Sulochrin Inhibits Eosinophil Degranulation

HIROSHI OHASHI, MASAHARU ISHIKAWA, JUNKO ITO, AKIHIRO UENO, GERALD J. GLEICH†, HIROHITO KITA†, HIROYUKI KAWAI and HIROMI FUKAMACHI

Pharmaceutical Research Laboratory, Kirin Brewery Co., Ltd.
† Department of Immunology, Mayo Clinic and Mayo Foundation

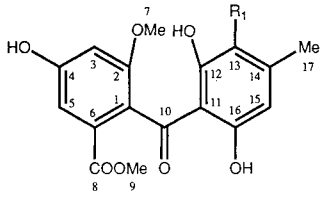
(Received for publication June 9, 1997)

Recent investigations suggest that eosinophils play an important role in allergic diseases such as bronchial asthma. In asthma, after immunoglobulin (Ig) E mediated reactions, eosinophils invade the tissue and degranulate cytotoxic granule proteins including major basic protein and eosinophil derived neurotoxin (EDN)[1]. As such cytotoxic proteins cause tissue injury to increase bronchial responsiveness, inhibitors of eosinophil degranulation would be useful for allergic diseases including asthma.

During the screening for inhibitors of eosinophil degranulation from microorganisms, we isolated sulochrin as an active substance from the culture filtrate of *Penicillium* sp. CS43 (Figure 1). Sulochrin was known as a metabolite of fungi[2,3] and has very weak antibacterial and antifungal activities[3]. We describe here that it has the property of eosinophil degranulation inhibition.

The fungus was cultivated on a rotary shaker at 25°C for 7 days in 500-ml Erlenmeyer flasks each containing 100 ml of medium which consists of malt extract 2%, glucose 2%, peptone 0.1% and agar 0.1%, pH 6. The filtrate of the fermentation broth (2 liters) was extracted twice with 1 liter of ethyl acetate at pH 3. The organic layer was concentrated *in vacuo* and subjected to silica gel column chromatography (Wako gel C-200) using $CHCl_3$-MeOH (50:1). After evaporation, the active portion was applied to preparative TLC ($CHCl_3$-MeOH=10:1) to give 30 mg of pure substance. The compound was identified as sulochrin judging from the data of MS, UV, ^1H NMR spectra, and they were consistent with literature values[3]. ^1H NMR and ^{13}C NMR data are summarized in Table 1. Forthermore, a monochloro derivative was obtained from this broth, and identified as monochlorosulochrin[3].

Fig. 1. The structures of sulochrin and monochlorosulochrin.



	R_1
Sulochrin	H
Monochlorosulochrin	Cl

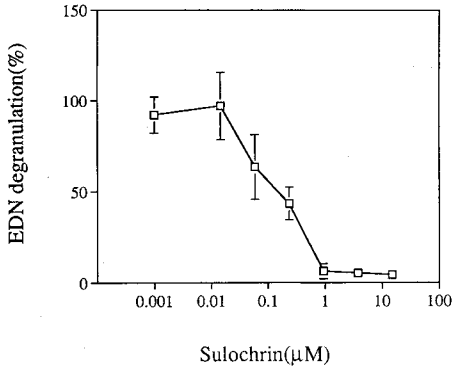
Eosinophils were obtained from normal volunteers with a magnetic cell separation system (MACS) as previously described[4]. Human secretory immunoglobulin A coupled to cyanogen bromide-activated Sepharose 4B beads (sIgA-beads) was used as stimulus for EDN degranulation from eosinophils. sIgA-beads are known as one of the strongest stimuli for eosinophil degranulation. Figure 2 shows the inhibitory activity of sulochrin for eosinophil degranulation, and the IC_{50} value was 0.1 μM. Sulochrin also inhibited eosinophil degranulation induced by PAF at similar concentrations (data not shown). Monochlorosulochrin also exhibited an inhibitory activity for eosinophil degranulation induced by sIgA-beads ($IC_{50}=0.3\,\mu$M). Then, we studied the effects of sulochrin on myeloperoxidase (MPO) degranulation from neutrophils and histamine release from mast cells. Neutrophils were obtained from normal volunteers, and MPO degranulation was induced by 1 μM fMLP (*N*-formyl-methionyl-leucyl-phenylalanine) in the presence of cytochalasin B (5 μg/ml)[5]. Human mast cells were obtained from culture of umbilical cord blood mononuclear cells as described by YANAGIDA *et al.*[6], and histamine release was induced by challenging with anti-IgE antibody after sensitizing with IgE. Sulochrin did

Table 1. ^{13}C and ^1H NMR data of sulochrin.

Position	^{13}C (ppm)	^1H (ppm)
1	126.2	
2	156.8	
3	103.4	6.67 (1H, d, J=2.1 Hz)
4	158.1	
5	107.1	6.89 (1H, d, J=2.1 Hz)
6	127.8	
7	55.9	3.63 (3H, s)
8	165.6	
9	52.0	3.60 (3H, s)
10	199.6	
11	109.1	
12, 16	161.9	
13, 15	107.5	6.07 (2H, s)
14	147.4	
17	21.6	2.14 (3H, s)
4-OH		9.98 (1H, br s)
12,16-OH		11.44 (2H, br s)

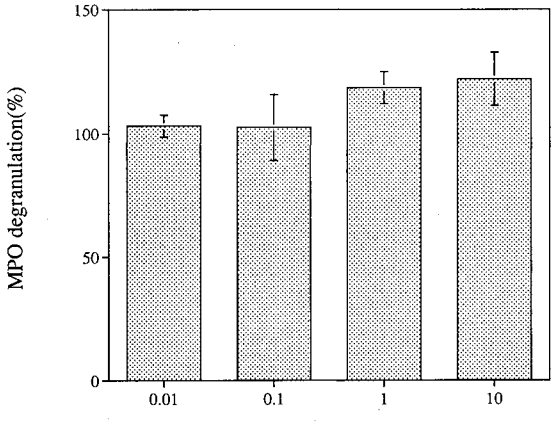
Chemical shifts are shown with reference to DMSO-d_6 (39.5 ppm for ^{13}C NMR, 2.49 ppm for ^1H NMR).

Fig. 2. The effect of sulochrin on EDN degranulation from eosinophils induced by sIgA-beads.



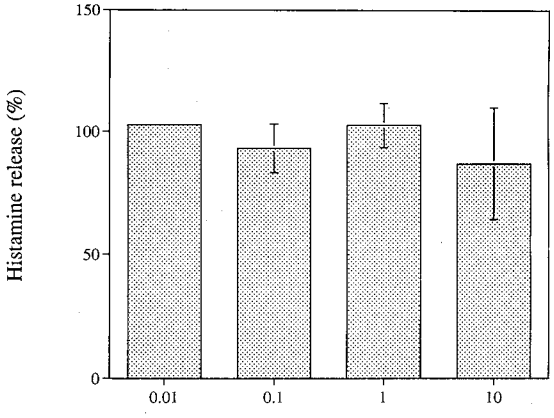
Eosinophils at 2×10^4 cells/well were incubated with various concentrations of sulochrin for 1 hour at 37°C, and then stimulated with sIgA-beads (cells : beads = 2 : 1). After additional incubation for two hours, the supernatants were collected and stored at −20°C until use. The amount of EDN in the supernatants were quantified by ELISA. Results are presented as EDN release, normalized to those obtained with cells in the absence of sulochrin. (Total content of EDN: 583 ± 58 ng/10^5 cells, EDN degranulation with sIgA-beads: 21 ± 4% of total content of EDN. Data are mean ± S.D., n = 3).

Fig. 3. The effect of sulochrin on MPO degranulation from neutrophils induced by fMLP.



Neutrophils at 2×10^4 cells/well were incubated with various concentrations of sulochrin for 30 minutes at 37°C, and then stimulated with fMLP (1 μM) and cytochalasin B (5 μg/ml). After additional incubation for 30 minutes, the supernatants were collected and stored at −20°C until use. The amount of MPO in the supernatants were quantified by RIA. Results are presented as MPO release, normalized to those obtained with cells in the absence of sulochrin. (Total content of MPO: 391 ± 38 ng/10^5 cells, MPO degranulation with fMLP plus cytochalasin B: 71 ± 12% of total content of MPO. Data are mean ± S.D., n = 3)

Fig. 4. The effect of sulochrin on histamine release from mast cells induced by anti-IgE.



Mast cells passively sensitized with IgE at 2×10^4 cells/well were incubated with various concentrations of sulochrin for 30 minutes at 37°C, and then stimulated with anti-IgE antibody. After additional incubation for 30 minutes, the supernatants were collected and stored −20°C until use. The amount of histamine in the supernatants were quantified by histamine analyser[6]. Results are presented as histamine release, normalized to those obtained with cells in the absence of sulochrin. (Total content of histamine: 57 ± 21 ng/10^5 cells, histamine release with anti-IgE antibody: 61 ± 11%. Data are mean ± S.D., n = 3)

not show any inhibitory activities for MPO degranulation from neutrophils and histamine release from cultured mast cells up to 10 μM (Figure 3, 4). Furthermore, sulochrin did not exhibit cytotoxicity against P388 cells, eosinophils, neutrophils and mast cells under the same conditions for degranulation experiments up to 10 μM (data not shown).

These results suggest that sulochrin is a specific inhibitor for eosinophil degranulation and has a possibility to be a good lead compound for an anti-allergic drug.

Acknowledgment

We wish to thank Dr. KUNIO TAKAHASHI (Meiji college of Pharmacy) and Dr. SHINSAKU NATORI (The Research Foundation for Pharmaceutical Sciences) for providing authentic sulochrin. We also thank Ms. MICHIKO MATUOKA for determining of genus of the fungus.

References

1) GLEICH, G. J.: Eosinophil granule proteins and bronchial asthma. Allergology International 45: 35~44, 1996
2) NISHIKAWA, H.: Uber sulochrin, bestandteil des myceliums von Oospora sulphurea = ochracea. Acta Phytochim. 11: 167~185, 1939

3) INAMORI, Y.; Y. KATO, M. KUBO, T. KAMIKI, T. TAKEMOTO & K. NOMOTO: Studies on metabolites produced by *Aspergillus terreus* var. *aureus*. I. Chemical structures and antimicrobial activities of metabolites isolated from culture broth. Chem. Pharm. Bull. 31: 4543～4548, 1983
4) KITA, H.; M. KATO, G. J. GLEICH & R. T. ABRAHAM: Tyrosine phosphorylation and inositol phosphate production: Are early events in human eosinophil activation stimulated by immobilized secretory IgA and IgG? J.Allergy Clin. Immunol. 94: 1272～1281, 1994
5) LABRO, M. T.; J. E. BENNA, N. CHARLIER, H. ABDELGHAFFAR & J. HAKIM: Cefdir (CI-983), a new oral amino-2-thiazolyl cephalosporin, inhibits human neutrophil myeloperoxidase in the exracellular medium but not the phagolysosome. J. Immunology 152: 2447～2455, 1994
6) YANAGIDA, M.; H. FUKAMACHI, K. OHGAMI, T. KUWAKI, H. ISHII, H. UZUMAKI, K. AMANO, T. TOKIWA, H. MITSUI, H. SAITO, Y. IIKURA, T. ISHIZAKA & T. NAKAHATA: Effects of T-helper 2-type cytokines, interleukin-3(IL-3), IL-4, IL-5 and IL-6 on the survival of cultured human mast cells. Blood 86: 3705～3714, 1995