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Effects of Cyclobuxine D on Carrageenin-induced Pleurisy and Croton Oil-induced Granuloma Pouch in Rats[1]

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ABSTRACT

Cyclobuxine D, a steroidal alkaloid, was extracted from *Buxus microphylla* var. *koreana Nakai*. The effects of cyclobuxine D on carrageenin-induced pleurisy and croton oil-induced granuloma pouch in rats was investigated and compared with those of aspirin, hydrocortisone and dexamethasone. Intrapleural injection of 2% carrageenin caused the accumulation of exudate. The rate of plasma exudation, measured by the exuded dye amounts for 20 min in the pleural cavity after intravenous injection of pontamine sky blue, showed a peak at 5 hr. Cyclobuxine D (5, 20 and 50 mg/kg, i.p.) suppressed dose-dependently the accumulation of the pleural exudate and the exudation of dye.

Among several methods used for screening and evaluation anti-inflammatory agents, granuloma pouch technic introduced by Hans Selye (Hans seyle, 1953) is considered as a simple and reliable method. An air pocket was produced in the subcutaneous tissue of the interscapular region by injection of 1 ml of 1% croton oil as irritant. Inflammatory exudate accumulated in the pouch during the succeding 14 days. Cyclobuxine D (5 and 20 mg/kg) decreased fluid volume in pouch and weight of pouch wall in granulomatous inflammation.

Key Words: Cyclobuxine D, Pleurisy, Granuloma Pouch

INTRODUCTION

Prostaglandins, particularly prostaglandin E_2, released during inflammation, contribute to the inflammatory response. Furthermore, their production is reduced by non-steroidal anti-inflammatory drugs (NSAID'S), now recognized to be the mechanism of action of these compounds (Eaking KE *et al.*, 1979; Higgs GS *et al.*, 1979)

Polymorphonuclear leukocyte (PMN) infiltration, which is also a characteristic response of inflammation, is reduced only with dose of NSAID higher than those required to inhibit the cyclooxygenase (Higgs *et al.*, 1979), suggesting that prostaglandins do not play a primary role in the recruitment of cells. Anti-inflammatory steroids, in addition to reducing the concentration of cyclooxygenase products, do prevent PMN infiltration. These steroids do not block cyclooxygenase directly but inhibit the enzymic liberation of the prostaglandin precursor, arachidonic acid, from cell-membrane phospholipids (Flower RJ *et al.*, 1979; Lewis PG *et al.*, 1975).

Granulomatous tissue model, originally called the granuloma pouch and in many recent papers re-named an air-bleb, involves the administration of an irritant into a subcutaneous pouch formed by air injection (Hans Seyle, 1953). Subsequently, a wall of proliferative tissue develops surrounding a pocket of exudate. PGE_2 and PGF_{2x} have been reported in 8-72 hr exudates of carrageenin pouch granulomata. One of major products transformed

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from arachidonic acid in vitro by 8 day granulation tissue was identified as thromboxane B_2, a stable metabolite of thromboxane A_2 with chemotactic activity (Higgs GA *et al.*, 1976).

Lee *et al.*, (1986) reported that cyclobuxine D, which was extracted from *Buxus microphylla* var. *koreana* Nakai, suppressed prostaglandins production and leukocyte migration in carrageenin-induced inflammation. In this paper, authors investigated the effects of cyclobuxine D on the accumulation of the exudate in combination with the plasma exudation in carrageenin-induced pleurisy and fluid volume in pouch and weight of pouch wall in rats croton oil-induced granuloma pouch.

MATERIALS AND METHODS

Carrageenin-induced pleurisy

Male Sprague-Dawley rats (180-200 g) were slightly anesthetized with ether, and 0.2 ml of 2% ι-carrageenin, suspened in sterile saline soultion, was injected into the right pleural cavity through a blunt edged 25 gauge needle (Katori M *et al.*, 1982). After injection of carrageenin, five rats were caged together and given food and water *ad libitum*. At given intervals, the animals were sacrified by exsanguination under ether-anesthesia.

To determine rates of plasma exudation from vascular vessels, pontamine sky blue (60 mg/kg, Tokyo Kasei) was injected intravenously under ether-anesthesia 20 min before the exanguination. Approximately 3 ml of blood was collected during exsanguination to measure the concentration of dye in serum. The pleural exudate was harvested after opening the chest and its volume was measured. The exudate was transfered to a tube containing 143 units of heparin (Riker Lab. Ind., Northridge) and centrifuged at 1,000 xg for 15 min. The dye concentrations in the exudate and the serum were measured spectrophotometrically by an absorbance at 630 nm. The rate of plasma exudation was expressed by the amount of dye exudated during 20 min, which was corrected in terms of serum dye concentration (1 mg/ml), as serum concentration of dye were different in individual rats.

Treatment of rats with drugs.

Aspirin (Jong Keun Dang, Seoul), which is an inhibitor of cyclooxygenase (Vane JR, 1971), was dissolved in 8% potassium citrate. Dexamethasone (Tae Pyung Yang, Youngin), which is an inhibitor of phospholipase A2 (Hammastrom S *et al.*, 1977), was dissolved in saline solution. Cyclobuxine D, which is extracted in our Lab. (Park YH *et al.*, 1984), was suspended in 5% arabia gum. All drugs were injected intraperitoneally at 0.1 ml/100 g body weight. Administration was performed 30 min before carrageenin.

Doses used were as follws; aspirin; 100 mg/kg, dexamethasone 1 mg/kg, cyclobuxine D; 5, 20 and 50 mg/kg.

Croton oil-induced granuloma pouch

Male Sprague-Dawley rats (130-150 g) were depilated in dorsal region. Granuloma pouches were made by the following procedure: 25 ml of air was injected deep into the loose connective tissue between the shoulder blades through a 26 gauge needle. This was immediately followed by the injection of 1 ml of 1% croton oil solution (diluted with castor oil) into the resulting air space, through the same needle (Hans Seyle, 1953; Lee SB, 1965). The wall of the air bubble began to thicken at about the fourth to sixth day. During the following days, the accumulation of exudate and the thickening of the granuloma-pouch wall proceeded constantly, The experiment was terminated on the 14th day after the croton oil injection.

Treatment of rats with drugs

In first experiment, drugs were administered subcutaneously into the loose connective tissue between the shoulder blades 24 hr before croton oil. Hydrocortisone (Jong Keun Kang, Seoul) and cyclobuxine D were suspended in 5% arabia gum. In other experiment, drugs were administered intramuscularly 1 hr before croton oil for 10 days. Dexamethasone was dissolved in saline solution.

Doses used were as follows; hydrocortisone; 10 mg/kg, dexamethasone; 0.5 mg/kg, cyclobuxine D ; 5 and 20 mg/kg.

Statistical analysis

Statistical analysis of the data was performed in each case according to Student's t-test. Significance was taken as $p<0.05$.

RESULTS

Effects of drugs on carrageenin-induced pleurisy

An injection of 0.2 ml ι-carrageenin into the right pleural cavity induced an accumulation of inflammatory exudate which began from 1 hr after the injection and reach a maximum at about 19 hr. To determine the rate of plasma exudation from vascular vessels, pontamine sky blue was injected intraveneously and the dye amount in the pleural exudate was measured. After the injection of carrageenin, the exuded dye markedly increased until 5 hr and decreased thereafter.

As shown in Fig. 1, pretreatment with cyclobuxine D (5, 20 and 50 mg/kg, i.p.) at 30 min before carrageenin caused a dose-dependent inhibition of the dye leakage. The inhibitory effect of cyclobuxine D on the dye leakage showed a peak at 5 hr. Cyclobuxine D (20 mg/kg) suppressed the dye exudation by over 50 per cent at 5 hr. Marked suppression of the accumulation of the pleural exudate was also observed until 7 hr.

The treatment with aspirin (100 mg/kg, i.p.), a cyclooxygenase inhibitor, significantly decreased the dye exudation until 5 hr, but did not at 7 hr. Dexamethasone (1 mg/kg, i.p.), a phospholipase A_2 inhibitor, significantly suppressed the amount of dye exuded and the accumultion of pleural exudate until 7 hr. Dexamethasone blocked completely the formation of inflammation induced by carrageenin (Fig. 2).

Effects of drugs on croton oil-induced granuloma pouch

Drugs suspension (0.2 ml) was injected into the loose subcutaneous tissue of the back paralleled with the vertebral column. Twenty-four hour later, 25 ml of air were injected deep into the loose connective tissue between the shoulder blades. This was immediately followed by the injection of 1 ml of 1% croton oil-into the resulting air space. The wall of the air bubble began to thicken and a hemorrhagic brownish fluid was beginning to fill the cavity at about the fourth to sixth day.

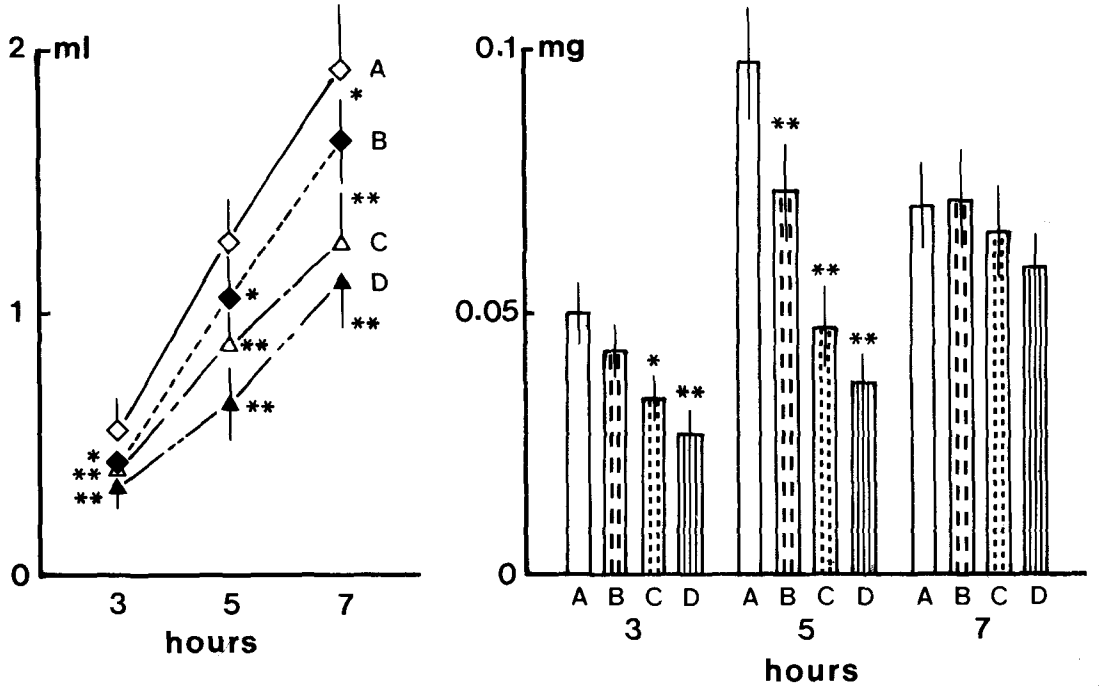

Fig. 1. Effects of cyclobuxine D (5, 20 and 50 mg/kg, i. p.) on the accumulation of the pleural exudate (left panel) and the exudation of dye (right panel). Each value indicates the mean ± S.E.M. of 5 rats.
A ; control, B ; cyclobuxine D 5 mg/kg, C ; cyclobuxine D 20 mg/kg, D ; cyclobuxine D 50 mg/kg.
* $P < 0.05$, ** $P < 0.01$

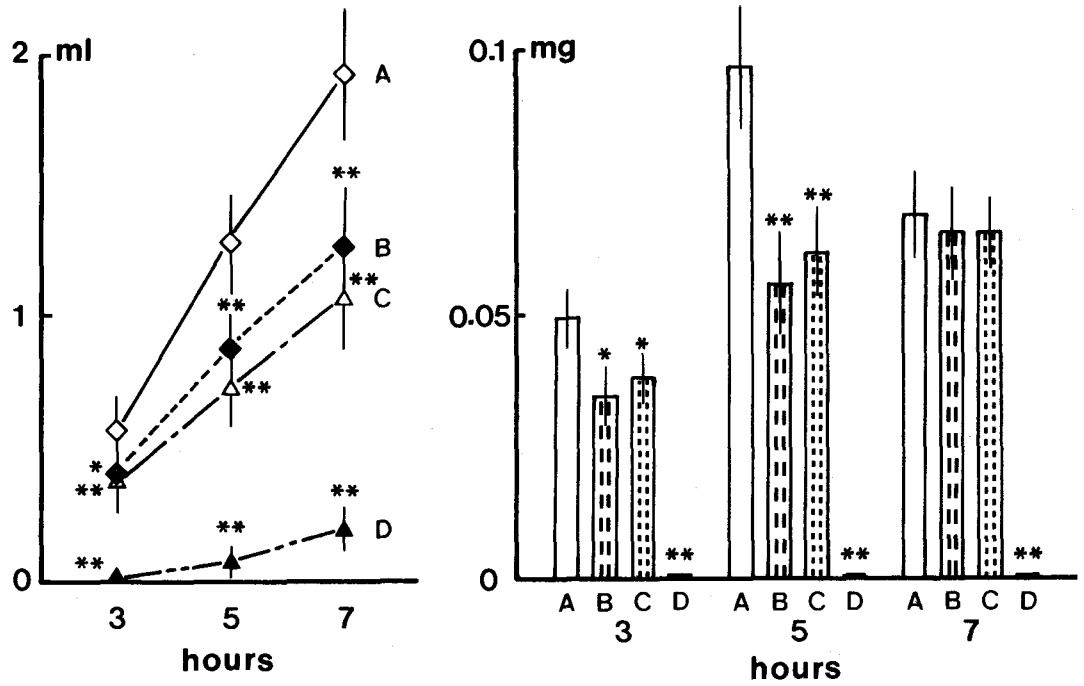

Fig. 2. Effects of cyclobuxine D, aspirin and dexamethasone on the pleural exudate (left panel) and the exudation of dye (right panel). Drugs were administered intraperitoneally. Each value indicates the mean ± S.E.M. of 5 rats. A ; control, B ; cyclobuxine D 20 mg/kg, C ; aspirin 100 mg/kg, D ; dexamethasone 1 mg/kg. * $P < 0.05$, ** $P < 0.01$

Table 1. Effects of drugs (s.c., before 24 hrs) on the croton oil-induced granuloma pouch in SD rats

Group	Dose (mg/kg)	N	Weight of pouch wall (gm)	Fluid in pouch (ml)
Control	–	7	4.84 ± 0.49	26.64 ± 7.25
Cyclobuxine D	5	7	3.09 ± 0.37**	12.33 ± 2.96**
Cyclobuxine D	20	7	2.39 ± 0.29**	4.74 ± 2.54**
Hydrocortisone	10	7	2.93 ± 0.33**	9.37 ± 2.28**

Each value indicates the mean ± S.E.M. of 7 animals.
Significant difference from control value (* $P < 0.05$, ** $P < 0.01$)

On 14th day after croton oil injection, the fluid volume in pouch and the weight of pouch wall in the untreated control group were 26.6±7.3 ml and 4.8±0.5 g, respectively. Cyclobuxine D (5 and 20 mg/kg) decreased dose-dependently the fluid volume in pouch and the weight of pouch wall. The fluid volume in pouch and the weight of pouch wall in the group treated with cyclobuxine D (20 mg/kg) were 4.7±2.5 ml and 2.4±0.3 g, respectively. Hydrocortisone also suppressed significantly the fluid volume in pouch and the weight of pouch wall (Table. 1).

In other experiement, drugs were administered intramuscularly 1 hr before croton oil injection for 10 days. In this experiment, cyclobuxine D and hydrocortisone also decreased significantly the fluid volume in pouch and the weight of pouch wall. Dexamethasone (0.5 mg/kg) prevented completely the development of granuloma pouch (Table 2).

Table 2. Effects of drugs (i. m., for 10 days) on the croton oil-induced granuloma pouch in SD rats

Group	Dose (mg/kg)	N	Weight of pouch wall (gm)	Fluid in pouch (ml)
Control	–	5	3.88 ± 0.41	24.71 ± 5.11
Cyclobuxine D	5	5	2.63 ± 0.35**	12.21 ± 2.80**
Cyclobuxine D	20	5	1.91 ± 0.31**	7.64 ± 1.69**
Hydrocortisone	10	5	1.16 ± 0.29**	4.02 ± 1.41**
Dexamethasone	0.5	5	ND	ND

Each value indicates the mean ± S.E.M. ND : not detected
Significant difference from control value (* $P < 0.05$, ** $P < 0.01$)

DISCUSSION

An intrapleural injection of 0.2 ml of 2% ι-carrageenin caused the accumulation of pleural exudate with leukocyte infiltration (Velo GP, *et al.*, 1973). The accumulation of exudate was attributed to plasma extravasation, which could be assessed by measuring the pontamine sky blue exuded into the pleural cavity. The amount of dye exuded for 20 min increased until 5 hr and then decreased gradually.

The increased levels of PGs and TXB_2 in the exudate during the first 5 hr exactly corresponded to the increased phase of the dye exudation (Harada Y *et al.*, 1983), Aspirin significantly inhibited the dye exudation until 5 hr, but did not at 7 hr (Fig. 2). These results clearly indicate that cyclooxygenase products were involved in the plasma exudation during the first 5 hr. Other investigators (Harada Y *et al.*, 1982; Ford-Hutchinson *et al.*, 1984) reported that the inhibitory effect on the plasma exudation might be explained by decreased infiltration of leukocytes, which release factors increasing vascular permeability such as leukotrienes.

When rats were pretreated with cyclobuxine D, which decreased prostaglandins production and leukocyte migration in carrageenin-induced non-allergic inflammation (Lee JH *et al.*, 1986), the dye exudation and the volume of pleural exudate were decreased (Fig. 1).

Dexamethasone, which reduce the availability of prostaglandin precursors (Hong *et al.*, 1976; Nijkamp *et al.*, 1976) due to a reduction in phospholipase A_2 activity, suppressed significantly the dye exudation and the accumulation of exudate (Fig. 2).

Granuloma pouch was developed by the administration of an irritant (croton oil) into a subcutaneous pouch formed by air injection. Granulomas are collections of macrophages. Fibroblasts and polymorphonucler leukocytes are seen in a granuloma (Robbins SL *et al.*, 1984). PGE_2, $PGF_{2\alpha}$ and TXB_2 have been reported in exudates of carraggenin pouch granulomata (Higgs GA *et al.*, 1976). The suggested sources of PGs include phagocytozing polymorphonuclear leukocytes and skeletal muscle surrounding the pouch.

Dexamethasone and hydrocortisone, which are phospholipase A_2 inhibitors, decreased significantly the fluid volume and the weight of pouch wall. Cyclobuxine D decreased dose-dependently the fluid volume and the weight of pouch wall in croton oil-induced granuloma pouch (Table 1 and Table 2). These effects of cyclobuxine D might be explained by decrease in infiltration of leukocytes which release factors increasing vasuclar permeability or/and inhibition of prostaglandins production.

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= 국문초록 =

흰쥐의 Carrageenin 유발 늑막염과 Croton oil 유발 육아종양에 미치는 Cyclobuxine D의 영향

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김정목, 김천숙, 차영덕, 김영석

Steroidal alkaloid인 cyclobuxine D의 carrageenin으로 유발한 늑막염과 croton oil로 유발한 육아종양에 대한 영향을 관찰하고 그 작용을 aspirin, dexamethasone과 hydrocortisone의 작용과 비교하였다. Carrageenin으로 유발한 늑막염에서 혈장 삼출정도는 pontamine sky blue을 정맥으로 투여하여 20분 동안 늑막염 삼출물로 삼출되어 나오는 양으로 측정하였다. Cyclobuxine D는 용량적으로 삼출물의 양과 20분 동안 삼출되는 pontamine sky blue의 양을 감소시켰다. Cyclooxygenase 억제제인 aspirin과 phospholipase A_2를 억제하여 결과적으로 cyclooxygenase와 lipoxygenase의 생성물을 차단하는 dexamethasone은 현저하게 carrageenin으로 나타나는 염증현상을 억제하였다.

Cyclobuxine D는 croton oil로 유발한 육아종양에서 종양 부위에 직접 투여하거나 근육내로 투여해도 pouch내 염증물 양과 pouch wall의 무게를 현저하게 억제하였다. Dexamethasone과 hydrocortisone은 육아종양의 형성을 현저히 억제하였으며, dexamethasone은 근육내로 투여하였을 때 육아종양 형성을 완전히 억제하였다.

이상의 결과에서 나타난 cyclobuxine D의 항염증 작용은 prostaglandins의 생성억제 또는 polymorphonuclear cell (PMN)의 유주 억제와 밀접한 관련이 있을 것으로 사려된다.