Pharm. Sci. Assiut Univ.
Vol. 2, PP. 151 - 161 (1978)

ANTI-INFLAMMATORY ACTION AND TOXICITY OF CERTAIN OXAMIDE DERIVATIVES

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The present study was aimed at evaluating the anti-inflammatory action and toxicity of seven N-[-1- (p-nitrophenyl)-1,3-dihydroxy isopropyl]-N`-(substituted)oxamides (I). The compounds are designed to include the following moieties : anthranilic and p-aminosalicylic acids and p-aminophenol in their basic structures. Two dose levels of these compounds were assessed for their anti-inflammatory property in rats by the trypan blue method and using acetyl salicylic acid as the reference anti-inflammatory agent. Median lethal doses of the compounds were determined in mice.

The possible correlation between structure and biological activity is suggested.

The unfailing attempts to find a potent analgesic agent have not been discouraged since the discovery of aspirin by Dreser in 1899[1]. The major impetus to research in this area is provided by recognition of the value and limitations of the available drugs specially for therapy of chronic inflammatory disorders.

Recently[2,3], a number of oxanilic acid amide derivatives were prepared and found to possess anti-inflammatory property equivalent to that of mefenamic acid but with greater reduction

site of each of the intradermal injections was determined. Concurrent control animals were treated at the same manner after an intraperitoneal injection of a 5% suspension of gum acacia.

2- Determination of median lethal doses :

Median lethal doses of the drugs under investigation and aspirin were determined by the method of Necolaev M.P.(1966)[5]. Groups of 6 mice were intraperitoneally injected with different doses of the drugs under investigation and 24 hours later the mortality was determined in each group of animals and the median lethal dose (LD_{50}) and its standard error calculated.

3- Animals and drugs :

Adult albino rats (150-200 g) and adult albino mice (18-22 g) of either sex were supplied locally.

Standard drugs used were : Acetyl salicylic acid (El-Nasr Co), Histamine Phosphate (Merck Sharp Co), Xylene (BDH), and Trypan blue (Prolabo Co).

Drugs under investigation were prepared at the Department of Pharmaceutical Chemistry, Assiut University, A R E.[6]. The seven compounds under investigation and their chemical structures are listed in table (1).

of their acute toxicity. These findings have justified the present attempt to evaluate the anti-inflammatory property of certain oxamide derivatives comprising the moieties of the most commonly clinically used anti-inflammatory drugs : p-aminophenol, anthranilic and p-aminosalicylic acids. Aspirin was also used in this study as a reference drug to compare the anti-inflammatory action and acute toxicity of the tested compounds.

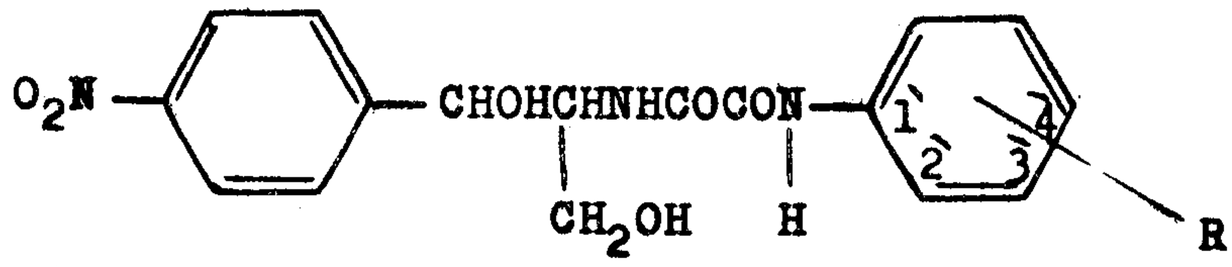
M E T H O D S A N D M A T E R I A L S

1- Evaluation of anti-inflammatory activity :

Anti-inflammatory action was determined by the trypan blue method as modified by Golikov P.P., (1964)[4]. This method depends on the quantitative determination of the effects of drugs under investigation on the rate of capillary permeability disturbance caused by the intradermal injection of a phlogogenic substance such as histamine or xylene. The rate of capillary permeability is calculated as the time needed for the appearance of a blue colour, around the site of the intradermal injection of histamine or xylene, after an intravenous injection of the trypan blue dye.

Suspensions of the compounds under investigation and aspirin in 5% gum acacia were intraperitoneally injected into rats in 2 dose levels (50 and 100 mg/kg). Thirty minutes later, animals were fixed to a board with their abdomen upright and the hair around the abdomen was shaven. Trypan blue 1% solution (2 ml/kg) was intravenously injected into the femoral vein. After 10 minutes, histamine phosphate (0.02 ml of a 0.1% solution), xylene (0.02 ml), and saline solution (0.02 ml) were intradermally injected into the abdominal skin. The time needed for the appearance of a blue colour around the

Table (1) : Chemical Structure and Configuration of the 7 Oxamide Derivatives under Investigation.



(I)

N-[1-(p-nitrophenyl)-1,3-dihydroxy isopropyl]-N'-(substituted)oxamide .

Compound	Configuration	R
Ia	L-threo-(+)	2' -COOH
Ib	DL-threo-(±)	2' -COOH
Ic	D-threo-(-)	3' -OH, 4' -COOH
Id	L-threo-(+)	3' - OH, 4' -COOH
Ie	DL-threo-(±)	3' - OH, 4'-COOH
If	D-threo-(-)	4' - OH
Ig	D-threo-(+)	4' - OC_2H_5

R E S U L T S

Anti-Inflammatory Activity :

The different oxamide derivatives under investigation exhibited a variable degree of anti-inflammatory action against inflammation induced either by histamine or xylene.

Table(2)presents the degree of the anti-inflammatory action of the seven compounds together with aspirin when injected intraperitoneally at 2 dose levels.

ANTI-INFLAMMATORY ACTION

Table (2) : The Anti-Inflammatory action* of 50 and 100 mg/kg of the Compounds Against Inflammation Induced by Histamine and Xylene in Rats.

Compound	Anti-Inflammatory Action of 50 mg/kg of the Compound Against :		Anti-Inflammatory Action of 100 mg/kg of the Compound Against :	
	Histamine	Xylene	Histamine	Xylene
Control (5% Gum Acacia)	2.14 ± 0.10	2.41 ± 0.11	2.19 ± 0.09	2.45 ± 0.11
Aspirin	2.86** ± 0.17	2.96* ± 0.12	3.24** ± 0.24	3.49** ± 0.23
Ia	2.13 ± 0.05	2.50 ± 0.06	2.15 ± 0.08	3.40** ± 0.28
Ib	2.37 ± 0.19	2.52 ± 0.22	2.49* ± 0.07	2.65 ± 0.19
Ic	2.94** ± 0.19	3.17* ± 0.30	3.20** ± 0.18	3.31** ± 0.26
Id	2.16 ± 0.06	2.88* ± 0.03	2.33 ± 0.14	3.47** ± 0.05
Ie	2.39 ± 0.09	2.44 ± 0.28	2.59* ± 0.08	2.64 ± 0.16
If	2.71* ± 0.15	2.95* ± 0.13	2.95** ± 0.16	3.01* ± 0.14
Ig	2.03 ± 0.18	2.58 ± 0.20	2.52 ± 0.22	2.75 ± 0.16

* Anti-inflammatory action is expressed as the time in minutes(mean ± standard error)taken for the appearance of the trypan blue dye at the site of intradermal injection of either histamine phosphate(0.02 ml of a 0.1% solution)or xylene(0.02 ml).

* Significant result(p = 0.05)

** Significant result(p = 0.01)

The order of effectiveness of the 50 mg/kg dose against histamine or xylene-induced inflammation is consistent for certain compounds. Compounds Ic, aspirin and If showed the highest and significant action in both types of inflammation. However, the rest of the compounds of the lower degree of effectiveness showed different orders of activity.

On the other hand, the anti-inflammatory action of the 100 mg dose showed different orders of effectiveness against histamine or xylene type of inflammation. Therefore, while aspirin, compounds Ic, If and Ie showed the highest and significant action against histamine-induced inflammation, yet aspirin, compounds Id, Ia, Ic and If produced the highest and significant effect against xylene-induced inflammation.

It is also apparent that the order of effectiveness at both dose levels is not exactly matching.

It is also evident that doubling the dose has improved the anti-inflammatory action of these compounds. In support of this observation is the finding that while the 50 mg dose failed to elicit a significant anti-inflammatory action in compounds Ia, Ib, Ie and Ig, the 100 mg dose produced significant action in all the compounds with the exception of compound Ig which failed to produce any significant effect at the 2 dose levels against both types of inflammation.

Acute Toxicity of the Investigated Compounds :

Acute toxicity study was carried out on the compounds and aspirin. Their median lethal doses and standard error were determined and are listed in table (3).

It is evident that all the tested compounds have higher lethal doses when compared with aspirin. Besides, compounds Ic, Id and If which demonstrated the highest significant anti-inflammatory activity are the least toxic compounds if we consider the order of lethality in terms of the magnitude of median lethal doses. Lethality and effectiveness are thus dissociated from each other.

Table (3) : The median lethal doses in mice and the relative margin of safety* of the compounds under investigation.

Compound	LD_{50} (mg/kg) ± S.E.	Relative margin of safety	
		LD_{50}/50 mg/kg	LD_{50}/100 mg/kg
Aspirin	533.33 ± 68.31	10.66	5.33
Ia	1033.33 ± 44.10	20.66	10.33
Ib	1066.67 ± 88.19	21.34	10.67
Ic	1833.33 ± 69.92	36.66	18.33
Id	1666.67 ± 97.75	33.34	16.67
Ie	1466.67 ± 74.54	29.34	14.67
If	1633.33 ± 63.25	32.66	16.33
Ig	1600.00 ± 93.09	32.00	16.00

* Relative margin of safety is the ratio of LD_{50} to the two dose levels (50 and 100 mg/kg) of the tested compounds used to determine their anti-inflammatory action.

The relative margin of safety of these compounds is tentatively calculated by dividing the corfesponding LD_{50} by the two effective doses used in this study (table 3). It is quite evident that compounds Ic, Id, Ie and If possess the highest margin of safety.

D I S C U S S I O N

This study revealed that at least six of the seven oxamide derivatives possess some degree of anti-inflammatory activity. The effectiveness of most of these compounds was almost comparable to that of aspirin as in case of compounds Ia, Ib, Ic, Id, Ie, and If and in certain cases superior as in case of compound Ic at the 50 mg dose level. Aspirin as well as compounds Ic and

If were effective against inflammation induced by both histamine and xylene. However, certain compounds as If and Ie were effective only, at the 100 mg dose level, against the histamine-induced inflammation, whereas compounds Ia and Il were effective only against xylene-induced inflammation. If we assume that the types of inflammation induced by histamine and xylene are different we may suggest that compounds Ia and If must have a more general anti-inflammatory properties while the other compounds have a more specific anti-inflammatory property.

The order of activity of the compounds was found to vary when the dose was increased from 50 to 100 mg/kg. Some of the compounds which were insignificantly effective at the lower dose demonstrated a significant effect at the higher dose level (compounds Ia, Ib, and Ie).

The activity of the tested compounds was also found to be related to some extent to their configuration. However, no consistency could be concluded of the relation of biological activity to certain types of configuration. Thus, whereas compounds with D activity like compounds Ic and If were found to be of high anti-inflammatory activity, compound Ig was practically inactive in this regards. Other compounds with L or DL activity were also found to be effective. This may most probably, be attributed to the incorporation of biologically-active moieties in the molecules of the compounds under investigation.

The inclusion of certain moieties possessing anti-inflammatory properties as anthranilic and p-aminosalicylic acid derivatives resulted in compounds retaining this property. Besides, compound If which includes p-aminophenol in its structure also possessed

potent anti-inflammatory action although compound Ig and other clinically available p-aminophenol derivatives are known to be devoid of this property[1].

Acute toxicity study in mice indicated that all the tested compounds were less toxic than aspirin. Of interest also is the finding that activity and toxicity could be dissociated. Thus, compounds Ic, Id, and If which possessed the highest anti--inflammatory activity were found to be also the least toxic; whereas aspirin was found to combine both a high anti-inflammatory activity and the highest lethality.

Relating the median lethal doses of these compounds to the effective doses used in this study demonstrated the superiority of most of them as anti-inflammatory agents with relatively low degree of toxicity as compared with aspirin.

The marked anti-inflammatory effectiveness and the relative low toxicity of most of the tested compounds necessitate a more thorough screening of these compounds for other pharmacological actions which are known to be inhereted in the different commonly used anti-inflammatory groups of drugs.

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دراسة التأثير المضاد للالتهابات وسمية بعض مشتقات الاوكساميد

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جامعة اسيوط

تم فى هذا البحث تقييم التأثير المضاد للالتهابات وكذلك السمية الحادة لسبعة مركبات مشتقة من ن ـ ١ ـ (بارانيترو- فينيل) ـ١ر٣ـ ثنائى هيد روكسى الايزوبروبيل ـ ن ـ اوكسامايد مستبدل.

لقد صممت هذه المركبات بحيث تشتمل على مجموعات موجودة فى كثير من الأدوية المستعملة والمعروفة كعلاج للالتهابات ومن أمثلة هذه المجموعات مايلى :

٢ـ امينو حمض البنزويك ، ٤ـ امينو حمض البنزويك ، ٤ـ امينو حمض السالساليك

اجريت دراسة التأثير المضاد للالتهابات لهذه المركبات فى جرعتين مختلفتين على الفئران البيضاء البالغة بطريقة التروبان الازرق اما السمية الحادة فقد أجريت على الفئران البيضاء الصغيرة البالغة مع المقارنة بالاسبرين فى كل حالة اعطيت هذه المركبات والاسبرين فى صورة معلقات فى محلول ٥٪ من صمغ الاكاشيا بطريق الحقن فى الغشاء البريتونى .

ولقد اثبتت الدراسة ان لثلاثة مركبات من بين هذه المجموعة تأثير مضاد للالتهابات ساو التأثير الاسبرين واقل سمية عنه بحوالى ١ر٣ ـ ٤ر٣ مرة . كذلك اسفر البحث عن موجود علاقة بين التركيب الفراغى والتأثير البيولوجى لهذه المركبات.