Inhibitory Effects of Anti-Inflammatory Drugs on Interleukin-6 Bioactivity

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Interleukin-6 (IL-6) is known as a proinflammatory cytokine involved in immune response, inflammation, and hematopoiesis. Inhibitory effects of anti-inflammatory drugs on IL-6 bioactivity using IL-6-dependent hybridoma have been evaluated. Three out of 16 nonsteroidal anti-inflammatory drugs (NSAIDs) showed IC₅₀ values of less than 100 μ M, which were in the order of oxyphenylbutazone hydrate (IC₅₀=7.5 μ M)>meclofenamic acid sodium salt (31.9 μ M)>sulindac (74.9 μ M). Steroidal anti-inflammatory drugs (SAIDs) exhibited significant inhibitory effects at 100 μ M on the IL-6 bioactivity, and their inhibitory potencies were in the order of budesonide (IC₅₀=2.2 μ M)>hydrocortisone 21-hemisuccinate (6.7 μ M), prednisolone (7.5 μ M), betamethasone (10.9 μ M)>dexamethasone (18.9 μ M) and triamcinolone acetonide (24.1 μ M). The results would provide an additional mechanism by which anti-inflammatory drugs display their anti-inflammatory and immunosuppressive effects at higher concentrations.

Key words anti-inflammatory drugs; IL-6 inhibitor; oxyphenylbutazone hydrate; budesonide

Interleukin-6 (IL-6) is a proinflammatory cytokine which is produced by both lymphoid and nonlymphoid cells.¹⁾ The receptor for IL-6 is composed of two polypeptide chains called α -subunit and β -subunit.^{1,2)} The α -subunit is the ligand-binding chain with a molecular weight of approximately 80 kDa, and is also known as gp80.^{2,3)} The β -subunit is known as gp130 and is the signal-transducing chain of the receptor complex.^{2,4)} The gp130 is shared by a family of cytokines including oncostatin M (OSM), leukemia inhibitory factor (LIF), IL-11, cardiotrophin-1 (CT-1) and ciliary neurotrophic factor (CNTF).^{5–7)}

IL-6 is a multifunctional cytokine acting in various cells. It induces the differentiation of B cells to antibody-producing plasma cells, T-cell growth and differentiation, the differentiation of myeloid leukemic cell lines into macrophages, megakaryocyte maturation, the neuronal differentiation of PC12 cells, development of osteoclasts, and acute-phase protein synthesis in hepatocytes.^{8–14)} IL-6 acts as a growth factor for myeloma/plasmacytoma, keratinocytes, mesangial cells, renal carcinoma, and Kaposi's sarcoma, but inhibits the growth of myeloid leukemia cell lines and certain sarcoma cell lines.^{15,16)} Thereby, IL-6 plays important roles in the pathogenesis of a variety of diseases, in particular, chronic inflammation, autoimmune diseases, viral infection, and malignancies.^{1,2)}

In this study, inhibitory effects of anti-inflammatory drugs on IL-6 bioactivity were investigated. Oxyphenylbutazone hydrate exhibited the highest inhibitory effect among nonsteroidal anti-inflammatory drugs (NSAIDs) and budesonide among steroidal anti-inflammatory drugs (SAIDs).

MATERIALS AND METHODS

Materials Anti-inflammatory drugs, RPMI-1640, and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Co., Inc. (St. Louis, MO, U.S.A.). Fetal bovine serum (FBS) and *N*-[2-hydroxyethyl]piperazine-*N'*-2-ethanesulfonic acid (HEPES)

were purchased from GIBCO BRL Products (Gaithersburg, MD, U.S.A.) and rhIL-6 from Wako Pure Chemical Ind., Ltd. (Osaka, Japan).

Culture of Hybridoma MH60/BSF-2 The murine B cell hybridoma dependent on IL-6 for its growth was cultured in RPMI (10.4 mg/ml RPMI-1640, 2.48 mg/ml HEPES, 100 units/ml benzylpenicillin potassium, 100 μ g/ml streptomycin sulfate, pH 7.1) containing 10% FBS and 1 unit/ml rhIL-6 at 37 °C with 5% CO₂.¹⁷⁾

IL-6 Bioassay One hundred microliter of 1×10⁴ MH60/BSF-2 cells, 50 μ l of 1.2 unit/ml rhIL-6, and 50 μ l of the medium with or without sample were added to a 96-well microplate (Nunc, Roskilde, Denmark). After incubation at 37 °C with 5% CO₂ for 48 h, the cell growth was measured by optical density (OD) at wavelength 570 nm using the MTT method. Effect on IL-6 bioactivity by the sample is represented as inhibition %, $[1 - (\text{sample OD}_{570} / \text{control OD}_{570})] \times 100$, compared with the control. Data are expressed as means $\pm$ S.E.M., and their significant differences from the controls were analyzed by Student's *t*-test.

IL-3 Bioassay and Cytotoxicity BAF/BO3 cell line was cultured in RPMI containing 10% FBS and 20 units/ml rmIL-3. Proliferation of BAF/BO3 by rmIL-3 was measured as an IL-3 bioassay.¹⁸⁾ IL-6-independent MH60 cell line was established by gradually decreasing IL-6 in RPMI containing 10% FBS for passage of MH60/BSF-2.¹⁸⁾ Effect on the growth rate of IL-6-independent MH60 cells in RPMI containing 10% FBS without IL-6 was viewed as the cytotoxicity of the sample.

RESULTS AND DISCUSSION

NSAIDs are well known as inhibitors of prostaglandin synthesis. However, the mechanism may not account for all pharmacological effects of NSAIDs. It was reported that indomethacin and diclofenac up-regulated the production of TNF, IFN- γ and IL-2 released from Th1 cells but down-regulated the production of IL-4 and IL-6 released from Th2

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cells.¹⁹⁾

Five NSAIDs of diclofenac sodium salt, sulindac, meclofenamic acid sodium salt, mefenamic acid and oxyphenylbutazone hydrate exhibited significant inhibitory effects at 100 μM on IL-6 bioactivity (Table 1). Diclofenac sodium salts and sulindac at 100 μM exhibited 42% and 79% inhibition on the cytokine bioactivity, respectively, but etodolac, indomethacin, and tolmetin sodium salt in the acetate subfamily of NSAIDs did not inhibit. Both meclofenamic acid sodium salt and mefenamic acid in the fenamate subfamily exhibited >95% and 37% inhibition at 100 μM , respectively. Oxyphenylbutazone hydrate exhibited >95% inhibition at 100 μM on IL-6 bioactivity, but phenylbutazone in the pyrazoline subfamily did not inhibit. None of NSAIDs in carboxylate, propionate and oxicam subfamilies tested in this study showed significant inhibitory effects on IL-6 bioactivity. Oxyphenylbutazone hydrate inhibited the IL-6 bioactivity in a dose-dependent manner, and showed an IC_{50} value at the concentration of 7.5 μM (Fig. 1). Based on the IC_{50} values, inhibitory potency on IL-6 bioactivity was in the order of oxyphenylbutazone hydrate>meclofenamic acid sodium salt>sulindac>diclofenac sodium salt and mefenamic acid (Table 1).

SAIDs are corticosteroids exerting profound effects on the inflammatory and immune responses.²⁰⁾ Corticosteroid responses are initiated by binding to a specific receptor, and mediated by the cascade of cellular events involving synthe-

sis of new proteins. It was reported that SAIDs inhibited the production of TNF, IL-1, IL-2, IL-6, IFN- γ chemokine, and GM-CSF, but increased the number of receptors of IL-1 and IL-6.²⁰⁾

All SAIDs exhibited significant inhibitory effects at 100 μM on IL-6 bioactivity (Table 1). Based on the IC_{50} values, inhibitory potency on IL-6 bioactivity was in the order of budesonide>hydrocortisone 21-hemisuccinate, prednisolone, betamethasone>dexamethasone, triamcinolone acetonide>beclomethasone dipropionate. Budesonide inhibited the IL-6 bioactivity in a dose-dependent manner, and showed an IC_{50} value at the concentration of 2.2 μM (Fig. 1).

Among anti-inflammatory drugs showing >50% inhibition on IL-6 bioactivity at 100 μM , all except sulindac and budesonide inhibited the IL-3 bioactivity with potency in the order of prednisolone, hydrocortisone 21-hemisuccinate, dexamethasone>oxyphenylbutazone hydrate, triamcinolone ace-

Fig. 1. Dose-Dependent Inhibitory Effects on IL-6 Bioactivity by Oxyphenylbutazone Hydrate and Budesonide

Samples are oxyphenylbutazone hydrate (open circle) and budesonide (solid circle). Data are represented as inhibition %, mean $\pm$ S.E. of three independent tests, and their significant differences from the control are * $p < 0.001$.

Table 2. Inhibitory Effects on IL-3 Bioactivity and Cytotoxic Effects on IL-6-independent MH60 Cells

Anti-inflammatory drug	IC_{50} (μM)	
	IL-3	Cytotoxicity
Nonsteroidal anti-inflammatory drugs		
Sulindac	>100	>100
Meclofenamic acid sodium salt	48.6	92.7
Oxyphenylbutazone hydrate	35.2	78.5
Steroidal anti-inflammatory drugs		
Betamethasone	51.0	>100
Budesonide	>100	>100
Dexamethasone	24.9	>100
Hydrocortisone 21-hemisuccinate	21.4	>100
Prednisolone	16.0	>100
Triamcinolone acetonide	39.9	>100

IL-3 bioactivity was measured by proliferation of BAF/BO3 cells in the presence of rIL-3. Cytotoxicity was measured by inhibitory effects on the growth rate of IL-6-independent MH60 cells in RPMI containing 10% FBS without IL-6. Data are represented as 50% inhibition (IC_{50}) at micromolar concentration.

Table 1. Inhibitory Effects on IL-6 Bioactivity

Anti-inflammatory drug	Inhibition (%) at 100 μM	IC_{50} (μM)
Nonsteroidal anti-inflammatory drugs		
Carboxylate		
Aspirin	<0 $\pm$ 3	>100
Acetates		
Diclofenac sodium salt	42 $\pm$ 2*	>100
Etodolac	19 $\pm$ 3	>100
Indomethacin	19 $\pm$ 1	>100
Sulindac	79 $\pm$ 1*	74.9
Tolmetin sodium salt	8 $\pm$ 6	>100
Propionates		
Fenoprofen calcium salt	16 $\pm$ 2	>100
Flurbiprofen	26 $\pm$ 3	>100
Ibuprofen	<0 $\pm$ 1	>100
Ketoprofen	<0 $\pm$ 4	>100
Naproxen sodium salt	18 $\pm$ 4	>100
Fenamates		
Meclofenamic acid sodium salt	>95 $\pm$ 1*	31.9
Mefenamic acid	37 $\pm$ 3*	>100
Pyrazolines		
Oxyphenylbutazone hydrate	>95 $\pm$ 1*	7.5
Phenylbutazone	26 $\pm$ 4	>100
Oxicam		
Piroxicam	12 $\pm$ 1	>100
Steroidal anti-inflammatory drugs		
Beclomethasone dipropionate	45 $\pm$ 2*	>100
Betamethasone	93 $\pm$ 2*	10.9
Budesonide	>95 $\pm$ 4*	2.2
Dexamethasone	>95 $\pm$ 1*	18.9
Hydrocortisone 21-hemisuccinate	92 $\pm$ 2*	6.7
Prednisolone	>95 $\pm$ 3*	7.5
Triamcinolone acetonide	86 $\pm$ 4*	24.1

IL-6 bioactivity was measured by proliferation of hybridoma MH60/BSF-2 in the presence of rhIL-6. Data are represented as inhibition %, mean $\pm$ S.E. of three independent tests, and their significant differences from the control are * $p < 0.001$.

tonide>meclofenamic acid sodium salt and betamethasone (Table 2). None of the compounds except oxyphenylbutazone hydrate and meclofenamic acid sodium salt showed cytotoxic effects on IL-6-independent MH60 cells (Table 2).

Several types of potential pharmaceutical agents which inhibit IL-6 synthesis or signaling have been reported including tenidap, suramin, suplatast tosylates, herbal extract of *Stephania tetrandra*, and marine extract of *Erylus placenta*.²¹⁾ Tenidap is a NSAID-like agent with an inhibitory effect on IL-6 production and exhibited inhibitory effects on acute-phase response and rheumatoid arthritis, for which IL-6 is a major mediator.²²⁾

In this study, inhibitory effects of anti-inflammatory drugs on IL-6 bioactivity were demonstrated. Oxyphenylbutazone hydrate among NSAIDs exhibited the highest inhibitory effect with an IC_{50} value of $7.5 \mu M$, and budesonide among SAIDs showed an IC_{50} value of $2.2 \mu M$. These results would provide an additional mechanism by which the drugs exert their anti-inflammatory and immunosuppressive effects.

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