EDITORIAL

Study of Some Toxic Effects of Diminazine in Vitro

Taj Eldin, IM* [1]; Mohamed, OY [2]; Mohamed, AH [3] and Elhadi, MA[4]

[1] Faculty of Pharmacy, Department of Pharmacology, University of Gezira, Wad Medani, Sudan
[2] Faculty of Pharmacy, University of Khartoum
[3] Medicinal and Aromatic Plants Research Institute, National Centre for Research P.O. Box 2404, Khartoum Sudan
[4] Faculty of Pharmacy, Department of Chemistry and Pharmacognosy, University of Gezira, Wad Medani, Sudan
* Corresponding author, omdataj64@gmail.com

Abstract:

Background: The trypanocidal agent, diminazine have been shown to produce some toxic effects such as itching, hypotension and gastrointestinal disturbances among the migratory domestic animals, especially in camels during the dry season in South-western Sudan.
Objective: The present study was an attempt to explain some toxic effects of diminazine in vitro.
Methods: A number of qualitative and quantitative experiments have been done to elucidate these mechanisms using different concentrations of diminazine.
Results: Incubation of different concentrations of diminazine with rat lung chops and their intraperitoneal administration, produced incubates that potently stimulated the isolated guinea-pig ileum. The obtained contractions were dosedependent and effectively blocked by the anti-histamine, diphenhydramine. The yielded incubated mixtures when developed on paper chromatography with authentic samples showed two spots with R_f values and colours similar to those of heparin and histamine when sprayed with ninhydrin or exposed to iodine vapour. The extent to which diminazine released histamine was determined by measuring the concentrations of the released histamine using the three-point assay. The addition of EDTA, diltiazem and dinitrophenol separately to the incubated mixtures indicated that the release of histamine and the accompanied heparin was calcium-and energy-dependent, most probably by exocytosis and damaging the lung and peritoneal mast cells.
When tested on isolated rabbit jejunum, diminazine was found to potentiate the effect of histamine with pA1/2 value of 5.4 ± 0.13 compared to 5.6 ± 0.15 for aminoguanidine, the standard diamine oxidase inhibitors.
Conclusion: Diminazine was found to have a potent histamine releasing capacity. These findings indicated that the severe toxic effects produced by diminazine might be due to its ability to release histamine and/or potentiating its effects.

Key words: Diminazine aceturate, Histamine, Diamine oxidase inhibition.

EDITORIAL

Introduction:

Trypanosomiasis is a disease that affects both human and animal in all tropical areas. The disease has a major economic importance in Africa; especially in tsetse endemic areas [(1)]. In Sudan, trypanosomiasis was first described in 1904 in donkeys and camels in Bahr Elgazal and in cattle in Malut in the Upper Nile and in some other parts of the country. During the dry season of the year 1983, there was an outbreak of trypanosomiasis among the migratory domestic animals, especially in camels [(2)]. The use of antitrypanosomal drugs for cure and prophylaxis of trypanosomal infection is one of the several techniques used in controlling trypanosomal infection in man and animals. Treatment and prophylaxis of trypanosomiasis in cattle, sheep and goats is dependent upon the most important three compounds; diminazine aceturate, homidium and isometamidium [(3)]. The treatment of livestock with standard therapeutic doses of diminazine (3.3-7 mg/kg) sometimes results in signs of toxicity [(4)]. In camels, however, a single dose of 7 mg/kg can be highly toxic. Diminazine is also relatively toxic in dogs [(5)]. Experimentally, intramuscular administration diminazine in doses exceeding 10 mg/kg, once or repeatedly, results in severe signs of disturbances in the gastrointestinal tract, musculoskeletal, respiratory and nervous systems; hypotension and itching are also observed in both camels and dogs [(6)]. In laboratory rodents, hypotension is commonly observed [(7)]. At necropsy, gross and histopathological lesions have been described in the liver, urinary bladder, lungs, heart and brain of dogs and camels.

The current study was carried out to explain the mechanisms of some toxic effects of diminazine in vitro.

Materials and Methods:

Qualitative Bioassay

Different concentrations (10, 20, 40, 80, 160, 320 µg/ml) of the trypanocidal agent, diminazine (Berenil, Hoechst Co. Ltd) were used and incubated separately with rat lung chops. The obtained incubates were separately assayed using guinea-pig ileum preparation and oscillograph (Harvard Apparatus Limited). The resultant contractions were effectively blocked by the antihistamine diphenhydramine maleate (100 ng, Sigma Chemical Co.) [(8)]. Histamine (Sigma Chemical Co) and heparin (Rotexmedick, GMPH, Germany) were used as authentic samples and co-chromatographed with incubates on paper (Whatman No. 1); n-butanol, acetic acid and water (BAW 4:1:5) were used as solvent system. **Quantitative Bioassay**

EDITORIAL

Also another concentrations of diminazine (10, 20, 40, 80, 160, 320 µg/ml) were incubated separately with rat lung chops and the obtained mixtures were assayed against standard known concentrations of histamine (5, 10, 20, 40 ng/ml) using the guinea-pig ileum preparation and oscillograph. The same concentrations of diminazine were administered intraperitoneally to rats and the obtained incubated mixtures were assayed by the same previously mentioned method. The chelating agent EDTA (5 ng/ml), diltiazem (20 mg/ml) and dinitrophenol (5 ng/ml) were added separately to the incubated mixtures to elucidate the mechanism by which diminazine may release histamine.

Aminoguanidine, in different concentrations (0.1, 0.2, 0.4, and 0.8 µg/ml) and diminazine in concentrations of (1, 2, 4 and 8 µg/ml) were studied separately in isolated rabbit jejunum to determine their effects on the enzyme, diamine oxidase, by measuring their pA1/2 values. [8]

Statistical Analysis

Data were expressed as means ± standard error of means (SEM). Each value is the mean of three experiments.

Results and Discussion:

The biological assays of the different incubated mixtures of diminazine in high concentrations (80, 160, and 320 µg/ml), using guinea-pig ileum preparation, resulted in a concentration-dependent contraction of guinea-pig ileum. The various responses produced by the different incubates of diminazine on the guinea-pig ileum were effectively blocked by the anti-histamine, diphenhydramine (100 ng/ml). Moreover, paper chromatographic analysis predominantly showed the presence of two brown spots after exposure to iodine vapour and ninhydrin spray. The two brown spots have the same R_f values as authentic histamine and heparin, which indicated that there may be a release of histamine by diminazine accompanied by release of heparin from the rat lung mast cells. Diminazine may facilitate the process of exocytosis of the content of the mast cell granules, which may result in degranulation of the mast cells and the release of histamine and heparin [9]. This may explain the mechanism by which diminazine induced this release. The addition of low concentrations of diminazine (10 and 20 µg/ml) to the rat lung chops may result in slight release of histamine; this was evident as very weak contractions were produced when the incubated mixtures were assayed using guinea-pig ileum preparation and oscillograph. These contractions were also blocked by diphenhydramine (100 ng/ml).

As shown in Table 1, the incubated mixtures of higher concentrations of diminazine (40, 80, 160 and 320 µg/ml) resulted in different contractions when assayed separately against histamine using guinea-pig preparation and oscillograph. The rate of histamine release increased as the concentrations of diminazine increased. These responses were also blocked by diphenhydramine (100 ng/ml).

EDITORIAL

Table 1: Mean Histamine Release from Rat Lung Mast Cells by Different Concentrations of Diminazine

Diminazine Concentrations (µg/ml)	Histamine Released by Diminazine Concentrations (ng/gm) wet weight of lung tissues)
40	27±0.16
80	48±0.14
160	65±0.135
320	115±0.11

The release of histamine from rat peritoneal mast cells by diminazine occurred in a concentration-dependent manner when different incubates of diminazine were assayed separately against histamine using guinea-pig ileum preparation and oscillograph. The responses produced by diminazine were inhibited by diphenhydramine (100 ng/ml), (Table 2). It was also shown that diminazine caused more release of histamine from rat lung chops and peritoneal mast cells. This release of histamine may explain some of the toxic effects of diminazine such as itching and hypotension and gastrointestinal disturbances.

Table 2: Mean Histamine Release from Rat Lung Peritoneal Mast Cells by Different Concentrations of Diminazine

Diminazine Concentrations (µg/ml)	Histamine Released by Diminazine Concentrations (ng/gm.wt)
20	13±0.23
40	23±0.19
80	42±0.15
160	73±0.13

The chelating agent ethylene diamine tetra acetic (EDTA) inhibited the release of histamine from the rat lung mast cells when the different lower concentrations (10, 20, 40, and 80 µg/ml) of diminazine were incubated separately with rat lung tissue. No responses were produced when the different incubated mixtures of diminazine were assayed using guinea-pig ileum as the presence of EDTA inhibits the exocytotic release of histamine since calcium ions are necessary for exocytosis [(9)]. In the current study, the release of histamine is a calcium dependent process.

The addition of the calcium channel blocker, diltiazem, to the chopped tissues in the presence of diminazine seems to retard the release of histamine, where no

EDITORIAL

contractions were produced when different incubated mixtures were assayed separately using guinea-pig ileum preparation. Thus the presence of diltiazem in the incubate may have some direct effect on the tissue or may affect the calcium channels or calcium content within the tissue, and that confirmed the previous results obtained with EDTA.

Furthermore, the addition of dinitrophenol (inhibitor of ATP synthesis) to the different incubates of diminazine showed inhibition of histamine release from rat lung mast cells, as no contractions or responses were obtained when different incubates of diminazine were assayed separately using the guinea-pg ileum preparation. This indicated that the release of histamine is energy-dependent process.

It was also noticed that the addition of diminazine at the concentrations (1, 2, 4, and 8 µg/ml) potentiated the responses of half-submaximal dose o histamine. This indicated that diminazine also have inhibitory effects on the enzyme diamine oxidase.

It was early reported that diminazine at doses similar to those releasing histamine may also seem to inhibit acetylcholinesterases, which may result in increasing acetylcholine concentrations that may explain some of the toxic effects produced by diminazine [(10)].

Toxicity studied have been associated to either the release of or an interaction with autacoids such as serotonin and histamine and cholinergic agents by the trypanocidal drugs within the host tissue. [(11-14)]

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EDITORIAL

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