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A 28 days oral toxicity of Dronedarone in Wistar Rats (*Rattus norvegicus*)

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ABSTRACT

The aim of the present experiment was to study clinical signs, haemato-biochemical alterations and pathomorphological changes induced by Dronedarone administration in rats. In present study, 48 Wistar rats (5-8 weeks old) were divided in to four different groups with equal numbers of male and female. Group I rats (Corn oil) severed as vehicle control. Group II, III and IV rats were administered Dronedarone @ 50, 100 and 200 mg/kg b.wt. respectively, orally daily for 28 days. The blood samples were collected on day 29 of the study from all the animals from retro-orbital plexus under carbon dioxide anaesthesia. All animals were euthanized on day 29 of the study using carbon dioxide asphyxiation. The haematological parameter viz. Hb, erythrocyte count, PCV, MCV, MCHC, eosinophils and basophiles percent and biochemical parameters such as total bilirubin and urea revealed significant ($P < 0.05$) alteration as compared to control group animals. Gross morphological changes include congestion in lung and dilatation of uterus while microscopic changes were characterized by individual cortical cell necrosis in thymus, focal to multifocal thickened alveolar septa in lung and dilatation of uterine lumen. Changes of gross and microscopic were spontaneous or incidental finding. Based on above finding it can be concluded that sub-acute exposure to Dronedarone administered at the dose rate up to 200 mg/kg/day by oral route over a period of 28 days did not produce any appreciable changes.

Keywords: Biochemical, Dronedarone, Haematological, Histopathology, Wistar rats

Efficient cardiac contraction depends on the sinus rhythm and atrioventricular as well as inter and intraventricular synchronization, giving the integrity of the cardiac conduction pathway and well organized excitation-contraction coupling. Arrhythmias are a major public health concern and represent a significant and increasing economic burden for healthcare systems. The most common forms of arrhythmia leading to a high risk of cardiac morbidity and mortality are atrial fibrillation (AF) and ventricular tachycardia/fibrillation (VT/VF). From the clinical viewpoint, arrhythmias are divided into two types: functional and organic arrhythmias. Organic arrhythmias are due to heart disease or structural disorders as stated by Mokhber *et al.* (2001). Horses are one of the most commonly examined animals in terms of assessing the function of the heart and detecting anomalies (Alidady *et al.,* 2002). Common causes of arrhythmias in horses include excitement, fever, toxaemia, colic, electrolyte imbalance, congenital defects, myocarditis, and valvular heart disease (McGuirk and Reef, 2002).

Currently amiodarone is one of the most widely used and most effective antiarrhythmic drugs with little proarrhythmic potential. However, during chronic usage amiodarone and its active metabolite desethylamiodarone can cause serious extra cardiac adverse effects, including effects on the thyroid. Therefore, a safer and effective antiarrythmic agent needs to be developed (Patel *et al.,* 2009). Dronedarone is a newer non-iodinated benzofuran derivative with class I, II, III, and IV antiarrhythmic properties: it blocks sodium channels at rapid pacing rates, lengthens the duration of cardiac action potentials and refractoriness, has Ca^{2+} antagonist activity, and has non-competitive anti-adrenergic activity. Dronedarone has a structural

resemblance to amiodarone, with two molecular changes, i.e. it lacks the iodine moiety and it has a methane sulfonyl group that decreases lipophilicity, resulting in a shorter half-life and lower tissue accumulation (Van Beeren *et al.,* 2003). The most significant differences in Dronedarone from amiodarone are the removal of iodine and the addition of a methane sulfonyl group. The former deletion is postulated to result in little or no thyroid toxicity and the latter addition is said to decrease lipophilicity. Because it is less lipophilic than amiodarone, Dronedarone accumulates less in tissue and has a small volume of distribution. Dronedarone has an elimination half-life of only 13–19 h.

There is an urgent need to study the toxicity of a newer drug like Dronedarone which is not studied at all. Looking to the paucity of literature and very meager information available on toxicity of Dronedarone in laboratory animals in India and abroad, the present study was carried out in Wistar rats.

MATERIALS AND METHODS

Ethical approval

The study protocol followed the ethical guidelines of CPCSEA on the proper care and use of laboratory animals and had been approved by the Institutional Animal Ethics Committee.

Study area

The present study was carried out in the Department of Pharmacology and Toxicology, CRO, Cadila Pharmaceuticals Limited, 1389, Trasad Road, Dholka – 387 810 and Department of Pathology, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar India.

Animals

A total of 48 healthy male and female rats of 5-8 weeks old were selected after physical and behavioral veterinary examination obtained from Breeding Section, Animal House, Cadila Pharmaceuticals Limited, 1389 Trasad Road, Dholka – 387 810. The weight range of all the selected animals was falling within ± 20% of the mean for each sex at the time of initiation of treatment.The rats were selected for this study as per the recommendation in Schedule Y drug and cosmetics (II Amendment) rules, Ministry of Health and Family Welfare, Government of India, January 20th, 2005.

Experimental design

After 5 days of acclimation period all 48 (24 M + 24 F) rats were sex wise randomly divided into four groups on the basis of body weight. Each group consists of 6 male and 6 female animals. Group I served as control and received only vehicle Corn oil till 28 days of dosing period. Group II, III and IV received Dronedarone at dose of 50 mg/kg (Low Dose), 100 mg/kg (Mid Dose) and 200 mg/kg (High Dose) respectively orally every day with 5 mL disposable syringe fitted with 16-G stainless steel rats feeding needle till 28 days of dosing period.

Analysis

The rats were marked on different body parts with picric acid solution prepared in water. The group no., cage no., sex of the animal and animal number was mentioned on cage card. The rats were housed in propylene cages under standard laboratory conditions with standard food and water *ad libitum*. The feed was provided *ad libitum* throughout the study period, except over night (16-20 hours) fasting, prior to pre-termination blood collection. The dark and light cycle was maintained using automatic timer to provide 12 hour light and 12 hour dark.

Vehicle used for diluting Dronedarone to obtain the desired concentration was Corn oil. A repeated dose 10-days range finding study was conducted before commencement of the main study. Based on the results of range finding study, for current study 50 mg/kg b.wt. (Group II/Low Dose), 100 mg/kg b.wt. (Group III/ Mid Dose) and 200 mg/kg b.wt. (Group IV/High Dose) dose levels were selected. According to group wise required amount of Dronedarone was weighed mixed thoroughly with the mortar and pestle to attain desired concentration. The constant dose volume used for all the dose group throughout the study period was 10 ml/ kg b.wt.

Observations

Clinical Signs and Sympatomatology

All animals were observed daily for any mortality, abnormal physical or behavioral changes. The body weight of each rat was recorded one day before initiation of treatment (Day 0) and at weekly intervals throughout the period of study. The last body weight was recorded one day prior to blood collection, exactly before keeping the animals for overnight fasting (Day 28). The feed intake and left over of each rat was calculated at every week throughout the period of study (28 days). The last feed left over was taken on day 28 of the study exactly before keeping the animals for fasting.

Parameters tested

All the haematology parameters viz. Total RBC Count, PCV, Haemoglobin, MCV, MCH, MCHC, Platelets, Total WBC Count, Differential Leucocytes Count were analysed from whole blood using fully automated haematology analyser Advia-120 (Bayer) by using RBC/PLT Reactif GR/PLT, Ery/Thromo RBC/PLT reagent. Biochemical parameters viz. ALT, AST, ALP, serum total bilirubin, serum total protein, serum total albumin, serum urea and serum creatinine were measured by kit from Roche diagnostic, whereas, serum glucose, serum cholesterol and serum triglyceride were measured by kit from Randox on Hitachi 902 Automatic analyzer (Boehringer manheim, Japan). After 28 days all remaining animals were euthanized and necropsy findings were made by systemic approach. For histopathological examinations, tissues from brain, eyes, thymus, adrenal gland, lung, heart, aorta, oesophagus, stomach, duodenum, jejunum, ileum, colon, rectum, liver, kidney, urinary bladder, epididymis, testes, spleen, ovary, uterus, skin, mesenteric lymph node and skeletal muscle were collected in 10% formalin and preserved for processing. The absolute organs weight of liver, kidneys (both the kidneys weighed together), spleen, heart, lungs, adrenals, brain, testes (in male) and uterus (in female) were recorded using analytical balance (Make: Mettler Toledo). The formalin fixed tissues were processed in automatic vaccume tissue processor (LEICA ASP 300) and then paraffin blocks of all the tissues were prepared in automatic tissue embedding station. Sections were cut at 5-7 microns thickness with microtome machine on the glass slide. Thereafter all the slides were stained with haematoxylin and Eosin (H&E) stain (Luna, 1968) in automatic staining machine (LEICA AUTO STAINER XL).

Statistical analysis

The statistical analysis of data generated on various parameters was subjected to statistical analysis using completely randomized design (CRD) (Snedecor and Cochran, 1980) and using CD values compared the treatment means. Since, the CD permits comparison of two consecutive treatment mean after arranging treatment mean in ascending or descending order, it was thought worthwhile to compare treatment mean with all other treatment mean (Overall comparison). Hence, Duncan's New Multiple Range Test (DNMRT) (Steel and Torrie, 1984) was used for the same.

RESULTS AND DISCUSSION

Male and female rats of all four groups did not exhibit any attributable symptoms or even mortality up to the 28 days oral administration of Dronedarone. No sex related difference was noticed. The parameters such as feed consumption, body weight, and organ weight showed no significant variations in treated groups compared with control group. In the present study, haematology in Dronedarone treated rats in general revealed significant ($P < 0.05$) decrease in Hb concentration (low dose and mid group), PCV percent (low, mid and high dose group), total RBC count (low, mid and high dose group) and MCHC (g/dl) (low dose group) while significant ($P < 0.05$) increase in eosinophils per cent in mid and high dose group male rats as compared to their control group males. Female rats of high dose group revealed significant ($P < 0.05$) decrease in MCV (fl) and Basophiles (%), PCV (%) in mid and high dose group, whereas, MCHC (g/dl) was significantly ($P < 0.05$) increase in high dose group. Anemia as observed in present study was also reported with amiodarone (Nishimura *et al.,* 1989; Cohen *et al.,* 1992), which may decrease the number of circulating erythrocytes by impaired erythropoiesis and/or accelerated erythrocyte death (Arpin *et al.,* 1991). Amiodarone triggers phosphatidylserine exposure, one of the hallmarks of suicidal erythrocyte death or eryptosis (Nicolay *et al.,* 2007). MCHC is a measure of the concentration of haemoglobin in a given volume of packed red blood cells. So, decrease in the MCHC might be due to decrease in haemoglobin content of red blood

cells including biological variation. According to (Poitout – belissent and Mccartney, 2010) change in eosinophils and basophiles count is infrequent in toxicological studies unless the test material is haemopoietic growth factor or a cytokine. It is also pertinent to note that till date, there are no published reports available regarding haematological alteration in Dronedarone administered rats. Biochemical parameters such as total bilirubin was found to be significantly ($P < 0.05$) decreased in male of mid dose group while urea was significantly increased in high dose group females. As in present study though mid dose group male showed significant decrease in total bilirubin there was no any change in high dose group, hence it had been concluded that this change was not treatment related. Urea and creatinine are waste products of protein metabolism that need to be excreted by the kidney, therefore marked increase in serum urea as noticed in this study, indicate functional damage to the kidney but histological examination of kidney revealed no any changes related to kidney damage in any of the experimental animals. There was no significant alteration in the values of other biochemical parameters in Dronedarone treated rats as compared to the control group animals.

There were no appreciable macroscopic lesions in any rats treated with various doses of Dronedarone except congestion of lung in only one rat belonging to Group III and dilatation of uterus in one female belonging to Group IV. Microscopic findings in general were not appreciable in any organ except lung, thymus and uterus. Four rats belonging to Group IV receiving the highest dose of Dronedarone in lung showed thickened inter alveolar septa which could be explained by the increased interstitial collagen fiber deposition and marked cellular infiltration with lymphocytes, neutrophils, eosinophils, and macrophages. Such type of lesions was also reported previously with Amiodarone (Zidan, 2011). Other findings in lungs were also observed in control group animal, so, it should be considered as incidental findings. One rat of high dose group showed multifocal individual cell necrosis in thymic cortex. There were no appreciable microscopic changes in the uterus of rats belonging to Group II and III. Group IV (high dose group) rats also showed no lesion except dilatation of lumen in one case only.

CONCLUSION

The absence of appreciable microscopic changes in various organs of different groups (Dronedarone treated) especially in high dose group suggest that the oral administration of Dronedarone up to 200 mg/kg b.wt. for a period of 28 days in rats is safe as compared to amiodarone, a potent anti arrhythmic agent.

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