

## Ranitidine treatment inducing methemoglobinemia in male Wistar rats

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*Drug idiosyncrasy is an adverse event of unknown etiology that occurs in a small fraction of people taking a drug. The histamine-2 (H<sub>2</sub>)-receptor antagonist ranitidine causes idiosyncratic reactions in patients. To investigate the hypothesis that ranitidine could induce hematological toxic effects, the drug was administered intraperitoneally (ip) to two of six groups of 200-220 g male Wistar rats (n=6). Group I received as single dose of saline solution (NaCl, 200 µL) Group II received 200 µL of NaCl, ip, at 0, 24, 48 and 72 h, Group III (controls of the vehicle) received as single dose of dimethyl sulfoxide (DMSO, 200µL), ip, Group IV (controls of the vehicle) received 200 µL of DMSO, ip, at 0, 24, 48, and 72 h, Group V received a single dose of 100 mg/kg of ranitidine in 200 µL of DMSO ip, Group VI received 50 mg/kg of ranitidine, in 200 µL of DMSO, ip, at 0, 24, 48, and 72 h. Erythrograms, leucograms were measured; percent methemoglobin content of the blood was analyzed spectrophotometrically. Methemoglobinemia increased to a significant extent following ranitidine treatment, in both study groups. White blood cell and neutrophil counts showed a discrete reduction following either regime of treatment. A marginal mobilization of the neutrophil pool probably occurred. Following the multiple dose regime, leukocyte counts increased, but their distribution maintenance profile remained the same. The administration of ranitidine to rats induces methemoglobinemia in the administered doses, suggesting that from a certain concentration and dosage scheme the substance can be methemoglobinemia inductor.*

### Uniterms

- Ranitidine
- Methemoglobinemia
- Hematologic abnormalities

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## INTRODUCTION

Adverse drug reactions of unknown etiology that occur in a small fraction of the treated population are

defined as idiosyncratic. These reactions are typically unpredictable, show no obvious relation to dose, and display a variable time to onset in relation to start of drug therapy. Idiosyncratic reactions cause not only human

suffering but also sometimes result in curtailing use of otherwise effective therapeutic agents (Luyendyk *et al.*, 2003).

Idiosyncratic drug responses are commonly thought to arise either from drug metabolism polymorphism or from an allergic response to a drug or its metabolite(s). However, for the majority of drugs, supporting evidence for either of these hypotheses is lacking (Luyendyk *et al.*, 2003).

Histamine H<sub>2</sub>-receptor antagonists are widely used in the treatment of gastrointestinal diseases related to gastric acid hypersecretion and cytoprotective effects (Penston, Wormsley, 1986; Aymard *et al.*, 1988; Munoz-Arrebola *et al.*, 1989). Ranitidine, one of such antagonists was first marketed in 1981; since then, many patients have been treated with this drug, and much experience regarding its safety has been gathered (Vial *et al.*, 1991).

A wide array of ranitidine side effects of infrequent incidence has been reported; among them, are several cases of mixed hepatitis of which very few have been well-documented. Ranitidine-associated acute hepatitis has been estimated to occur in less than 1 per 100,000 patients (Penston, Wormsley, 1986). Following long-term treatment of prepyloric or duodenal ulcer with 2 x 150 mg of ranitidine daily, a total of 6 adverse effects were observed in 5 (18.7%) out of 32 patients (Meryn *et al.*, 1983).

Mikawa *et al.* (1999), found that while cimetidine and famotidine slightly reduced O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> production by neutrophils, in a dose-dependent manner, ranitidine failed to do so, and did not appear to have any deleterious effect on neutrophil function, an important consideration for its use in severely ill patients.

In a study using Sprague-Dawley rats, ranitidine was administered orally at doses of 30, 100, 300 and 1000 mg/kg. Animals surviving this dose, presented increased salivation, decreased body weight gain, increased water consumption, increased urinary Na and K excretion, as well as increased serum albumin content and increased liver, kidney and heart weights (Takeuchi *et al.*, 1983).

A case of leukocytosis and eosinophilia has been reported by Gelwan *et al.* (1986), in a patient receiving ranitidine therapy.

Nakada *et al.* (1996) concluded that acute renal failure is one of the risk factors of ranitidine neurotoxicity, and that increased sensitivity of the central nervous system to the drug may contribute towards its toxicity in renal failure.

Luyendyk *et al.* (2003) in co-treatment of rats with LPS and ranitidine resulted in the expected hepatocellular damage as marked by increases in serum ALT and AST activities and cholestatic injury as increases in serum GGT activity, likely to cause idiosyncratic reactions.

In view of the various reports on adverse effects of

ranitidine, we decided to test the drug at respectively single and multiple dose regimes, for effects on hematological parameters and percent increase of methemoglobin in the male Wistar rat.

## MATERIAL AND METHODS

### Animals

Ranitidine was administered intraperitoneally (ip) to six groups of 6 animals each, of 200-220 g male Wistar rats (n=6). Group I received a single dose of saline solution (NaCl, 200 µL) Group II received 200 µL of NaCl, ip, at 0, 24, 48 and 72 h, Group III (controls of the vehicle) received as single dose of dimethyl sulfoxide (DMSO, 200 µL), ip, Group IV (controls of the vehicle) received 200 µL of DMSO, ip, at 0, 24, 48, and 72 h, Group V received a single dose of 100 mg/kg of ranitidine (Glaxo-Wellcome, S.Paulo, Brazil) in 200 µL of DMSO ip, Group VI received 50 mg/kg of ranitidine, in 200 µL of DMSO, ip, at 0, 24, 48, and 72 h. Two hours following treatment, animals were anesthetized with ethyl ether, and blood was collected from the aorta, and used for the determination of hematological parameters and methemoglobinemia.

### Analytical procedures

Hematological measurements included: manual red cell and leukocyte counts (in Neubauer hemocytometer), hematocrit (microhematocrit method), hemoglobin (cyanomethemoglobin method), calculations of red indices, differential leukocyte count (in blood smears stained with Leishman) and reticulocyte count (with the supravital dye brilliant crezyl blue) (Coates, 1995).

All blood samples were assayed for methemoglobin levels relative to hemoglobin levels, by the spectrophotometer technique of Grossman and Jollow (1988) and Coleman *et al.* (1991).

### Statistical analyses

Data were analyzed by ANOVA and by multiple post-test comparisons according to Tukey-Kramer, using the GraphPad InStat and Statgraphics software, the level of significance being set at <0.05.

## RESULTS

No significant differences were observed when we compare the groups that received saline solution (NaCl) in single doses and multiple doses with the groups that

received DMSO (Table I). We compare then the animals that received DMSO and those that received the drugs. Within 2 hours following the administration of a single dose of 100 mg/kg or of 50 mg/kg in multiple doses of ranitidine, rapid, acute methemoglobinemia resulted, showing values of  $5.80 \pm 0.84\%$  and  $6.50 \pm 0.84\%$ , respectively compared to control values of  $1.19 \pm 0.21$  and  $4.22 \pm 0.80\%$ , respectively (Table I). Methemoglobin levels increased significantly after administration of either concentration, suggesting a dose-dependent methemoglobinemic toxic effect of ranitidine (Figure 1).

However, no significant differences in hematological parameters of the red or white cell series was observed in the groups studied (Tables I and II, respectively).

## DISCUSSION

Ranitidine is a  $H_2$ -receptor antagonist that has been in use for the treatment of gastrointestinal diseases for over a decade, thanks to the lack of serious adverse reactions and a long required dosage interval (Fischer, Counteur, 2001).

Adverse reactions to drugs can be divided into two categories. The first occurs when the reaction bears no relation to the mode of action of the drug and occurs well within the intended dosage range. This type of reaction is often termed idiosyncratic, idiopathic or hypersensitivity.

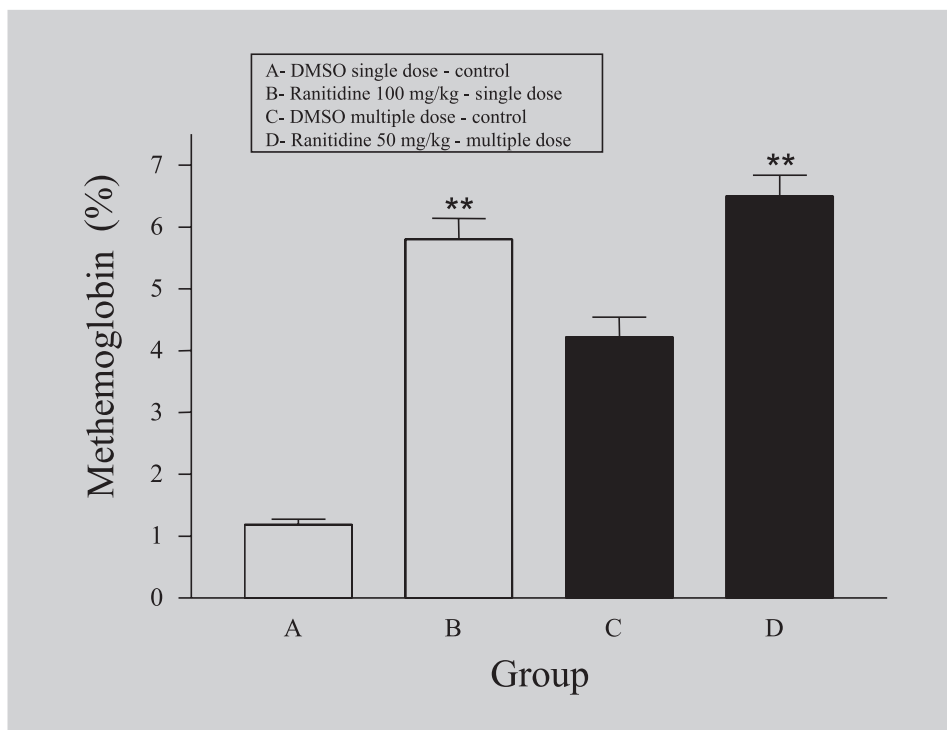
A widely used drug associated with idiosyncratic hepatotoxicity is the histamine-2 ( $H_2$ )-receptor antagonist ranitidine (Luyendyk *et al.*, 2003).

Lewis (1987) after having given ranitidine to tens of millions of patients, reported that its hepatic toxicity supports such a low incidence, and that ranitidine-induced hepatic injury is presumably due to hypersensitivity. Few clinical reports implicate ranitidine as the cause of a predominately cholestatic hepatitis, which resembles ascending cholangitis, by predominantly presenting fever and rigors. Eosinophil infiltration in the liver and a response to rechallenge in some cases, suggest an immunological mechanism for its pathogenesis. A direct toxic action is also probable:  $H_2$ -blockers are toxic to isolated hepatocytes *in vitro*, and also inhibit bile flow. Granulomatous infiltration of the liver has been shown to be evoked by a number of drugs, including ranitidine. This effect sometimes occurs

**TABLE I** - Comparison of hematological parameters (red series) for ranitidine 100 mg/kg administered in single dose and ranitidine 50 mg/kg administered in multiple doses with control groups (DMSO).

Hematological parameters	Control (n=6)	Control (n=6)	Control (n=6)	Ranitidine 100	Control (n=6)	Ranitidine 50
	NaCl Single dose mean $\pm$ SD	NaCl Multiple dose mean $\pm$ SD	DMSO Single dose mean $\pm$ SD	(n=6) Single dose mean $\pm$ SD	DMSO Multiple dose mean $\pm$ SD	(n=6) Multiple dose mean $\pm$ SD
Erythrocytes (millions/mm <sup>3</sup> )	5.25 $\pm$ 0.27	5.30 $\pm$ 0.40	5.00 $\pm$ 0.12	5.10 $\pm$ 0.19	4.98 $\pm$ 0.37	4.95 $\pm$ 0.41
Hematocrit (%)	42.50 $\pm$ 1.85	46.30 $\pm$ 2.10	44.33 $\pm$ 1.21	45.50 $\pm$ 1.87	44.6 $\pm$ 1.23	43.5 $\pm$ 2.42
Hemoglobin (g/dl)	14.9 $\pm$ 1.20	14.25 $\pm$ 0.65	14.7 $\pm$ 0.80	14.51 $\pm$ 0.40	14.03 $\pm$ 1.57	13.66 $\pm$ 1.31
Mean corpuscular hemoglobin (pg)	28.57 $\pm$ 1.85	27.35 $\pm$ 0.95	29.03 $\pm$ 1.58	28.23 $\pm$ 0.48	28.11 $\pm$ 1.67	27.51 $\pm$ 1.29
Mean corpuscular hemoglobin concentration (g/dl)	32.00 $\pm$ 1.52	30.10 $\pm$ 1.12	33.00 $\pm$ 1.09	31.57 $\pm$ 0.87	30.50 $\pm$ 1.35	29.91 $\pm$ 1.01
Mean corpuscular volume (fl)	87.63 $\pm$ 1.20	88.50 $\pm$ 1.15	89.63 $\pm$ 1.41	89.43 $\pm$ 1.56	91.12 $\pm$ 1.67	90.08 $\pm$ 2.67
Reticulocytes (%)	3.25 $\pm$ 0.85	3.15 $\pm$ 1.70	3.08 $\pm$ 0.30	2.50 $\pm$ 1.22	2.89 $\pm$ 0.87	2.91 $\pm$ 0.43
Methemoglobin (%) single dose	1.54 $\pm$ 0.13	3.95 $\pm$ 0.35	1.19 $\pm$ 0.21	5.80 $\pm$ 0.84 **	4.22 $\pm$ 0.80	6.50 $\pm$ 0.84 **

Ranitidine 100 mg/kg -ranitidine in single dose, Ranitidine 50 mg/kg-ranitidine in multiple dose, SD-standard deviation. \*\*-significance at 1% level ( $p < 0.01$ ).



**FIGURE 1** - Effect of ranitidine on percent methemoglobin levels in the rats treated with ranitidine 100 mg/kg in single dose and with ranitidine 50 mg/kg in multiple dose. \*\* $p < 0.01$  for the comparison between B and D with controls groups A and C, respectively.

**TABLE II** - Comparison of hematological parameters (white series) for the ranitidine 100 mg/kg administered in single dose and ranitidine 50 mg/kg administered in multiple dose with control groups (DMSO).

Hematological parameter	Control (n=6) NaCl Single dose mean $\pm$ SD	Control (n=6) NaCl Multiple dose mean $\pm$ SD	Control (n=6) DMSO Single dose mean $\pm$ SD	Ranitidine 100 (n=6) Single dose mean $\pm$ SD	Control (n=6) DMSO Multiple dose mean $\pm$ SD	Ranitidine 50 (n=6) Multiple dose mean $\pm$ SD
Leukocytes (mm <sup>3</sup> )	6.465 $\pm$ 1.12	7.155 $\pm$ 1.955	6.833 $\pm$ 1.07	7.320 $\pm$ 1.899	6.979 $\pm$ 989.15	8.567 $\pm$ 900.40
Neutrophil (mm <sup>3</sup> )	2.135 $\pm$ 865.25	2.578 $\pm$ 955.60	2.713 $\pm$ 996.35	2.685 $\pm$ 948.69	2.317 $\pm$ 807.15	2.243 $\pm$ 675.53

Ranitidine 100 mg/kg -ranitidine in single dose, Ranitidine 50 mg/kg-ranitidine in multiple dose, SD-standard deviation

in the absence of other hepatic abnormalities, but is more commonly associated with an acute hepatitis or cholestatic reaction to the drug. However, some of these reactions are accompanied by a generalized granulomatous reaction.

Other reported side effects have associated ranitidine with hematological abnormalities including granulocytopenia, thrombocytopenia and hemolytic anemia. Agranulocytosis or neutropenia, although of rare occurrence may be severe, even life-threatening. Adverse reactions to drugs are thought to be a common cause of neutropenia; however their study is difficult because they are not predictable and lack of an animal fit for their study.

Autoimmune thrombocytopenia is also related to

treatment with ranitidine. The appearance of this condition calls for the interruption of all medication given to the patient; should this not be possible, drugs in use should be changed. In general, platelet counts rise within 7 days, but this effect may be delayed; patients should be handled in a standard manner. Once a patient has demonstrated sensitivity to the drug, its future use should be carefully avoided.

Choo *et al.* (1994) demonstrated that hemolytic anemia associated with ranitidine is of rare occurrence. Such toxicity is uncommon, but may be exceedingly severe leading to a rapidly fatal outcome in otherwise healthy patients.

The second set of adverse reactions is a predictable intensification of the intended pharmacological response caused by the use of high doses or overdoses of the drug. Such reactions are in general experimentally reproducible, and dose dependent. In the present study, in order to determine hematological alterations caused by ranitidine, we administered the drug in concentrations of 100 mg/kg (single dose) and of 50 mg/kg (multiple doses), and evaluated hematological parameters in the rat.

There was no significant difference in the red blood cells series. In our study, we could find no evidence that ranitidine induce hemolytic anemia. Only one animal presented evidences of hemolytic crisis (not shown). Discrete increment of leukocyte count was observed, but differences in the white blood cells series were not significant. The levels of methemoglobin were significantly increased in comparison to those of control groups.

## CONCLUSION

The results obtained showed that ranitidine can cause increased methemoglobinemia in the administered doses, suggesting that from a certain concentration and dosage scheme the substance can be methemoglobinemia inductor.

## RESUMO

### Metemoglobinemia induzida pela ranitidina em ratos

*A idiossincrasia a fármacos é um evento adverso de origem desconhecida, que acontece em poucas pessoas. É conhecido que a ranitidina, um antagonista de receptores H<sub>2</sub>, causa reações idiossincrásicas. Para investigar uma possível indução da ranitidina em relação a hemotoxicidade, o fármaco foi administrado intraperitonealmente (i.p.) em dois de seis grupos de ratos machos Wistar (n=6), pesando entre 200-220 g. O grupo I recebeu 200µL de solução salina (NaCl) por via intraperitoneal (i.p.), o grupo II recebeu 200µL de NaCl durante quatro dias consecutivos, o grupo III (controle do veículo,) recebeu 200 µL de dimetilsulfóxido (DMSO) em dose única, o grupo IV (controle do veículo) recebeu 200 µL de DMSO, durante quatro dias consecutivos, o grupo V recebeu 100 mg/kg de ranitidina em 200 µL de DMSO em dose única, o grupo VI recebeu 50 mg/kg de ranitidina em 200 µL de DMSO durante quatro dias consecutivos. Realizou-se eritrograma, leucograma e determinação da porcentagem de metemoglobina por espectro-*

*fotometria. A porcentagem de metemoglobinemia aumentou de forma significativa em ambos os grupos tratados. A contagem de neutrófilos mostrou uma discreta redução nos regimes de tratamento, ocasionada provavelmente pela mobilização marginal dos mesmos. No regime de doses múltiplas, observou-se aumento no número de leucócitos, mas seu perfil de manutenção de distribuição permaneceu o mesmo. A ranitidina induziu a formação de metemoglobinemia nas doses administradas, sugerindo que a partir de uma certa concentração e ou esquema de dosagem, o fármaco pode ser um indutor do processo.*

*UNITERMOS: Ranitidina. Metemoglobinemia. Anormalidades hematológicas.*

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