Matkovic *et al.*, 1996). Persistent and chronic hyperglycemia leads to oxidative stress that in turn causes the depletion of antioxidative defense system thereby leading to generation of free radicals (Coskun *et al.*, 2005; Ihara *et al.*, 1999). Streptozocin-induced diabetes has also been shown histopathologically (Ali *et al.*, 2015; Coskun *et al.*, 2005; Das, Padayatti, Paiuiose, 1996) wherein fibrotic growth and derangement of islet cells have been described.

Although, risperidone treatment did not induce hyperglycemia, but pancreatic damage and pancreatitis were depicted by elevated amylase levels. Similarly, olanzapine showed weight loss, hyperglycemia, hyperamylasemia, hyperlipasemia, derangement of beta cells and fibrotic growth as reported by our laboratory (Shah *et al.*, 2015).

Studies have shown that M₃ receptor antagonism results in insulin insufficiency which might be attributed to ultimate damage. It may, however, be assumed that olanzapine and risperidone might be involved in the generation of free radicals at the level of pancreas, in the same pattern, but with low intensity as streptozocin (Weston-Green, Huang, Deng, 2013).

There are very limited data available that describe the role of olanzapine and risperidone in causing oxidative stress and generation of free radicals, which are also contradictory, in the sense that typical antipsychotic

(Haloperidol) causes oxidative stress but atypical antipsychotic drugs do not (Ng *et al.*, 2008). On the other hand, risperidone has been reported to be associated with oxidative stress (Ng *et al.*, 2008; Pillai *et al.*, 2007) while olanzapine plays a protective role against the oxidative stress in schizophrenic patients (Pillai *et al.*, 2007). The data which show oxidative stress in animals or humans employing pancreas specific markers are still needed to be evaluated.

In our study, antipsychotics-induced pancreatic damage is a step forward towards exploration of mechanistic pathway, wherein oxidative stress could be the major cause of pancreatic damage in olanzapine- and risperidone-treated rats, in the same pattern as streptozocin.

CONCLUSION

Olanzapine and risperidone caused fibrotic growth, necrosis and derangement of the islet cells of pancreas, which was more pronounced in rats treated with streptozocin as compared to treatment with olanzapine and risperidone.

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