Pancreatic response after treatment with 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper in rats

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Received 18 January 2014; Accepted 12 June, 2015

3,5-Dimethylpyrazole (U-6245) is a hypoglycemic and antilipolytic agent used in the treatment of diabetes mellitus. Complex 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper was prepared in 2009. This study indicated that 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper has hypoglycemic properties by decreasing glucose level in blood but caused histopathological changes in the pancreas rats. Rats treated with 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper for 2, 4 and 6 weeks revealed inflammatory infiltration in the pancreas, marked cytoplasmic vacuoles, congestion of blood vessels with edema and haemorrhage, as well as, some of the degenerated cells showed necrosis. Large spaces were detected in some areas due to degeneration of cells. Sera of animals treated with 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper in the present study revealed a significant decrease in serum glucose after 2, 4 and 6 weeks of treatment.

Key words: Hypoglycemic agent, glucose, pancreas, necrosis.

INTRODUCTION

The pancreas is an organ composed of endocrine and exocrine tissue. The endocrine portion is the islets of langerhans, contains β-cells that secrete insulin hormone. Insulin stimulated the uptake, storage and use of glucose by tissues. These activities result in a decrease in the level of blood glucose (Butler, 1995). Diabetes mellitus is a disease that leads to complications including heart disease, kidney failure and nerve damage (Butler, 1995). The search of highly efficient therapeutic drugs for the prevention and treatment of diabetes are now one of the main objectives of modern medicine (Richard et al., 2010).

3,5-Dimethylpyrazole (U-6245) is a hypoglycemic agent that decrease the level of glucose in blood and are used in the treatment of diabetes mellitus and is antilipolytic agent that inhibits lipolysis. 3,5-dimethylpyrazole markedly depressed plasma fatty acid and blood sugar after 15 min to 3 h of its administration. The mechanism of hypoglycemic activity of 3,5-dimethylpyrazole is not the same as insulin. The action...
3,5-dimethylpyrazole is similar to that of insulin in that it increases glucose oxidation and decreases plasma free fatty acids of intact rats as reported for insulin and decrease blood glucose. 3,5-dimethylpyrazole is unlike insulin in that it is not effective in lowering blood sugar of eviscerated rats which respond to insulin (George and William, 1965).

Various pyrazole derivatives exhibit various pharmacological properties as antioxidant, antivirus, antidepressant, anti-inflammatory and anti-tumor effect by inhibiting enzymes which play an important role in cell division (Kroigaard et al., 2010; Manuel et al., 2012). The ligand 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane (C_{18}H_{28}N_{4}O_{5}-Cu) was prepared by adding a solution of 1,5-Bis (3,5-dimethylpyrazol-1-yl)-3-oxapentane (0.262 g, 1 mmol) in 2 ml of acetone to a suspension of Cu(CH_{3}CoO)_{2} H_{2}O (0.199 g, 1 mmol) in 2 ml of the same solvent and stirring the mixture for 24 h at room temperature. Green crystals formed were filtered and dried in vacuo (Potapov et al., 2007). Complex 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper was prepared by the reaction of ligand and copper (11) acetate monohydrate in acetone solution (Potapov et al., 2009). The proposed structure for this compound was confirmed by UV-Vis and IR spectroscopy, molar conductivity measurements and elemental analysis data. The present study aim to investigate the side effects induced by 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper on the blood and pancreas of albino rats.

**MATERIAL AND METHODS**

**Animals**

Healthy adult male albino rats (*Rattus norvegius*), approximately three months old and weight (130±5) g were used in the present study. The animals were kept under constant condition of temperature for at least two weeks before the experimental period. Animals were maintained on a standard diet, manufactured especially for laboratory purposes, obtained from Atimida Company for national development. Water was available ad libitum. Animals were kept under constant temperature (30±2°C) and the humidity was 45±5% with 12:12 light-dark cycle.

**Chemical materials**

1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper (C_{18}H_{28}N_{4}O_{5}-Cu) was dissolved in 0.9% mammalian saline (9 gm sodium chloride dissolved in 1000 ml distilled water) and injected intraperitoneally (ip) at dose 12 mg/kg body weight rat/ day for 2, 4 and 6 weeks (Donati et al., 2008).

**Experimental design**

The animals were divided into 3 groups.

**Control group**

Animals of this group (25 rats) were injected intraperitoneally by 0.9% mammalian saline (9 gm sodium chloride dissolved in 1000 ml distilled water) and they were sacrificed after different times parallel to that of treated groups throughout the whole experimental period.

**Group 1**

Animals of this group (24 rats) were only one time injected intraperitoneally (ip) by 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper freshly dissolved in saline (12 mg/kg b.w.). Animals were sacrificed after 30 min, 1.45 h, 2.30 h and 24 h of single injection.

**Group 2**

Animals of this group (24 rats) were injected daily for 6 weeks intraperitoneally (ip) by 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper freshly dissolved in saline (12 mg/kg bw/ day). Animals were then sacrificed 2, 4 and 6 weeks after beginning of the treatment, 8 animals in each period were sacrificed 2 h after injection.

**Histological preparation**

For light microscopic studies, immediately after scarification, pancreas was removed carefully and quickly fixed in 10% neutral formalin, washed in running tap water for 12h, fixed and stored in 70% ethyl alcohol. Tissue pieces were dehydrated in ascending series of ethyl alcohol (70%, 80%, 90% and two changes 100%), cleared in two changes of xylene and embedded in molten paraplast paraffin (mp.50 to 58°C). Sections of 5 microns thickness were cut using rotary microtome (Leica, Model Rm 2125, Germany), and mounted on clean slides without using any adhesive medium. For histological examination, sections were stained with Ehrlich’s haematoxylin and counterstained with eosin (Lillie and Fulmer, 1976).

**Biochemical analysis**

Blood samples were taken from portal vein and left to coagulate at 37°C in incubator, centrifugated at 174
g/min for 15 min (1550 r.p.m) with centrifuge (Shanghai Surgical Instruments Factory, Model 9 to 1). Sera were stored at -20°C until further analysis. Specimens from control and all treated groups were obtained for examination from starting the experiment. Serum glucose was determined according the method of Bergmeyer et al. (1974).

Statistical analysis
All biochemical results were expressed as mean ± standard error. The results were analyzed statistically utilizing computer program (Excel). The criterion for statistical significance was set at P<0.05.

RESULTS

A- Histological observations

Control group
The endocrine pancreas consist of islets of langerhans which secrete hormones and the exocrine pancreas contains many secretory units called acini arranged around a system of ducts. The ducts combine to form the large pancreatic duct. The pancreas contains thousands of acini, each acinus composed of pyramid-shaped secretory cells (acinar cells) which produce and release digestive enzymes. The nucleus of the acinar cell is located at the base of the cell (Figure 1).

Treated groups
Rats treated with 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper exhibited many histopathological changes in pancreas. Pancreatic sections after 2 weeks of treatment showed inflammatory infiltration around widened pancreatic duct. Mild congestion, mild edema and haemorrhage of blood vessels were observed. Degeneration and vacuoles of the acinar cells and the islets of langerhans were also evident (Figures 2 and 3). Pancreatic sections after 4 weeks of treatment showed marked inflammatory infiltration around widened pancreatic duct and blood vessels with haemorrhage. Marked degeneration, vacuoles and karyolysis of the acinar cells and the islets of langerhans were also observed (Figures 4 and 5). Examination of pancreatic sections after 6 weeks of treatment showed increased congestion of blood vessels with haemorrhage. Marked degeneration, vacuoles, apoptosis and loss of pancreatic acinar cells, and the spaces between the islets and ducts enlarged and became filled with fibroblast-like cells. Most of the cells had condensed nuclei (pyknotic cells) with marked cytoplasmic vacuoles and lost normal pancreatic structure (Figures 6 and 7).

Biochemical results (Serum glucose (mg/dl))
Intraperitoneal administration of 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper to rats decreased serum glucose level after 30 min and 1.45 h, then beginning to rise slowly towards normal values after 2.30 h and reach normal value after 24h of single injection (Figure 8). While animals daily treated for 2, 4 and 6 weeks revealed a significant decrease in serum glucose compared with control group (Figure 9).

DISCUSSION
The study results indicated that treating rats with 1,5-Bis (3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper caused many histopathological alterations in the pancreas structures, inflammatory infiltration, swelling and marked enlarged vacuolated cytoplasm in pancreatic cells, congestion of blood vessels with edema and haemorrhage, as well as, some of the degenerated cells showed karyorrhexis, pyknosis and area of necrosis. Large spaces were detected in some areas due to degeneration of cells. Similarly, Yamada et al. (1987) and Choia et al. (2013) suggested that ethanol-pyrazole treated rats induced pancreatic injury even at an early stage and accelerates the collagen metabolism in the pancreas. Pyrazole induced hepatotoxicity and oxidative liver injury in mice (Wang et al., 2011; Bae et al., 2012). Administration rats by 3,5-dimethylpyrazole at dose 12 mg/kg body weight caused hypoglycemia and stimulation of the endocrine pancreas (Locci and Bergamini, 1983). The antilipolytic agent 3,5-dimethylpyrazole inhibits insulin release in isolated rat islets (Masiello et al., 2002). Ritva et al. (1983) reported that centrilobular necrosis was observed among rats treated with pyrazole at dose 200 mg/kg. Rats injected intraperitonealy with pyrazole at dose 200 mg/kg body weight, once per day for 2 days, pyrazole produced swelling of mitochondria and induced liver histopathology and liver injury (Yongke et al., 2005). Pyrazole enhanced liver injury, oxidative stress, indication of pathological changes, apoptosis, necrosis; positive staining with apoptotic morphology casually appeared at 3 h and increased at 8 h of treatment (Lu and Cederbaum, 2006).
Examination of sera of animals after single intraperitoneal administration of 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper to rats revealed that serum glucose level decreased after 30 min and 1.45 h then beginning to rise slowly after 2.30 h and reach normal value after 24 h of single
Figure 1. Sections in the pancreas of control rats showing the normal pancreatic structure; where the pancreatic acini (thin arrow) arranged beside the islets of langerhans (thick arrow). Pancreatic ducts (double rod) were also evident (H&E stain, ×100).

Figure 2. Sections in the pancreas of rats treated with 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper for 2 weeks showing inflammatory infiltration around widened pancreatic duct (thick arrows) and blood vessels (double rod). Degeneration and vacuoles of the acinar cells (thin arrow) and the islets of langerhans (double head arrow) were also evident (H&E stain, ×100).
Figure 3. Sections in the pancreas of rats treated with 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper for 2 weeks showing inflammatory infiltration around widened pancreatic duct (thick arrows). Degeneration and vacuoles of the acinar cells (thin arrow) and the islets of langerhans (double rod) were also evident (H&E stain, ×400).

Figure 4. Sections in the pancreas of rats treated with 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper for 4 weeks showing inflammatory infiltration around widened pancreatic duct and blood vessels with edema and haemorrhage (thick arrows). Marked degeneration and vacuoles of the acinar cells (thin arrow) and the islets of langerhans (double head arrow) were also evident (H&E stain, ×100).
Figure 5. Sections in the pancreas of rats treated with 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper for 4 weeks showing inflammatory infiltration around widened pancreatic duct (thick arrows). Marked degeneration, vacuoles and karyolysis of the acinar cells (thin arrow) and the islets of langerhans (double rod) were also evident (H&E stain, ×400).

Figure 6. Sections in the pancreas of rats treated with 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper for 6 weeks showing marked inflammatory infiltration around widened pancreatic duct and blood vessels with edema and hemorrhage (thick arrows). Marked degeneration, vacuoles, karyolysis and pyknosis of the acinar cells (thin arrow) were also evident (H&E stain, ×100).
Figure 7. Sections in the pancreas of rats treated with 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatomopper for 6 weeks showing marked degeneration, vacuoles and pyknosis of the acinar cells (thin arrow) and lost normal pancreatic structure were also evident (H&E stain, ×400).

Figure 8. Effect of single intraperitoneal administration of 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatomopper on serum level of glucose (mg/dL). (**) highly significant in comparison with control group.
injection. Donati et al. (2006), reported that the antilipolytic agent DMP (12 mg/Kg bw) was injected intraperitoneally to rats 3 h before their sacrifice caused rapidly lowering free fatty acids (FFA), glucose and insulin plasma levels for almost 3 h. Locci et al. (1985), suggested that the effects of the 3,5-dimethylpyrazole on free fatty acids plasma levels were exhausted in 3 h. After this time, the free fatty acids plasma levels began to rise slowly towards normal values and full restoration required 8 h.

Examination of sera of animals treated with 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper for 2, 4 and 6 weeks revealed a significant decrease in serum glucose. Several investigators also obtained similar results. In this concern, Bergamini et al. (1987), showed similar results of the administration of antilipolytic drugs 3,5-dimethylpyrazole (DMP) to rats induced with significant decrease in plasma FFA in 15 min, glucose and insulin (Donati et al., 2008).

CONCLUSION

This study indicated that 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper has hypoglycemic properties by decreasing glucose level in blood but caused histopathological changes in the pancreas rats. Results obtained in this study can be used for the search of new drugs based on 1,5- Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper in medical therapy for treatment diabetes, but after special chemical modifications.

Conflicts of interest

The authors declare that they have no conflicts of interest.

REFERENCES


