Anti-Inflammatory Activity of Furosemide and Spironolactone+

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Abstract

Two diuretics - furosemide and spironolactone - were investigated for anti-inflammatory activity in two models of inflammation, namely, exudative phase (carrageenin-induced rat hind paw oedema) and proliferative phase (cotton pellet-induced granuloma in mice). Phenylbutazone, a non steroidal anti-inflammatory agent was used as a standard drug for comparison. In both the models of inflammation, furosemide showed statistically significant anti-inflammatory activity. Spironolactone, however, failed to exhibit significant anti inflammatory activity in the model for acute inflammation though it was very effective in chronic inflammation.

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Introduction

Although a large number of anti-inflammatory agents are in clinical use, their precise mechanism of action remains exclusive. Known anti-inflammatory agents have been examined for a common biochemical or physicochemical property as a basis of their action. The most widely accepted mode of action of non-steroidal anti-inflammatory drugs (NSAID) is that of inhibition of prostaglandin synthesis[1,2] and the latter has been discussed in detail[3]. Other possible modes of action of NSAID have also been described[4,5,6,7,8,9].

However a number of compounds which do not find place clinically have

exhibited anti-inflammatory activity in laboratory tests. Some compounds - derivatives of sulfonamides - have also been reported to have anti-inflammatory activity[10]. Based on these observations, hydrochlorothiazide - a benzothiazide diuretic belonging to benzenedisulfonamide group was studied in our laboratory and showcd significant anti-inflammatory activity[11].

It seemed therefore interesting to find out if furosemide. a diuretic related to benzothiazide structurally as well as functionally and spironolactone, an aldosterone antagonist, structurally and functionally unrelated to benzothiazide, would exhibit any anti inflammatory activity.

Materials and Methods

Anti-inflammatory activity was tested in two models of inflammation, namely (1) the exudative phase of inflammation in albino rats using carrageenin as the phlogistic agent[12] and (2) the proliferative phase of inflammation using the cotton pellet-induced granuloma test [13] in albino mice. In both the tests, phenylbutazone, a nonsteroidal anti-inflammatory drug (NSAID) was used as a standard drug for comparison.

In the carrageenin-induced oedema method, male albino rats (Haffkin strain) in groups of 6 animals each, weighing between 240-310 g were used. The animals were fasted for 18 h while water was allowed ad lib. The test drugs as well as phenylbutazone were dissolved in freshly prepared 2% gum acacia solution and were administered orally, 1 h prior to injection of 0.1 ml of 0.5% freshly prepared carrageenin suspension in the rat hind paw. The control group received only 2% solution of gum acacia. The paw volumes measured by the micropipette method[14] 3 h after the injection of carrageenin.

In the cotton pellet-induced granuloma method, albino mice in groups of 6 animals each, weighing between 25-35 g were used. Pellets of surgical cotton weighing 10± 1 mg were sterilized in a hot air oven at 150°C for 2 h and implanted subcutaneously in only one axilla of each mouse, under light ether anaesthesia. The drugs were administered daily for 7 days, the first dose being given 30 min prior to implantation of the pellet. Twenty four hours after the last dose, the pellets were carefully dissected out under light ether anaesthesia. The pellets were then dried in hot air oven at 150°C for 2 h and weighed after cooling; the difference between the two readings being taken as the weight of the granuloma.

Results

It can be seen from Table I that phenylbutazone administered orally exhibited statistically significant activity

against carrageenin-induced oedema in all the three doses whereas furosemide was effective only in doses of 40 mg/kg and 80 mg/kg. It appears that the anti-inflammatory activity of furosemide is maximal at a dose of 40 mg/kg since doubling the dose did not increase the activity. However, spironolactone failed to exhibit any significant anti-inflammatory activity in this model of acute inflammation.

Table II shows the effect of these drugs in the cotton pellet granuloma test in mice. As expected, phenylbutazone exhibited significant activity. Furosemide also was effective in all the three doses used. What is striking, however, is that spironolactone, which failed to show any significant effect in the model for acute inflammation, exhibited highly significant activity in this model of chronic inflammation.

Discussion

The results of this study show that whereas furosemide has significant activity in both models of inflammation, spironolactone is effective only in proliferative phase of inflammation.

It is unlikely that the anti-inflammatory effect of furosemide is due to its diurectic effect because another potent diuretic, mannitol administered in a dose which produced a diuretic effect comparable to 200 mg kg of hydrochlorothiazide, failed to have any anti-inflammatory effect in both models of inflammation[11]. Furosemide has been shown to produce decrease in tolerance to carbohydrate. Diabetogenic effect of furosemide has also been reported[15]. Hyperglycaemia has been associated with anti-inflammatory activity[7] and possibly, this could explain the observed action of furosemide.

The experiments with spironolactone have brought out an interesting finding, in that spironolactone which is devoid of any significant anti-inflammatory activity in the exudative phase (model of acute inflammation) shows highly significant anti-inflammatory activity against the proliferative phase of inflammation (chronic inflammation).

Failure of spironolactone to show activity in the model of acute inflammation is probably related to its pharmacokinetics Spironolactone as well as its active metabolite canrenone are extensively bound to plasma proteins and there is considerable enterohepatic circulation[16]. Spironolactone is also reported to have a relatively slow onset of action, requiring 2-3 days for maximum effect and a similarly slow diminishment of action over 2-3 days on discontinuation[16]. It is therefore easy to visualise that anti-inflammatory effect of spironolactone will become evident on chronic administration over seven days rather than with a single dose.

TABLE I: Effect of Phenylbutazone (PBZ), Furosemide (FUR) and Spironolactone (SP) on Carrageenin-Induced Oedema.

Group (n = 6)	Drug mg/kg (po)	Carrageenin-Induced oedema mean paw volume (ml) ± SE	P value
Control	–	0.413 ± 0.05	
PBZ	40	0.354 ± 0.07	< 0.05
	60	0.266 ± 0.06	< 0 01
	80	0.188 ± 0.05	< 0.01
FUR	20	0.43 ± 0.03	N.S.
	40	0.233 ± 0.05	< 0.01
	80	0.233 ± 0.05	< 0.01
SP	50	0.386 ± 0.17	N.S.
	100	0.363 ± 0.06	N.S.
	200	0.393 ± 0.05	N.S.

n = Number of animals in each group
N.S. = Not Significant

TABLE II: Effect of Phenylbutazone (PBZ), Furosemide (FUR) and Spironolactone (SP) on Cotton Pellet Granuloma

Group (n = 6)	Drug mg/kg (po)	Mean weight of granuloma (mg) ± SE	P value
Control	–	28.43 ± 1.68	
PBZ	20	15.14 ± 2.23	< 0.01
	40	14.94 ± 2.87	< 0.01
	80	13.84 ± 1.4	< 0.01
FUR	20	18 20 ± 2.13	< 0.01
	40	17.69 ± 1.07	< 0.01
	80	9.65 ± 0.69	< 0.01
SP	50	18 5 ± 1.35	< 0.01
	100	17.21 ± 1.79	< 0.01
	200	14.8 ± 1.47	< 0.01

n = Number of animals in each group
N.S, = Not Significant

Although furosemide and spironolactone have shown significant anti-inflammatory activity, their clinical utility as anti-inflammatory agents would offer no greater advantage over the currently available drugs in view of their numerous side effects.

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