ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 May; 20 (5): 405 – 408
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical

Systemic anti-inflammation by synthetic interleukin-1 blockers

George C Y CHIOU[1], Shirley X L LIU[2] ([1]*Department of Medical Pharmacology and Toxicology, College of Medicine, Texas A & M University, College Station TX 77843, USA*; [2]*Department of Pharmacology, Nanjing Institute of Materia Medica, Nanjing 210009, China*)

KEY WORDS carrageenan; inflammation; non-steroidal anti-inflammatory agents; OB-101; OB-186; aspirin; ophthalmic solutions

ABSTRACT

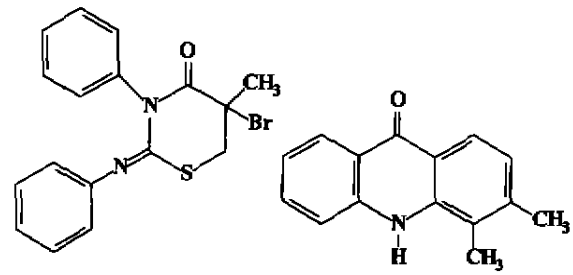
AIM: To study the systemic anti-inflammatory actions of interleukin-1 (IL-1) blockers, OB-101 and OB-186. **METHODS**: Prevention of palm swelling induced by carrageenin injection was used as an animal model of systemic anti-inflammation efficacy. **RESULTS**: Both OB-101 and OB-186 ($10 - 30\ mg \cdot kg^{-1}$) were approximately 10 – 30-fold more potent than aspirin ($300\ mg \cdot kg^{-1}$) to inhibit carrageenin-induced systemic inflammation. The LD_{50} of OB-101 and OB-186 were at least $20\ g \cdot kg^{-1}$ ig, indicating that they were extremely safe agents with a therapeutic index (LD_{50}/ED_{50}) of at least 2000. **CONCLUSION**: These IL-1 blockers are extremely safe systemically and are useable for the treatment of systemic inflammation such as rheumatoid arthritis.

INTRODUCTION

Extensive research has been conducted in the field of non-steroidal anti-inflammatory drugs (NSAID) because of serious side effects induced by corticosteroidal anti-inflammatory agents[1,2]. Although NSAID of arachindonate metabolite-related drugs have a long history of research and development, they produced limited success mainly because the potency of these drugs is relatively low and their numerous untoward side effects are not eliminated[3-5].

Recently, the interleukin-1 (IL-1) receptor antagonist (IL-1ra) has been shown to produce potent antagonizing effects on various inflammatory diseases[6]. Since IL-1ra is a peptide compound, it is too unstable for clinical uses. In order to solve the problem of its short half-life, synthetic organic compounds, which produce non-competitive inhibition of IL-1 receptors, have been developed[7-12]. They are found to be potent anti-inflammatory agents in the eyes[7-12] and are able to prolong the effective period of filtration surgery in glaucoma treatments[13].

Since inflammation diseases in the eye should be the same as in the systemic organs as far as the mechanism of the disease's inductions are concerned, it is hoped that the drugs (IL-1 blockers) which can inhibit uveitis ought to be able to antagonize systemic inflammatory responses as well. To prove this point, a standard animal model, a carrageenin injection into the rat palm, was used to test the prevention of palm swelling by IL-1 blockers, 5-bromotetrahydro-5-methyl-3-phenyl-2-phenylimino-4-*H*-1,3-thiazine (OB-101) and 3,4-dimethyl-9(10*H*)acridone (OB-186) using aspirin as a positive standard. The safety of IL-1 blockers was reaffirmed by using LD_{50} measurement and the Draize test.



OB-101 OB-186

MATERIALS AND METHODS

Materials OB-101 and OB-186 were synthesized with the published methods[13,14]. Carrageenin was

[1] Correspondence to Prof George C Y CHIOU. Phn 1-409-845-2817. Fax 1-409-845-0699. E-mail gchiou@tamu.edu
Received 1998-09-09 Accepted 1999-03-19

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 May; 20 (5)
E-mail aps@server shcnc.ac.cn Phn/Fax 86-21-6474-2629

purchased from Sigma Chemical (St Louis, MO) and aspirin was obtained commercially. Carrageenin was dissolved in sterile 0.9 % sodium chloride solution.

Carrageenin-induced inflammation rat model Forty female Sprague Dowley rats weighing 164 ± 16 g were used for the experiments. The test and control groups were assigned randomly. Carrageenin (0.1 mL of 1 % solution) was injected into the plantar surface of the rat hind paw to induce inflammation. Ten minutes before carrageenin injection, 8 mL of water was administered ig. OB-101, OB-186, and aspirin were grounded with Tween 80 and then suspended into distilled water. OB-101, OB-186, or aspirin were injected ip 3 times at time 0, 8, and 16 h before the injection of carrageenin. The changes in volume of the inflamed edematous foot were measured by the volume of water displaced and recorded at 0, 0.5, 1, 2, 4, and 6 h after the carrageenin injection.

LD_{50} determination For each dose, 20 mice of either sex, weighing 18 – 20 g were used to determine the LD_{50} according to the method of Litchfield and Wilcoxon[15]. Animals were housed in an animal room at 25 ℃ and 70 % relative humidity. The light cycle was set for 12 h light and 12 h darkness.

OB-101 and OB-186 were grounded with Tween 80 and then suspended in 1 % CMC (carboxymethyl cellulose). The suspension was administered ig at 20 $g \cdot kg^{-1}$ and the animals were observed for 7 d.

Eye irritation test Draize test[16] was followed for the determination of eye irritation. Before the Draize test, it was already known that OB-101 and OB-186 did not produce skin irritation in guinea pigs. So, Draize test was used to show the safety rather than the toxicity of drugs in the rabbit eyes in this experiment.

Six New Zealand albino rabbits of either sex, weighing 2 – 2.5 kg were used in each group. Animals were housed individually in cages at 25 ℃ and 70 % relative humidity. The light cycle was maintained at 12 h light and 12 h dark for 7 d after the instillation of OB-101, OB-186, or vehicle as controls.

OB-101 (0.1 %) and OB-186 (0.1 %) were suspended in 6 % Me_2SO, 6 % PEG 400, 10 % Tween 80, and 78 % saline. Fifty microliters of the test compound was instilled into the cul de sac of the right eye as treated and the vehicle to the left eye as control. The eye lids were gently held together for 10 s to prevent the loss of the materials. Animals showing eye irritation, ocular defects or pre-existing corneal injuries were not used for the experiment. The eyes were examined with ophthalmoscopy at 0, 1, 3, 5, 7, and 24 h, and 2 d, 3 d, 5 d, and 7 d after the eyedrop instillation. The scores of the Irritation Table were recorded and calculated according to the Draize Test[16].

Statistical analysis All data were presented as $\bar{x} \pm s$ and analyzed with Student's test for unpaired samples and two-way analysis of variance.

RESULTS

Marked inflammation was observed when carrageenan was injected into the rat hind paw which was significantly inhibited by ip aspirin 300 $mg \cdot kg^{-1}$ (Tab 1). Similar degrees of anti-inflammatory actions were observed with OB-101 and OB-186 at much lower doses (10 $mg \cdot kg^{-1}$ and 30 $mg \cdot kg^{-1}$ ip), indicating that OB-101 and OB-186 are approximately 10 – 30-fold more potent than aspirin to antagonize carrageenin-induced inflammation (Tab 1, 2). At 3 $mg \cdot kg^{-1}$ ip, both OB-101 and OB-186 still showed significant reduction of carrageenin-induced inflammation; yet the degree of inhibition was much weaker than that of OB-101 and OB-186 at 10 $mg \cdot kg^{-1}$ and 30 $mg \cdot kg^{-1}$ or aspirin at 300 $mg \cdot kg^{-1}$ ip (Tab 1, 2).

The LD_{50} of both OB-101 and OB-186 were extremely high, at least 20 $g \cdot kg^{-1}$, which is equivalent to (1400 g)/(70 kg) for man. Since the ED_{50} were approximately 10 – 30 $mg \cdot kg^{-1}$, the therapeutic index (LD_{50}/ED_{50}) would be as high as (20 000 mg) ÷ (10 mg) = 2000.

The ocular irritation was also very low and negligible as can be seen from the results of the Draize test (Tab 3).

DISCUSSION

Although OB-101 and OB-186 were shown to be effective in inhibiting ocular inflammation induced by lens protein, endotoxin, and IL-1[7-12], they were not tested in a systemic inflammation model so far. Their effectiveness in inhibiting carrageenin-induced inflammation indicates that these compounds could be used

Tab 1. Effects of aspirin and OB-101 on carrageenin induced inflammation. $n = 8$. $\bar{x} \pm s$.
$^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* control. $^{d}P > 0.05$, $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* aspirin.

Treatment	Inflammation responses/mL			
	0 h	2 h	4 h	6 h
Control	0.100 ± 0.005	0.218 ± 0.030	0.342 ± 0.035	0.368 ± 0.045
OB-101 (3 mg·kg^{-1})	0.098 ± 0.005^{ae}	0.220 ± 0.025^{af}	0.276 ± 0.030^{cf}	0.298 ± 0.070^{bf}
OB-101 (10 mg·kg^{-1})	0.096 ± 0.010^{ae}	0.144 ± 0.030^{cd}	0.202 ± 0.045^{ce}	0.226 ± 0.040^{ce}
OB-101 (30 mg·kg^{-1})	0.084 ± 0.005^{bd}	0.104 ± 0.015^{cd}	0.182 ± 0.015^{ce}	0.198 ± 0.025^{cd}
Aspirin (300 mg·kg^{-1})	0.074 ± 0.005^{b}	0.116 ± 0.030^{c}	0.122 ± 0.030^{c}	0.184 ± 0.015^{c}

Tab 2. Effects of aspirin and OB-186 on carrageenin induced inflammation. $n = 8$. $\bar{x} \pm s$.
$^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* control. $^{d}P > 0.05$, $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* aspirin.

Treatment	Inflammation responses/mL			
	0 h	2 h	4 h	6 h
Control	0.098 ± 0.004	0.213 ± 0.032	0.341 ± 0.032	0.365 ± 0.042
OB-186 (3 mg·kg^{-1})	0.096 ± 0.005^{ad}	0.207 ± 0.009^{af}	0.257 ± 0.037^{cf}	0.285 ± 0.032^{cf}
OB-186 (10 mg·kg^{-1})	0.094 ± 0.004^{ad}	0.141 ± 0.019^{ce}	0.176 ± 0.009^{cf}	0.204 ± 0.019^{cd}
OB-186 (30 mg·kg^{-1})	0.093 ± 0.005^{ad}	0.111 ± 0.009^{cd}	0.156 ± 0.042^{cd}	0.191 ± 0.028^{cd}
Aspirin (300 mg·kg^{-1})	0.072 ± 0.028^{a}	0.106 ± 0.013^{c}	0.120 ± 0.032^{c}	0.180 ± 0.019^{c}

Tab 3. Rabbit irritation responses by 0.1 % OB-101 suspension or 0.1 % OB-186 suspension after the eyedrop instillation. Scores are means of 6 eyes.

Treatment	0 h		1 h		3 hh		5 h		7 h		1 d		2 d		3 d		5 d		7 d	
	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
0.1 % OB-101 suspension																				
Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Iris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva																				
a. Redness	0	0	0	0.1	0	0.1	0.1	0	0.1	0	0	0.1	0.1	0.1	0.1	0	0.1	0.1	0.1	0
b. Chemosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
c. Discharge	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0.1	0	0.1	0.1	0	0.1	0	0	0.1	0.1	0.1	0.1	0	0.1	0.1	0.1	0
0.1 % OB-186 suspension																				
Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Iris Conjunctiva	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva																				
a. Redness	0.1	0.17	0	0.17	0	0.17	0.1	0.1	0.17	0.1	0.1	0.1	0.1	0.17	0.17	0.17	0	0.25	0	0.1
b. Chemosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
c. Discharge	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	0.1	0.17	0	0.17	0	0.17	0.1	0.1	0.17	0.1	0.1	0.1	0.1	0.17	0.17	0.17	0	0.25	0	0.1

Cornea = degree of opacity; Iris = degree of iritis; Conjunctiva = redness, chemosis, and discharge; Total = Cornea, iris, and conjunctiva together; R = right eye (test); L = left eye (control).

not only for ocular inflammation but also for systemic inflammation. Most excitingly, these agents were 10 – 30 times more potent than aspirin to inhibit carrageenin-induced inflammation. In general, NSAID of arachidonate-related agents are less potent than corticosteroids in inhibiting inflammation which prevents them from replacing corticosteroids in the clinics. With the new class of compounds, IL-1

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 May; 20 (5)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

blockers, it is hopeful that OB-101 and OB-186 could be used to replace corticosteroids in the treatment of various inflammatory diseases.

In addition to drug efficacy, the safety of drugs is very important to determine the clinical value of new drugs. The reasons that NSAID are not able to replace corticosteroids in inflammation treatment are not only that their efficacies are low, but also the side effects are serious. It is exciting to note that OB 101 and OB 186 are devoid of any serious side effects detectable. No eye irritation was observed with the Draize test in the rabbit and the LD_{50} is at least 20 $g \cdot kg^{-1}$ in mice. Since the ED_{50} of these compounds for posterior uveitis[7-12] and carrageenin-induced palm edema are approximately 10 $mg \cdot kg^{-1}$, the apparent therapeutic index (LD_{50}/ED_{50}) can be as high as 2 000 (20 000 mg/10 mg). There are very few drugs which can show such a high safety margin for disease treatment.

In short, a new class of anti-inflammatory agents, IL-1 blockers, have been invented to inhibit inflammation effectively both in the local and systemic tissues and organs. These compounds are not only potent but also safe with an unusually high therapeutic index.

REFERENCES

1 Chiou GCY, Chang MS. Ocular inflammation induced by lens protein and its prevention with new agents. In: Bazan NG, editor. New trends in lipid mediator research, lipid mediators in eye inflammation.
Basel: Karger; 1990. p 94-109. 405-408
2 Masuda K. Anti-inflammatory agents; non-steroidal anti-inflammatory drugs. In: Sears ML, editor. Pharmacology of the eye.
New York: Springer-Verlag; 1984. p 539-51.
3 Braquet P. Handbook of PAF and PAT antagonists.
Boca Raton: CRC Press; 1991. p 97-258.
4 Melli M, Parente L. Cytokines and lipocortins in inflammation and differentiation.
New York: Wiley-Liss; 1990. p 183-94.
5 Dawson MM. Lymphokines and interleukins.
Boca Raton: CRC Press; 1991. p 82-105.
6 Chiou GCY, Liu SXL. Novel non-arachidonate mediated, non-steroidal anti-inflammatory agents.
Expert Opinion Inv Drugs 1994; 3: 725-32.
7 Chiou GCY, Yao QS, Chang MS, Okawara T. Prevention and treatment of ocular inflammation with a new class of non-steroidal anti-inflammatory agent.
J Ocular Pharmacol 1994; 10: 335-47.
8 Chiou GCY, Yao QS, Okawara T. Prevention of ocular inflammation induced by lens protein, endotoxin and interleukin-1 with synthetic IL-1 blockers.
J Ocular Pharmacol 1994; 10: 577-86.
9 Chiou GCY, Chen JZ, Xuan B, Yamasaki T, Okawara T. Antagonism of interleukin-1 (IL-1) induced uveitis with synthetic IL-1 blockers.
J Ocular Pharmacol Ther 1997; 13: 427-33.
10 Xuan B, Chiou GCY, Yamasaki T, Okawara T. Anti-uveitis and inhibition of fibroblast-like corneal and conjunctival all cultures by interleukin-1 blockers, CK 125, CK 126 and CK 128. Ophthalmic Res 1998; 30: 180-8.
11 Chiou GCY, Xuan B, Yamasaki T, Okawara T. Anti-inflammation and anti-fibroblastic actions of interleukin-1 blockers. J Ocular Pharmacol Ther 1998; 14: 375-88.
12 Xiao JG, Schwade N, Chiou GCY. Mechanisms of antagonism of interleukin-1 (IL-1) by synthetic IL-1 blockers. J Ocular Pharmacol Ther 1995; 11: 369-78.
13 Schwade N, Chiou GCY. Effects of interleukin-1 blockers on ophthalmic wound healing in rabbit model of trabeculectomy. J Ocular Pharmacol Ther 1995; 11: 125-34.
14 Okawara T, Nakayama K, Furukawa M.
Facile formation of 1,3-disubstituted-2,3,5,6-tetrahydro-2-thioxopyrimidin-4(1H)-ones and 2-N,3-disubstituted-2,3,5,6-tetrahydro-2-imino-1,3-thiazin-4-ones from thioureas and β-haloacyl halides.
Chem Pharm Bull 1983; 31: 507-12.
15 Litchfield JT, Wilcoxon F. A simplified method of evaluating dose-effect experiments.
J Pharmacol Exp Ther 1949; 96: 99-113.
16 Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes.
J Pharmacol Exp Ther 1944; 82: 377-90.

5

合成白细胞介素-1阻滞药的全身性抗炎作用

邱春億[1], 劉學麗[2] R979.5
([1]*Department of Medical Pharmacology and Toxicology, College of Medicine, Texas A & M University, College Station TX 77843, USA*;
[2]南京药物研究所药理室, 南京 210009, 中国)

关键词 角叉菜胶; 炎症; 非甾类消炎药; OB-101; OB-186; 阿司匹林; 眼药水

(责任编辑 周向华)