NOVOBIOCIN, AN ANTAGONIST OF LEUKOTRIENE B_4

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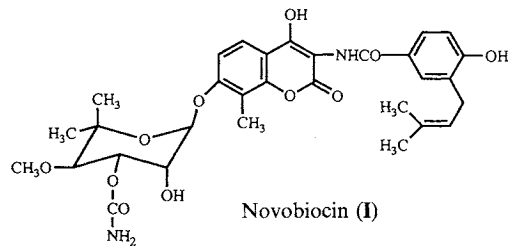
(Received for publication June 19, 1992)

Leukotriene B_4 ((5*S*,12*R*)dihydroxy-6,14-*cis*,8,10-*trans*-eicosatetraenoic acid, LTB_4), a potent activator of polymorphonuclear leukocytes (PMNLs) is known to be an endogenous mediator of several inflammatory diseases such as psoriasis[1] and ulcerative colitis[2]. In the course of our screening for new antagonists of LTB_4, we have isolated WS6629 from the cultured filtrate of *Streptomyces* sp. No. 6629. The structure elucidation studies of WS6629 demonstrated that WS6629 is identical with novobiocin (**I**). Novobiocin is an antimicrobial agent, which was first isolated as cathomycin from the broth of *Streptomyces spheroides* in 1955[3]. However, LTB_4 antagonistic activity of the compound is not known. Here, we wish to describe the isolation of WS6629, its identification with novobiocin, and show the unique anti-inflammatory activities of the compound.

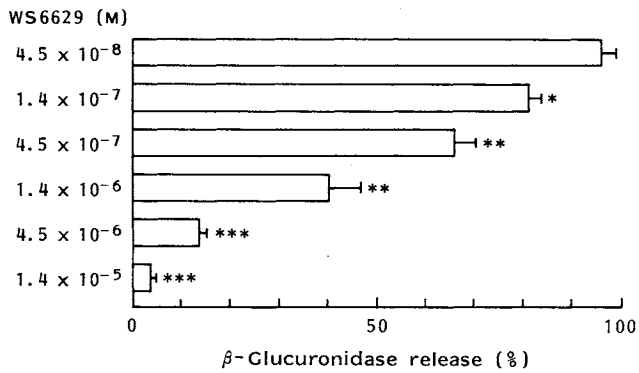


Novobiocin (**I**)

Streptomyces sp. No. 6629 was cultivated in a 30-liter jar fermenter containing 20 liters of a medium consisting of glycerol 3%, sucrose 1%, soybean powder 1%, chicken meal 1%, dried yeast 0.5% and $CaCO_3$ 0.3% for 4 days at 30°C under airation of 20 liters/minute and agitation of 200 rpm. The amount of WS6629 in the fermentation broth was monitored by LTB_4-induced PMNL degranulation assay as previously described[4].

The fermentation broth (15 liters) was filtered, and the filtrate was passed through a column of polymeric adsorbent (Diaion HP-20, Mitsubishi Chemical). The column was washed with water and the adsorbent was eluted with methanol. The eluate was concentrated under reduced pressure and the residue was partitioned between ethyl acetate and water. The ethyl acetate fraction was subjected to column chromatography on silica gel (Kieselgel 60, Merck). The column was washed with chloroform and active compound was eluted with chloroform-methanol (20 : 1). After evaporation to dryness, the residue was applied to a reverse phase column (YMC-GEL ODS-A, Yamamura Chemical). The column was washed with 70% aqueous methanol

Fig. 1. Effect of WS6629 on LTB_4-induced β-glucuronidase release in rabbit PMNLs.



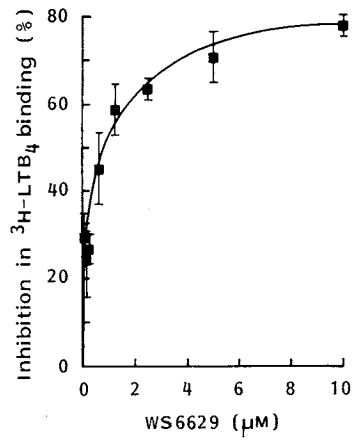
PMNL suspensions (5×10^6 cells/ml) were incubated with WS6629 or vehicle for 5 minutes at 37°C. LTB_4 (7×10^{-8} M) together with cytochalasin B (10^{-5} M) were then added and incubated for 5 minutes at 37°C. The amount of β-glucuronidase released from the cells were quantitated. The results were expressed as percent of the enzyme release from WS6629-treated cells (means $\pm$ SEM, $n=3$) when compared to the release from vehicle-treated cells. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

and the desired compound was eluted with 90% aqueous methanol. The eluate was concentrated to give 320 mg of WS6629 as a crystalline form.

The crystalline WS6629 exhibits mp 156~158°C and is optically active, $[\alpha]_D^{25}$ −62.8° (*c* 1.0, EtOH). These physico-chemical properties and the IR spectrum data (data not shown) of WS6629 are identical with the data from novobiocin reported by the Upjohn group[5]. ^1H and ^{13}C NMR analysis also supported the structure of WS6629 to be **I**.

In the PMNL degranulation assay[4], WS6629 significantly decreased the LTB_4-induced PMNL degranulation in a dose-dependent manner without showing any agonistic activity (Fig. 1). The IC_{50} value was 7.7×10^{-7} M. In contrast, WS6629 did not show any inhibitory effect on the degranulation induced by platelet activating factor (PAF, 7×10^{-7} M) or formyl-methionyl-leucyl-phenylalanine (FMLP, 7×10^{-10} M) at concentrations up to 10^{-4} M.

Fig. 2. Effect of WS6629 on ^3H-LTB_4 binding to PMNL membranes.



The membrane suspension (from 2×10^6 cells) and 7.5 nM of ^3H-LTB_4 in the presence of WS6629 or vehicle were mixed and incubated at 4°C for 30 minutes. Filtration method was used to separate bound from free ligand. The results were expressed as percent inhibition of the binding (means±SEM of 3 experiments).

^3H-LTB_4 receptor binding assay was performed as described previously[4]. As shown in Fig. 2, WS6629 blocked ^3H-LTB_4 binding to PMNL membrane. The IC_{50} value was 1.0×10^{-6} M. These results suggested that WS6629 is a specific antagonist of LTB_4.

We next evaluated WS6629 on arachidonic acid (AA)-induced mouse ear edema. In this animal model, leukotrienes are involved in the edema formation[6]. Five-week-old male Balb/c mice were used. AA was dissolved in acetone at a concentration of 25 mg/ml. Twenty microliters of AA solution was applied by an automatic pipette to the inner surface of the left ear. The right ear received 20 μl acetone. WS6629 was applied topically in acetone 15 minutes prior to AA treatment. When WS6629 was applied orally, the compound was suspended in 0.5% methylcellulose solution and given 30 minutes prior to AA treatment. Edema measurements were taken with a dial thickness gauge. The thickness of the left and right ears were measured at 90 minutes after AA application. Ear edema was calculated by subtracing the thickness of the right ear (vehicle control) from left ear (treated ear). STUDENT's *t*-test was used to determine statistical significance. Regression analysis was used to calculate ED_{30} value. WS6629 significantly inhibited AA-induced ear edema at a dose from 0.1 mg/ear when given topically (Table 1). However, the inhibition did not

Table 1. Effect of WS6629 on arachidonic acid-induced ear edema in mice.

Route	Dose	*n*	Increase in ear thickness (means±SEM, ×0.01 mm)	Inhibition (%)
Topical	Control	15	20.9±0.7	0
	0.1 (mg/ear)	10	18.8±0.6*	10.2
	0.32	10	15.8±0.9***	24.5
	1.0	10	15.9±1.0***	24.0
	3.2	5	11.6±1.4***	44.6
Oral	Control	10	21.0±0.5	0
	32 (mg/kg)	5	20.2±0.7	3.8
	100	10	17.3±0.8***	17.6
	320	7	13.3±1.0***	36.7

WS6629 was administered topically 15 minutes, and orally 30 minutes prior to AA treatment. Ear edema was measured 90 minutes after AA treatment.

* $P<0.05$, *** $P<0.001$.

exceed 50% with doses up to 3.2 mg/ear. The compound by an oral route also inhibited the ear edema in a dose-dependent manner at doses from 32 to 320 mg/kg. The ED_{30} value was 220 mg/kg when WS6629 was administered orally.

Novobiocin is a clinically useful antibiotic, but the anti-LTB_4 or anti-inflammatory activity of the compound has not previously been reported. This is the first report of novobiocin as a LTB_4 antagonist.

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