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Inhibitory effect of disodium cromproxate on superoxide anion (O_2^-) generation and membrane potential changes in stimulated neutrophils[1]

WU Ruo-Shu[2], Ushio SANKAWA

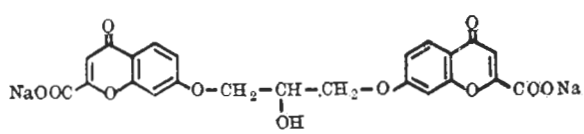
(*Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo Bunkyo-Ku, Tokyo 113, Japan*)

ABSTRACT Disodium cromproxate is an antiallergic agent. This drug (0.5-2 mmol/L) inhibited O_2^- production by neutrophils induced by FMLP and PMA. However, the inhibition of FMLP-induced O_2^- generation was more pronounced than that induced by PMA. Disodium cromproxate also counteracted the changes in membrane potential in neutrophils induced by either FMLP or PMA. The actions of disodium cromproxate differed from those of propranolol, as propranolol had no antagonistic action on membrane potential changes induced by FMLP and PMA.

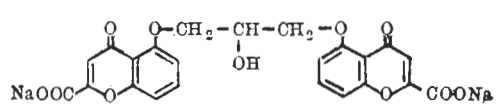
KEY WORDS disodium cromproxate; superoxide anions; membrane potentials; propranolol; neutrophils

Disodium cromproxate (SCP), an isomer of disodium cromoglycate, was first synthesized by Fisons pharmaceutical Ltd in 1967. Liang *et al*[1] synthesized SCP using a simplified procedure. SCP was found to have anti-allergic actions. It significantly inhibited passive cutaneous and passive lung anaphylaxis. It also inhibited degranulation and the release of histamine and serotonin from mast cells, as well as significantly decreasing phosphodiesterase activity. Thus, the effects of compound 48/80 on cAMP of mast cells and the release of histamine and serotonin from mast cells were all reduced (data to be published). The therapeutic effects of SCP on vernal keratoconjunctivitis, allergic rhinitis and asthma were comparable to those of disodium cromoglycate.

When neutrophils were treated with different stimuli *in vitro*, molecular and functional changes took place in the plasma membrane of neutrophils, including mobilization of Ca^{2+}[2,3], phospholipid turnover[3-5] superoxide anion (O_2^-) generation[6,7] and changes in membrane potential[2,7,8]. The mobilization of Ca^{2+} and phospholipid turnover were also seen in mast cells activated by certain stimuli[9,10]. Hydrogen peroxide generation appears to stimulate normal mediator release in mast cells[11]. Since neutrophils are also involved in allergic inflammation, the effects of SCP on O_2^- generation and the membrane potential of neutrophils activated by different stimuli were studied.



Disodium cromproxate (SCP)



Disodium cromoglycate

Materials and methods

Reagents Phorbol myristate acetate (PMA) and *n*-formyl-methionyl-leucyl-phenylalanine (FMLP, Sigma) were dissolved in dimethyl sulfoxide (1 mg/ml) and di-

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[2]Visiting scholar from the *Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, China*

luted with Hank's solution just prior to each experiment. Ferricytochrome C (type Ⅲ, Sigma) and propranolol (Sigma) were dissolved in water. An ethanol solution of diS-C_3 (3,3-dipropylthiodicarbocyanin iodide, Nihon Kanko Shikiso Co) at 0.5 mg/ml was diluted to 10 fold with water to make a working solution before use. SCP was obtained from the Institute of Materia Medica of the Chinese Academy of Medical Sciences.

Preparation of neutrophils[6] Guinea pigs were given 20 ml of 2% casein ip. After 18-20 h, the peritoneal exudate was filtered through a nylon cloth and centrifuged. The pellet was then resuspended in Hank's solution. If the suspension was contaminated with erythrocytes, the RBC were lysed with ice-cold water, and then an equal volume of 1.8% NaCl was added. After centrifugation, the pellet was resuspended in Hank's solution. The suspension contained over 95% neutrophils.

Measurement of O_2^-[6] The reaction mixture contained 20 μl of 2 mmol/L ferricytochrome C, 20 μl of cell suspension (1×10^8 cells/ml) and various concentrations of drugs and was made up to a final volume of 2.45 ml with Hank's solution. The reaction mixture was preincubated at 37℃ for 5 min in a cuvette with a 1 cm light path following which 5 μl of an activating solution (FMLP or PMA 10 μg/ml) were added. The increase in absorbance (A) at 550 nm was followed with reference to 540 nm using a dual beam spectrophoto meter (Hitachi, model 557). The results are expressed as a percent of the control rate, which is the rate of ferricytochrome C reduction produced by FMLP or PMA in the absence of drugs. IC_{50} is expressed by 50% O_2^- production of the control as determined using the dose-response curve.

Measurement of membrane potential changes[6] The fluorescence intensity of diS-C_3 was recorded using a fluorophotometer (Hitachi 665-105) at 510 nm under an excitation wavelength of 460 nm. The reaction mixture containing 1 ml of Hank's solution, 5 μl of cell suspension (1×10^8 cells/ml) and 5 μl diS-C_3 (50 μg/ml) was preincubated in a cuvette at 37℃ for 5 min following which SCP and 10 μl of FMLP (10 μg/ml) or PMA (10 μg/ml) were added.

Results

Effects of SCP on O_2^- generation When neutrophils were preincubated with SCP or propranolol for 5 min before being challenged with FMLP and PMA, O_2^- production was inhibited. The degree of inhibition varied according to the drug concentration and the stimuli (FMLP, PMA). The inhibitory action of SCP on FMLP-stimulated O_2^- generation was more potent than that on the PMA-induced response. On the contrary, the inhibition by propranolol of O_2^- generation activated by PMA was more pronounced than that activated by FMLP. In general, in the cases of either PMA or FMLP-stimulated O_2^- production, the inhibitory action of SCP was weaker than that of propranolol. The IC_{50}

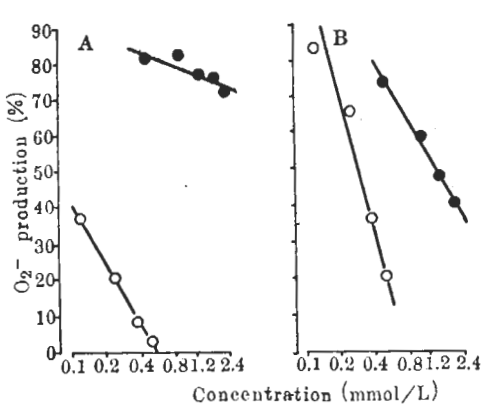

Fig 1. Inhibitory effects of disodium cromproxate (SCP, ●) and propranolol (Pro, ○). A) on phorbol myristate acetate (PMA) stimulated O_2^- generation of peritoneal neutrophils; B) on *n*-formyl-methionyl-leucyl-phenylalanine (FMLP) activated O_2^- generation of peritoneal neutrophils.

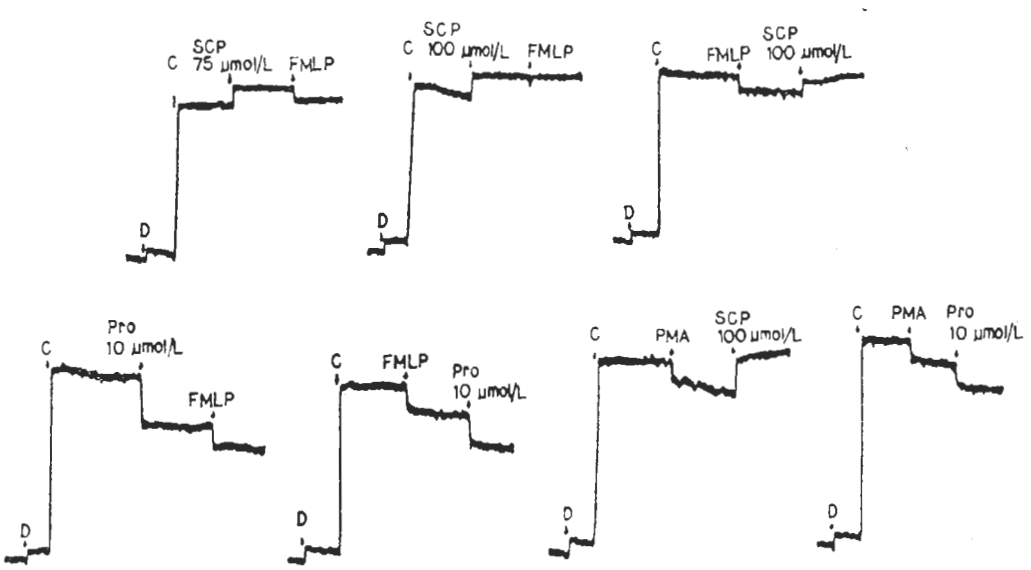

Fig 2. Effects of SCP and Pro on changes of membrane potential of neutrophils (cells, C) induced by FMLP and PMA. The membrane potential was measured by the change in fluorescence intensity of 3,3-dipropylthiodicarbocyanin iodide (diS-C_3, D).

of propranolol on FMLP-stimulated O_2^- production was 290 μmol/L, while that of SCP was 1.35 mmol/L (Fig 1).

Effects of SCP on membrane potential The changes in membrane potential of neutrophils were followed using the cyanine dye method. The fluorescence intensity was increased by SCP and decreased by propranolol. The addition of FMLP or PMA to the reaction mixture reduced the fluorescence intensity to a considerable extent. The degree of counteraction was dependent upon the drug concentration. The higher the concentration, the more marked was the inhibition. 100 μmol/L of SCP almost completely counteracted the action of FMLP (Fig 2). However, propranolol was devoid of this antagonistic action on FMLP and PMA activation.

Discussion

O_2^- production by neutrophils stimulated by various factors is the consequence of 2 separate processes: activation (trigger) and enzyme activity. Both activation and the enzyme mechanism are associated with the plasma membranes of granulocytes and the molecular and functional changes in the plasma membrane are modulated by some drugs[12]. The stimulating processes of FMLP and PMA on neutrophil O_2^- generation were quite different in terms of membrane phospholipid metabolism[4]. PMA stimulates certain sites relatively close to NAD(P)H oxidase[8], while FMLP stimulation is a more complicated process involving the action of phospholipid and protease activation[13]. Since the mediator release of mast cells also involves Ca^{2+} mobilization and phospholipid turnover in the plasma membrane, and because the inhibition of generation of oxygen free radicals may inhibit mediator release in actively secreting mast cells[14], it is quite possible that the anti-allergic drugs interfere with neutrophil O_2^- generation. The present study has demonstrated that SCP antagonizes O_2^- production and membrane potential changes in neutrophils stimulated by FMLP and PMA. The inhibition of O_2^- generation stimulated by FMLP was more pronounced than that induced by PMA. In contrast with SCP, the inhibitory action of pro-

pranolol on PMA-stimulated O_2^- production by neutrophils was stronger than that stimulated by FMLP. In addition, propranolol had no effects on membrane potential. The differences between SCP and propranolol may be due to differences in the intrinsic characteristics of the two drugs. Nevertheless, the inhibition of O_2^- production and the membrane potential of neutrophils by SCP is a new finding and involves another possible site of action.

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色羟丙钠对受刺激的多形核白细胞超氧阴离子生成及膜电位改变的抑制作用

关若钵[1]、　三川　潮
(*Faculty of Pharmaceutical Science, University of Tokyo, Tokyo 113, Japan*)

提要　色羟丙钠(disodium cromproxate)是一种抗变态反应药．该药0.5-2 mmol/L能抑制甲酰三肽(FMLP)及肉豆蔻乙酸(PMA)引起的多形核白细胞超氧阴离子生成，但其作用弱于普萘洛尔，色羟丙钠对FMLP活化的抑制作用强于对PMA活化的抑制作用，而普萘洛尔反之．色羟丙钠能对抗FMLP或PMA引起的白细胞膜电位的改变，而普萘洛尔无此对抗作用．

关键词　色羟丙钠；超氧化物阴离子；膜电位；普萘洛尔；嗜中性白细胞

[1]北京中国医学科学院药物研究所访问学者．

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