Pharmacological Reports
2007, 59, suppl. 1, 232–236
ISSN 1734-1140

Action of thioperamide in the immunosuppressive test in mice

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Abstract:
The present experiment was conducted to investigate the influence of thioperamide on the immunological system.
The study was performed on female albino Swiss mice. Mice were divided into group A – without adrenalectomy and five groups with adrenalectomy. In group B – 0.9% saline solution, in group C – prednisolone, in groups D, E and F – thioperamide (3, 15 and 45 mg/kg) were administered. All substances were given for 4 consecutive days. Then the mice were sacrificed, thymuses and spleens were weighted and thymocytes were selected.
Adrenalectomy caused an increase in thymus weight and lymphocyte number. Prednisolone prevented these changes. Thioperamide inhibited thymus enlargement and decreased the number of thymocytes but the changes were not so pronounced. The strongest influence of thioperamide on those parameters was observed in group E. No influence of thioperamide on spleen weight was noted.
Immunosuppressive properties of thioperamide were revealed but explanation of the exact mechanism requires further studies.

Key words:
thioperamide, immunosuppressive test, prednisolone, mice

Introduction

Histamine is present in almost all body tissues and plays an important biological role. It exerts important immunomodulatory effect through H_1-, H_2-, H_3- and H_4-receptors. Depending on the predominance of the type of histamine receptor, histamine may have proinflammatory or anti-inflammatory effects. Acting through the H_1-receptor, histamine has proinflammatory properties and is involved in the development of several aspects of antigen-specific immune response, including the balance of type 1 helper (Th1) T cells and type 2 helper (Th2) T cells. Histamine may also block humoral immune responses through an increase in the production of interferon. Histamine also induces the release of proinflamatory cytokines and lysosomal enzymes from human macrophages and modulates the activity of basophils, eosinophils, and fibroblasts. In addition, trough the H_1 receptor it plays a role in autoimmunity and malignant disease [3, 8]. In contrast to activation of H_1- and H_3-receptor, the stimulation of H_2-receptor can suppress antigen-presentation capacity [3]. Histamine, acting *via* H_2-receptors on monocytes/macrophages, suppresses the activity of a key enzyme in oxygen radical formation, the NADPH oxidase. By this mechanism, histamine protects NK cells and T cells against oxygen radical-induced dysfunction and apoptosis, and also maintains their activation by interleukin 2 (IL-2) and other lymphocyte activators [9].

Based on these data, it was suggested that substances modulating histamine synthesis, release and

action may possess also immunomodulatory properties. It is known that H_3-receptor antagonists (e.g. thioperamide and clobenpropit) increase histamine synthesis and release from the histaminergic neurons in the brain and peripheral cells [4, 10, 22]. The released histamine may subsequently exert its immunomodulatory action on immunological cells *via* H_1-, H_2- and/or H_4-receptors. Other data from *in vitro* studies reported a suppressive effect of thioperamide and clobenpropit on neoplastic cell proliferation [18].

Based on these findings, the present experiment was conducted to investigate *in vivo* the influence of thioperamide administration on the immunological system.

Materials and Methods

Animals

The experiment was performed on female albino Swiss mice bred in the animal house of the Department of Pathological Anatomy, Wrocław Medical University. The average mice weight in the groups at the beginning of the experiment was 23.6–25.8 g. Animals were housed up to five per cage, at 22°C and 40% humidity under 12 h light-dark cycle, with free access to water and standard food.

Chemicals

Thioperamide maleate *in subst.* (Sigma Aldrich, Germany), thiopental – amp. a 0.5 g (Biochemie, Austria), prednisolone sodium tetrahydrophthalate – amp. a 0.025 g (Jelfa, Poland), 0.9% natrium chloratum – amp. a 10 ml (Polpharma S.A., Poland).

Experiment

The experiment was carried out according to Santisteban's immunosuppressive test described earlier [16]. Mice were divided into six groups: A – without adrenalectomy, in which 0.9% saline solution was administered and five groups (B, C, D, E and F) in which adrenalectomy was conducted on day 0 of the experiment. In group B only 0.9% saline solution was administered, in group C prednisolone at a dose of 5 mg/kg was given, in groups D, E and F thioperamide was given at the doses of 3, 15 and 45 mg/kg, respectively. Saline solution and the studied substances were given intraperitoneally in a volume of 10 ml/kg, once a day in the morning from day 1st to 4th of the study. Mice were observed and weighted every day. The mice were sacrificed on the 5th day of the experiment. Thymuses and spleens were isolated and weighted. Based on these data, the relative weights of these organs (in relation to body weight) were assessed. Thymuses were subsequently ground and thymocytes were selected and counted in Thoma's chamber.

All the procedures followed in these experiments adhered to the ethical guidelines of Medical University in Wrocław and received approval of the Local Ethics Commission for Studies on Animals in Wrocław.

Statistical analysis

Data were presented as the mean value and SD. Differences between the control group and experimental groups were evaluated using one-way analysis of variance (ANOVA). The STATISTICA 6.0 software was used, $p < 0.05$ was considered to be statistically significant.

Non-relative weights were assessed in milligrams [mg]. Relative weight of thymuses and spleens were expressed as percentage of body weight [%]. Number of thymocytes was calculated in millions per 1 ml in Thoma's chamber and are also expressed as millions per 1 g of thymus weight.

Results

Detailed values (mean ± SD) of initial and final body weight, non-relative and relative weight of thymus and spleen and number of thymocytes are shown in Table 1.

In all examined groups, body weight loss at the end of experiment compared to the 0 day of the study was observed. In groups receiving prednisolone (group C), thioperamide at doses of 3 mg/kg (group D) and 45 mg/kg (group F) the loss of body weight was significant ($p < 0.001$).

Adrenalectomy caused an increase in non-relative and relative thymus weight (group B) in relation to the group of mice without adrenalectomy (group A)

Tab. 1. Effect of thioperamide on initial and final body weight, non-relative and relative thymus weight, thymocyte number and spleen weight in Santisteban's immunosuppressive test. Values are expressed as the mean and SD. Group A – mice without adrenalectomy receiving 0.9% saline solution, other groups – mice after adrenalectomy: B – receiving 0.9% saline solution, C – receiving prednisolone at a dose of 5 mg/kg, D, E and F – receiving thioperamide at doses of 3, 15 and 45 mg/kg, respectively. All substances were administered intraperitoneally. Significant comparisons between groups are mentioned in the text

Parameter		Groups					
		A N = 20	B N = 24	C N = 10	D N = 10	E N = 14	F N = 10
Body weight	Initial [g]	23.8 ± 1.7	23.6 ± 1.5	24.4 ± 0.9	24.7 ± 0.7	24.4 ± 2.0	25.8 ± 0.8
	Final [g]	23.5 ± 2.1	22.8 ± 2.1	21.5 ± 1.3	22.6 ± 1.1	23.7 ± 2.6	21.0 ± 2.2
Thymus weight	Non-relative [mg]	55.8 ± 18.8	75.0 ± 20.1	38.1 ± 11.1	65.3 ± 23.1	52.0 ± 13.1	57.8 ± 37.0
	Relative [%]	0.24 ± 0.08	0.33 ± 0.09	0.16 ± 0.05	0.26 ± 0.09	0.21 ± 0.06	0.23 ± 0.14
Thymocyte number	Non-relative [mln/ml]	115.0 ± 50.5	160.0 ± 53.9	41.0 ± 22.1	97.1 ± 47.8	87.4 ± 44.2	102.3 ± 77.5
	Relative [mln/g of thymus weight]	2042 ± 479.0	2170 ± 646.7	1003 ± 340.6	1440 ± 309.9	1650 ± 676.9	1746 ± 752.6
Spleen weight	Non-relative [mg]	121.8 ± 32.6	177.2 ± 52.9	108.0 ± 20.9	184.9 ± 47.3	182.9 ± 50.5	168.0 ± 56.9
	Relative [%]	0.52 ± 0.13	0.78 ± 0.24	0.44 ± 0.09	0.75 ± 0.19	0.75 ± 0.18	0.65 ± 0.25

(in both cases $p < 0.005$). Prednisolone administration (group C) prevented these changes. Differences in non-relative and relative thymus weights between group C and B were statistically significant in both cases ($p < 0.001$).

In groups receiving thioperamide, the inhibition of thymus enlargement was not so pronounced as in the group treated with prednisolone.

In all groups receiving thioperamide (D, E and F), like in the group treated with prednisolone, non-relative thymus weights were lower than in the group B. Only the difference between the group in which thioperamide was administered at a 15 mg/kg dose (group E) and the group B was statistically significant ($p \leq 0.005$).

Non-relative thymus weights in groups receiving thioperamide at all experimental doses (groups D, E and F) were greater than in the group receiving prednisolone (group C). Differences between groups in which thioperamide was administered at doses of 3 mg/kg and 15 mg/kg (groups D and E) compared to the group C were statistically significant ($p \leq 0.005$ and $p \leq 0.02$, respectively).

The results based on the relative thymus weight were quite similar to those achieved for non-relative weight.

Similarly, the number of thymocytes in the group of mice after adrenalectomy (group B) was statistically significantly greater than in the group of healthy mice (group A) ($p < 0.001$). As it was expected, the number of thymocytes after prednisolone administration (group C) was lower than in group B and even lower than in healthy animals (group A) ($p < 0.001$ in both cases). Thioperamide, at all examined doses, exerted similar effect to prednisolone. The number of thymocytes was significantly lower in groups receiving thioperamide at doses of 3 mg/kg (group D), 15 mg/kg (group E) and 45 mg/kg (group F) compared to the group B ($p \leq 0.005$, $p \leq 0.001$ and $p \leq 0.02$, respectively). The influence of thioperamide on thymocyte number was less pronounced than that of prednisolone. In all groups receiving thioperamide, the number of thymocytes was greater than in the group C ($p \leq 0.005$, $p \leq 0.005$ and $p \leq 0.05$, respectively).

Adrenalectomy caused an increase in non-relative and relative spleen weight (group B) in relation to the group of mice without adrenalectomy (group A) (in both cases $p < 0.001$). The lowest non-relative and relative weight of the spleen was observed in the group of mice after adrenalectomy receiving prednisolone (group C). The differences between groups C and B were statistically significant ($p \leq 0.001$ in both cases). No influence of thioperamide on spleen weight was observed, regardless of the dose.

Discussion

Nowadays many researches are focused on immunosuppressive activity of different compounds [15]. Im-

munomodulatory properties of imidazole derivatives (e.g. omeprazole or cimetidine) have been already described in several papers. There are a lot of different tests used to estimate immune response in experimental conditions [23]. In the Santisteban's test, omeprazole exerted immunosuppressive or immunostimulatory effect in a dose-dependent manner [19]. It is known that cimetidine is an immunostimulatory agent [21]. It increases the number of tumor infiltrating lymphocytes in colon cancer what is considered to be a good prognostic factor [1, 2].

The purpose of this study was to assess the influence of another imidazole derivative, thioperamide, on the immunological system. As a reference, a well-known immunosuppressive drug, prednisolone, was also used in the experiment. The immunosuppressive properties of thioperamide observed in the study were not as evident as those of prednisolone. The strongest effect was observed when thioperamide was used at 15 mg/kg dose.

Inhibition of thymus enlargement, thymocyte number and loss of body weight were used as indicators of the immunosuppressive effect of the studied substance. The greatest loss of body weight was observed in the group receiving thioperamide at the dose of 45 mg/kg. The mechanism of this phenomenon could be multifactorial. One of the potential mechanisms may involve the inhibition of food intake provoked by thioperamide. In our previous study anorectic properties of betahistine (agonist of H_1 and antagonist of H_3-histamine receptors) were demonstrated [20].

The mechanism of the immunosuppressive action of thioperamide has not been fully examined. It could be connected with an increase in histamine synthesis and release after H_3-histamine receptor blockade by thioperamide. The released histamine may subsequently exert its immunomodulatory action *via* various histamine receptors located on immunological cells. It was demonstrated that amounts of histamine released locally in tumor tissue are sufficient to affect proliferation and function of normal lymphocytes, exerting immunosuppressive effect. Because cimetidine counteracts this immunosuppressive effect, the involvement of H_2-receptors is postulated [2, 12–14].

Recently, a novel class of histamine receptor, named H_4 receptor, was described. It is most closely related to the H_3-histamine receptors [11]. The distribution of H_4 mRNA suggests that it may play a role in the regulation of immune function [17]. The H_4-receptor transcripts were found to be highly expressed in the tissues and cells implicated e.g. in inflammatory responses such as leukocytes, spleen, lung and liver [6]. Blockade of the H_4-histamine receptors on dentritic cells leads to a decrease in cytokine and chemokine production *in vitro*. Data suggest the potential therapeutic use of H_4-histamine receptors antagonists as anti-inflammatory drugs [7]. A full interpretation of the results of the present study is difficult because of the fact that thioperamide is now considered to be dual H_3/H_4-receptor antagonist [5]. Direct blockade of H_4-receptors by thioperamide may be also involved in anti-inflammatory and/or immunosuppressive action. Therefore, further studies with selective H_4-histamine receptor antagonists are necessary.

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Received:
March 12, 2007; in revised form: July 25, 2007.