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Short communication

Selected azaphenothiazines inhibit delayed type hypersensitivity and carrageenan reaction in mice

Jolanta Artym [a], Iwona E. Kochanowska [a], Maja Kocięba [a], Ewa Zaczyńska [a], Michał Zimecki [a,*], Małgorzata Jeleń [b], Beata Morak-Młodawska [b], Krystian Pluta [b]

[a] Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wrocław, Poland
[b] The Medical University of Silesia, School of Pharmacy with the Division of Laboratory Medicine, Department of Organic Chemistry, Jagiellońska 4, 41-200 Sosnowiec, Poland

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ABSTRACT

Several, previously selected azaphenothiazines, as strongly antiproliferative agents in *in vitro* models, were subjected to evaluation for their potential immunosuppressive effects in the model of delayed type hypersensitivity (DTH) to ovalbumin (OVA) in BALB/c mice and in foot pad inflammation induced by carrageenan in CBA mice. In the DTH model the compounds were given to mice intraperitoneally (*i.p.*) in 50 μg or 250 μg doses, 1 h before the elicitation of the response. In the carrageenan-induced foot pad inflammation the compounds were given *i.p.* in 50 μg or 250 μg doses, 24 h and 2 h before administration of carrageenan. Among the compounds, the significantly suppressive activities in both models were exhibited only by compound **5** (6-chloroethylureidoethyldiquino[3,2-b;2′,3′-e][1,4]thiazine) and compound **4** (6-acetylaminobutyl-9-chloroquino[3,2-*b*]benzo[1,4]thiazine). Structure-activity relationship, plausible mechanism of action and potential application in therapy of the compounds are discussed.

1. Introduction

Search for new drugs was recently accelerated because of constant increase of allergic and autoimmune diseases, as well as for organ transplant purposes. Presently available immunosuppressive drugs, although very effective, have undesirable side-effects, leading to, among others, to opportunist infections, lymphopenia, kidney dysfunction or hypertension, particularly upon prolonged treatment [1,2]. Hence, search for new classes of immunosuppressive drugs, presenting better therapeutic profiles, is a constant challenge.

Phenothiazines belong to a class of compounds demonstrating various biological activities, such as: antipsychotic, antihistaminic, antitussive and antiemetic [3]. Recently, new important properties of phenothiazines were found, such as antitumor, antibacterial and associated with them anti-multidrug resistance (MDR) activity, summarized in [4,5,6,7,8,9,10]. Neuroleptic phenothiazines are also strong modulators of the immune response, as for example chlorpromazine. The compound was found to suppress both the humoral and cellular immune responses [11,12].

Tricyclic phenothiazines attract considerable attention due to their significantly variable biological activities and interesting chemical features. Classical phenothiazines with aminoalkyl substituents are an important source of valuable drugs [3]. Modifications of these compounds have been conducted by introduction of new groups, mainly at thiazine nitrogen atom and by substitution of one or two benzene rings with homo- or heteroaromatic rings. Modifications of phenothiazines with azine rings originated azaphenothiazines. Such phenothiazines may contain not only a tricyclic ring system but also tetra- and pentacyclic ones with additional nitrogen atoms (from 1 to 4) in aromatic rings [13,14].

Recently we synthesized series of azaphenothiazines by replacing benzene rings with pyridine and quinoline rings [15–19]. These studies comprised screening of about 150 compounds for their potential biological activities, mostly in *in vitro* tests. Several compounds from that group strongly inhibited phytohemagglutinin A (PHA)-induced proliferation of human peripheral blood mononuclear cells and growth of tumor cell lines. In addition, some of them strongly suppressed lipopolysaccharide (LPS)-induced tumor necrosis factor alpha (TNF α) production in cultures of whole human blood. Importantly, the selected compounds did not affect the viability of human lymphocytes up to concentration of 100 μg/ml.

Among these compounds only five, showing best suppressive activities, were chosen for evaluation of their potential suppressive potential in mouse *in vivo* models. For this purpose we applied the models of delayed type hypersensitivity (DTH) to ovalbumin (OVA)

Abbreviations: anti-MDR, anti-multidrug resistance; cFa, complete Freund's adjuvant; DEX, dexamethasone; DMSO, dimethylsulfoxide; DTH, delayed type hypersensitivity; iFa, incomplete Freund's adjuvant; *i.p.*, intraperitoneally; LPS, lipopolysaccharide; MLR, mixed lymphocyte reaction; OVA, ovalbumin; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; *s.c.*, subcutaneously.

* Corresponding author.
E-mail address: zimecki@iitd.pan.wroc.pl (M. Zimecki).

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and carrageenan-induced foot pad inflammation. The results showed that only two compounds were significantly suppressive in these models. A possible explanation of their mechanism of action was delivered.

2. Material and methods

2.1. Mice

Female BALB/c and CBA mice were purchased from the breeding center of The Institute of Laboratory Medicine, Łódź, Poland, when 8–10 week old. The mice were kept at 12 h/12 h light/dark conditions, fed a granulated commercial food and filtered tap water *ad libitum*. The studies obtained a permission # 64/2015 of a local ethics committee from The Institute of Immunology and Experimental Therapy, Wrocław, Poland.

2.2. Reagents

Ovalbumin, carrageenan and DMSO were from Sigma, Freunds' complete (cFa) and incomplete (iFa) adjuvants were from BD Biosciences, US, dexamethasone (Dexaven®) from Jelfa, Poland, Vetergesic Vet® from Orion Pharma, Great Britain, and 0.9% saline solution from Polpharma SA, Poland.

2.3. The azaphenothiazines

The syntheses of the compounds from the appropriate pyridine and quinoline compounds followed by the introduction of the substituent at the thiazine nitrogen atom were described elsewhere [15–19]. The structures and biological characteristics of the compounds are presented in Table 1. For the experiments the compounds were initially dissolved in DMSO (1 mg in 100 μl DMSO), diluted in physiological saline for injection and given intraperitoneally (*i.p.*) to mice in volume of 0.2 ml.

2.4. Delayed type hypersensitivity

Mice (n = 8) were sensitized subcutaneously (*s.c.*) with 5 μg of OVA emulsified in cFa in the tail base. After 4 days the mice were challenged *s.c.* with 50 μg OVA in iFa in both hind footpads. Following next 24 h the footpad thickness was measured using a spring caliper. The compounds were administered to mice *i.p.*, at 50 μg and 250 μg dose, 1 h before the eliciting dose of antigen. Dexamethasone (DEX), as reference drug, was used in a dose of 30 μg, *i.p.*, 1 h before the eliciting dose of antigen. According to the ethics committee recommendations the mice were treated *s.c.* for the whole period of the experiment, every 8 h, with 0.1 mg/kg b.w. of Vetergesic Vet®. Background (BG) mice were not sensitized but received the challenging dose of OVA in iFa; the value from this non-specific inflammatory response was subtracted from the responses measured in sensitized mice. The results were presented as a mean value of antigen-specific increase of footpad thickness measured in 8 mice (16 measurements) and expressed in DTH units (one DTH unit = 0.01 cm) ± standard error (SE).

2.5. Carrageenan test

The compounds were given *i.p.* at doses of 50 μg or 250 μg per mouse 24 h and 2 h before carrageenan injection. DEX, as a reference drug, was used at a dose of 30 μg, *i.p.*, 24 h and 2 h before carrageenan injection. The mice (n = 8) were given 2% carrageenan solution (100 μg in 50 μl of 0.9% saline) *s.c.* into both hind foot pads and after 4 h the foot pad thickness was measured by means of a spring caliper. BG mice were injected with saline only; the thickness of these sites was subtracted from the responses measured in sensitized mice (injected with carrageenan). The results were presented as a mean value of antigen-specific increase of footpad thickness measured in 8 mice (16 measurements) and expressed in mm ± SE.

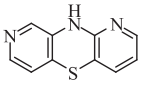
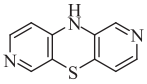
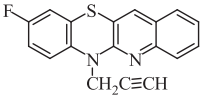
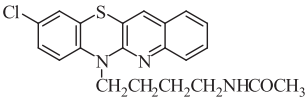
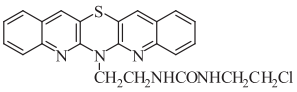
2.6. Statistics

The results are presented as mean values ± SE. Brown-Forsyth's test was used to determine the homogeneity of variance between groups. Due to non-constant variance, the data were analyzed using the non-parametric the Kruskal-Wallis' analysis of variance, followed by Dunn's test to estimate the significance of the difference between groups. Significance was determined at $p < 0.05$. Statistical analysis was performed using STATISTICA 7 for Windows.

3. Results and discussion

The studied compounds were evaluated for their potential activities in suppression of the eliciting phase of delayed type hypersensitivity to OVA (Fig. 1). The compounds were given *i.p.* to mice, in 50 μg and 250 μg doses, 1 h before administration of the eliciting dose of antigen (OVA). Control mice received 0.9% saline or DMSO in saline in concentrations corresponding to DMSO dilutions in solutions of the compounds. DEX (30 μg/mouse dose) was used as the reference drug. The results show

Table 1
The characteristics of the compounds.

#	Structure	Chemical name	Biological activity	Ref.
1		10*H*-1,8-diazaphenothiazine	Suppressive for PHA-induced human PBMC proliferation and growth of tumor cell lines	[19]
2		10*H*-2,7-diazaphenothiazine	Inhibitory for the humoral and cellular immune response in mice and TNF α production by human PBMC	[15]
3		9-Fluoro-6-propargylquino[3,2-*b*]benzo[1,4]thiazine	Suppressive for PHA-induced human PBMC proliferation, mixed lymphocyte reaction (MLR), tumor growth and LPS-induced TNF α production by human PBMC	[18]
4		6-Acetylaminobutyl-9-chloroquino[3,2-*b*]benzo[1,4]thiazine	Suppressive for PHA-induced human PBMC proliferation, tumor growth and LPS-induced TNF α production by human PBMC	[17]
5		6-Chloroethylureidoethyldiquino[3,2-b;2′,3′-e][1,4]thiazine	Strong suppression of tumor growth	[16]