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SUB ACUTE TOXICITY STUDY OF ONDANSETRON HYDROCHLORIDE IN WISTAR RATS BY NASAL ROUTE

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ABSTRACT

The objective of the present study is to evaluate the potential toxicity in wistar rats of the preclinical safety use of Ondansetron hydrochloride nasal spray. The study design involved 4 groups of 6 animals per each (male and female) at different dose levels. Ondansetron hydrochloride nasal spray has been administered in a constant volume of 2 ml/kg nasally for 28 days. No mortality was seen in any of the treatment groups during the course of study. Various physiological, hematological as well as biochemical parameters were studied and found not to be changed significantly, indicating that Ondansetron hydrochloride nasal spray is nontoxic even at higher dose levels in Wistar rats. Overall tolerability and safety profile of Ondansetron hydrochloride nasal spray is proved good and does not appear to carry the risk of serious adverse effects.

Keywords: Ondansetron hydrochloride, nasal spray, toxicity study, biochemical parameters.

INTRODUCTION

Nasal drug delivery has now been recognized as a very promising route for delivery of therapeutic compounds including Biopharmaceuticals. It has been demonstrated that low absorption drugs can be encountered by using absorption enhancers or increasing the drug residence time in the nasal cavity and that some mucoadhesive polymers can serve both functions. Over the years, the use of Ondansetron hydrochloride in the treatment of antiemetic has been very successful and its historic usage has been useful in drug discovery development.

Ondansetron hydrochloride is a 1,2,3,9-terahydro-9-methyl-(2-methyl-1-H-imidazol-1-yl) methyl -4H-carbazol-4-one, monohydrochloride [1,2]. Ondansetron hydrochloride is a short acting drug for the management of nausea and vomiting. Chemotherapeutic agents and radiotherapy cause release of 5HT in the small intestine initiating the vomiting reflex by activating vagal afferents via $5HT_3$ receptor. It blocks the initiation of these reflexes [3,4]. It has a short biological half life of 3.1 h. Ondansetron hydrochloride was commonly employed antiemetic agent in developing countries for the treatment with chemotherapeutic agents[5]. The popularity and availability of the remedies have generated concerns regarding the safety, efficacy and new drug delivery system [6]. They are considered as safer and less damaging to nasal epithelium. However, the lack of standardization has been a major concern regarding the use of Ondansetron hydrochloride nasal delivery system. Although nasal delivery may be considered to be safe, some are known to be nontoxic at low doses due to mucociliary clearance and others may have a potentially adverse effect after prolonged use [7]. The general public is largely unaware that adverse health effects can be associated with the use of the nasal drug delivery system [8]. Resulting from an overdose, the contaminated formulations have inherent toxicity of the new drug delivery system[9].

The aim of this study was to evaluate the safety profile of the Ondansetron hydrochloride nasal spray delivery system. After applied in a single dose by nasally to the several experimental animals; untreated by nasal of the test animals served as control. The degree of the irritation is read, scored at specified intervals and is further described to provide a complete evaluation of the effects. The duration of the study 28 days repeated dosing on Ondansetron hydrochloride nasal spray for selected biochemical and hematological parameters. Information derived from this test indicates the existence of possible hazards [10] and risks likely to arise from exposure of the nasal epithelium to the Ondansetron hydrochloride. It serves as a basis for classification and permits the selection of optimum dose levels for toxicity studies with repeated exposure by the Ondansetron hydrochloride[11]. Data on nasal irritation are required to support the registration of each manufacturing use product and end use product. OPPTS Harmonized test guideline: Acute nasal irritation 870.25000 [12].

MATERIALS AND METHODS

Study conduct

The study was conducted in central animal husbandry, Raja Muthiah medical college hospital

(RMMCH), Annamalai University, Tamil Nadu, India.

Animals

Total twenty four healthy wistar rats (12 male and 12 female rats, weight ranges from 180-220 g) were selected for the present study. All the animals were acclimatized to laboratory condition for a week before commencement of the experiment. The animals were grouped and housed in polycarbonate cages (6 in each cage) at controlled room temperature of 22^0C ($\pm3^0$C) and a relative humidity between 40 to 60 %, and a constant light dark schedule (12 hours light and 12 hours dark cycle). Animals were fed with Pellet feed supplied by Kalieshwari oil mills Ltd., and fresh water *ad libitum.* All procedures were reviewed and approved by the central animal husbandry, Raja Muthiah medical college hospital (RMMCH). All procedures were reviewed and approved by the institutional animal ethical committee (IAEC no.160/1999/CPCSEA/829). Annamalai University. Animals were divided into four groups of 6 animals each. The group I treated with vehicle (sterile water) was kept as control. Groups II, III and IV treated with 1.08, 3.24 and 10.8 mg/kg body weight corresponding to low, intermediate and high dose, respectively for 28 days according to body weight of each group rats. Exposed to Ondansetron hydrochloride through nasally for 28 days. Animals were observed twice daily for 28 days for any symptoms of toxic exposure to the Ondansetron hydrochloride. At the end of treatment, overnight fasted animals were sacrificed by cervical dislocation, the blood and tissue samples were collected on the 29th day [13]. Physical, physiological, neurological, hematological, biochemical, histological parameters, urinalysis, irritation of the nasal mucosa, gross necroscopy and histopathology of nasal epithelium, brain were measured in all treated groups as well as in the control group. The organs were quickly blotted, weighed on digital balance and processed for histological studies. These studies were evaluated at the end of the experiment.

Physical parameters

Physical parameters (body weight, food and water intake) and local injury were studied during treatment of animals. Mortality was also recorded during treatment of all groups. Physiological (body posture, respirator character, tremors, convulsions, vocalization, palpebral closure, mucous membrane, skin, fur, lacrimation, salivation, piloerction, response to handling), Neurological (number of rearing, gait, tail elevation, head position, pinna reflex, righting reflex, limb tone, abnormal tone, grip strength, loco motor activity). Urinalysis (color, blood, biliburibin, urobilinogen, ketone, protein, glucose, pH, specific gravity) and

Irritation of nasal mucosa (erythma and edema), Autopsy was done if animals died during the course of treatment.

Hematological

Blood samples were withdrawn from the orbital plexus of rats and collected in an EDTA coated vacuette tube and centrifuged at 3000 RPM for about 10 minutes to separate the serum for analysis. Blood samples were analyzed for routine hematological parameters (WBC, RBC, Hemoglobin (Hb), Haematocrit (HCT), Mean corpuscular volume (MCV) and Platelets. Blood cell count was done with blood smears. Hemogram was performed on SYSMEX-KX21, Hematology Analyzer (Beckman Coulter India, Ltd., Mumbai, India).

Biochemical parameters

Blood samples were collected by orbital plexus puncture and collected in plain vacuette tubes, centrifuged at 3000 RPM for about 10 min to separate the serum for biochemical analysis. Albumin, bilirubin, creatinine, alkaline phosphatase (ALP), aspartame (ASP), alanine aminotransferase (ALT), total protein, blood urea nitrogen (BUN) and blood sugar levels were estimated in plasma samples. All parameters were studied by using fully automated random access biochemical auto analyzer by using ERBA XL300 analytical kits.

Histological examinations

Since the nasal delivery bypass the first pass metabolism, brain and nasal epithelium were removed from the scarified animal and the brain sample was stored on 10% formalin solution and stored at -70^0C until analyzed for histological examination.

RESULTS

Physical parameters

There were no significant changes observed in physical, physiological, neurological parameters, urinalysis and irritation of the nasal mucosa in all groups of rats throughout the dosing period. No mortality was observed in all treatment groups throughout the dosing period. There was no significant change in the mean body weight of all the groups as compared with a control group on the 29th day.

Hematology

In male and female rats, no significant changes were observed in hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), platelet counts (PLT), Haemotocrit (HCT) and mean

corpuscular volume (MCV) in all the treated groups as compared to respective control group (Table 1).

Table 1: Hematological parameters in rats

Groups	Vehicle Control	Therapeutic Dose	Average Dose	High Dose
WBC×10^3 (M)	13.4±0.95	13.6±1.03	14.2±2.11	13.3±1.32
WBC×10^3 (F)	13.5±1.62	13.3±1.14	14.2±2.11	13.3±1.32
RBC×10^6 (M)	8.6±0.81	8.5±0.29	7.7±0.38	8.4±0.62
RBC×10^6 (F)	8.2±0.43	8.3±0.21	8.6±0.64	7.4±0.25
Hb gm/dl (M)	13.9±1.90	14.8±0.76	14.5±1.03	14.9±1.13
Hb gm/dl (F)	15.4±1.51	14.7±0.78	15.6±0.78	14.9±0.57
HCT% (M)	51.3±6.43	50.7±4.18	49.8±2.57	52.6±2.53
HCT% (F)	51.4±3.39	52.0±1.44	50.5±2.29	46.6±3.22
MCVμ^3 (M)	63.2±5.3	59.2±5.36	59.4±2.98	58.2±1.30
MCVμ^3 (M)	62.1±2.55	59.9±3.27	57.2±3.21	59.5±2.76
PLT×10^3 (M)	806.8±141.37	938.3±106.13	847.7±72.59	803.4±83.69
PLT×10^3 (F)	958.3±42.14	891.3±34.67	911.8±118.14	854.7±68.48

Biochemical parameters

In male and female rat groups, no significant changes were seen in total serum protein, albumin, urea (BUN), ALT, ALP, ASP activities, glucose, creatinine and bilirubin in all the groups as compared to respective control group (Table 2). However, changes were statistically insignificant.

Table 2: Biochemical parameters in rats

Groups	Vehicle Control	Therapeutic Dose	Average Dose	High Dose
Glucose(mg/dl)(M)	64.2±5.31	69.7±8.66	68.7±4.68	67.8±5.31
Glucose (mg/dl)(F)	64.7±7.99	66.0±5.40	62.7±3.50	68.2±4.58
Albumin(g/dl)(M)	3.9±0.37	3.8±0.29	3.5±0.12	3.8±0.16
Albumin (g/dl)(F)	3.7±0.12	3.6±0.23	3.4±0.20	3.6±0.13
Total serum protein(g/dL) (M)	6.8±0.58	6.8±0.53	6.8±0.45	6.3±0.16
Total serum protein(g/dL) (F)	6.8±0.61	6.8 ±0.31	6.5±0.23	6.5±0.40
ALP μ/L (M)	229.2±17.09	211.0±29.77	216.8±4.12	203.7±28.54
ALP μ/L (F)	174.3±32.32	140.7±39.13	171.5±28.61	139.7±15.64
ALT μ/L (M)	41.0±4.69	47.2±1.47	47.8±4.02	48.0±4.60
ALT μ/L (F)	43.5±2.51	45.3±6.71	44.0±2.1	41.7±9.93
AST μ/L (M)	171.5±16.92	166.5±12.74	161.0±16.57	160.2±10.8
AST μ/L (F)	175.5±15.6	173.8±9.02	153.8±7.99	164.8±8.06
Bilirubin (mg/dL)(M)	0.3±0.09	0.4±0.07	0.3±0.05	0.3±0.11
Bilirubin(mg/dL)(F)	0.3±0.03	0.3±0.17	0.3±0.09	0.3±0.05

Creatinine(mg/dL)(M)	0.7±0.12	0.7±0.07	0.7±0.08	0.6±0.07
Creatinine(mg/dL)(F)	0.6±0.06	0.7±0.11	0.6±0.08	0.6±0.07
BUN (mg %) (M)	16.8±1.17	16.5±1.38	16.2±1.17	17.0±1.10
BUN (mg %) (F)	16.7±1.51	15.8±1.60	16.2±0.98	16.8±0.75

Histological examination

There was no significant treatment related histopathological changes observed in organs of all the treated groups of male and female rats as compared to control group. Histopathological changes in nasal epithelium after treatment was presented in Fig. 1.

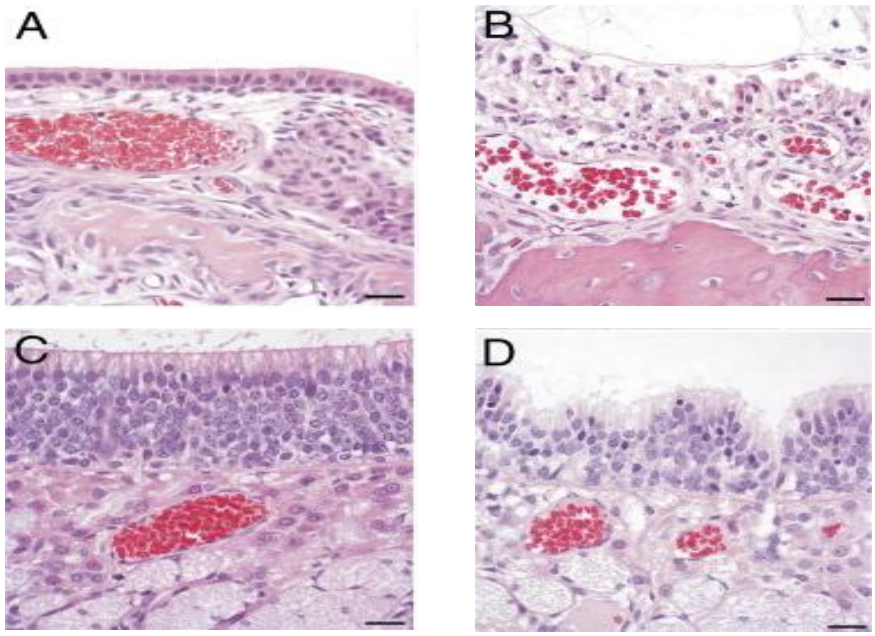

Figure 1: Histopathological changes in nasal epithelium after treatment. (A & B: Normal transition epithelium of the maxilloturbinate in an air control rat and C & D: Normal neuroepithelium of an air-exposed control rat).

Statistical analysis

Resulting data were represented as mean ± SD. Statistical data were analyzed by SAS System 8.2 between control *vs* all treated groups. $P<0.05$ was considered statistically Significant.

DISCUSSION

Ondansetron hydrochloride is an effective simple antiemetic drug. Intravenous administration of Ondansetron hydrochloride has been painful and shown adverse effects. Oral administration of an equivalent dose of Ondansetron hydrochloride and the target concentration achieved more rapidly and with less variability in plasma concentrations compared with enteral formulations[1,11]. In the present investigation, there was no signs of

local injury and inflammatory response at site of nasal epithelium in the treated groups of rat. No behavioral changes were observed during the study period in all the treatment groups. Increase in body weights and growth of treated animals of either sex were of similar pattern as in control groups. Blood was evaluated for hematological toxicity of Ondansetron hydrochloride Hemogram was estimated and results showed no deleterious effect on blood cell count, haemoglobin and other related parameters. There was no gross change in the vital organ collected after euthanization.

The Ondansetron hydrochloride eliminated through renal excretion, thus it is mandatory to estimate effects of Ondansetron hydrochloride on kidney functions. In the present study, biochemical parameters related to kidney function were evaluated and no significant differences were observed in blood urea, creatinine, glucose and proteins with respect to control. However, it has been reported that certain strains of rats that have high concentrations of day after a single, nonlethal dose of Ondansetron hydrochloride no signs of toxicity was found. There were no signs of toxicity was found in organs of histopathological analysis. Thus histopathological studies provides support to the safety data of other physiological, biochemical and hematological parameters of Ondansetron hydrochloride.

CONCLUSION

In summary, our data suggest that Ondansetron hydrochloride nasal spray is safe at high dose than intended to be used for human treatment as it indicates no clinically relevant alterations of any of physiological, hematological and biochemical parameters. In conclusion, our result provides support for safety profile of this potential drug. The data suggest that the Ondansetron hydrochloride is safe even at the maximum dose level and no significant effect was observed on any of physiological and biochemical parameters. Thus, Ondansetron hydrochloride nasal spray is an effective safe antiemetic nasal spray and possessing widely clinical application and worth for wide use.

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