Acta virol. **34** : 26—34, 1990

EFFECT OF MECLOMEN ON PRODUCTION AND ACTION OF HUMAN INTERFERON ALPHA

M. MAHAFZAH[1], S. STADLER[2], J. L. AMBRUS[2], K. CHADHA[3]

[1]Sister's of Charity Hospital, Department of Medicine, Buffalo, NY U.S.A., [2]Roswell Park Memorial Institute, Department of Pathophysiology, Buffalo, NY U.S.A., [3]Roswell Park Memorial Institute, Department of Molecular and Cellular Biology, Buffalo, N.Y. 14263 U.S.A.

Received August 30, 1988

Summary. — Meclomen (sodium meclofenamate) is a nonsteroidal anti-inflammatory drug (NSAID) with dual inhibition of both cyclooxygenase and lipooxygenase enzymes involved in arachidonic acid metabolism. Meclomen at nontoxic levels (10^{-5} to 10^{-8} mol/l) caused a 2—6 fold increase in interferon (IFN) production in lymphocytes induced by Sendai virus. This enhancement was observed in the case of all blood donors tested. Meclomen at the same concentration range had no effect on the antiviral activity of human IFN alpha. However, meclomen did show some enhancement of antiproliferative activity of human IFN alpha against human adenocarcinoma lung cells. Meclomen (10^{-5} mol/l) inhibited intracellular levels of prostagladin E_2 by over 90% within the first four hours. The results presented here strongly suggest that inhibition of intracellular levels of PGE_2 has no adverse influence upon the antiviral and antiproliferative activities of human IFN alpha.

Key words: *HuIFN-α*; *meclomen*; *antiproliferative activity of IFN-α*; *prostaglandins*; *nonsteroidal anti-inflammatory drug*; *cyclooxygenase and lipooxygenase*

Introduction

Interferons (IFNs) are a family of proteins with distinctive antiviral, antiproliferative and immunomodulatory activities. IFNs are synthesized by cells in response to certain inducers, e.g. viruses, double-stranded RNA and mitogens (Lengyel, 1982). The kinetics of IFN induction and production varies with different inducer-cell systems, with IFN appearing extracellularly generally within 4—12 hrs, maintaining maximum levels for about 12—18 hr, and then decreasing rapidly (Stewart, 1979).

Normal individuals can be classified into 3 groups, based on their ability to produce IFN-α upon induction of their WBCs with Sendai virus. The groups are: 1) low producers; 2) average producers; and 3) high producers (Ikossi-O'Connor *et al.*, 1981). WBC from any individual belonging to one

of the above mentioned groups generally exhibited the same pattern of IFN-α production over 3 years of observations (Ikossi-O'Connor, 1981). It has been documented that patients with malignant conditions, post-surgery patients, patients with AIDS are low IFN-α producers (Chadha *et al.*, 1984). Recently, we found that adding PGE_2 to the IFN-induction system inhibits the production of IFN-α (Ikossi *et al.*, 1986). Such an effect can be reversed by using indomethacin that inhibits PG synthesis (Ikossi *et al.*, 1986). Furthermore, we found that indomethacin enhances the IFN-α production in individuals that are low producers but has no effect on average and high IFN-α producers. Meclomen (sodium meclofenamate) is categorized among the non-steroidal anti-inflammatory drug (NSAID) family. In terms of decreasing potency in inhibiting PGE synthesis, meclomen was found to be 3 times more potent than indomethacin, 300 times more potent than oxyphenbutazone and 2750 times more potent than aspirin (Flower, 1974). Meclomen antagonizes PGE as well as leukotriens at the receptor sites (McLean and Glickman, 1983; Burka and Eyre, 1976) and inhibits activity of lipoxygenases (Buctor *et al.*, 1986). Whenever a PG synthesis inhibitor has been utilized to study the role of arachidonic acid metabolites in various physiological and pathological conditions, the results are often ambiguous and inconclusive (Thomas *et al.*, 1974; Sannoro *et al.*, 1976; Jubiz *et al.*, 1979). This is because arachidonic acid metabolism is converted into the leukotriens pathway whenever the prostaglandin pathway is inhibited and vice versa (Vermylin and Deckman, 1978). In the studies reported here, we have examined the effect of meclomen on IFN-α production and upon its antiviral and antiproliferative activities.

Materials and Methods

Media and reagents. Minimal essential medium (MEM) (Eagle, 1955) and RPMI-1640 supplemented with 500 μg/ml penicillin and 50 μg/ml streptomycin were used for cell culture work. The medium was supplemented either with 2% or 10% heat inactivated foetal calf serum. Foetal calf serum was purchased from Grand Island Biological Company, Grand Island, NY. Ficoll-Paque was purchased from Pharmacia Fine Chemicals, Piscataway, NJ. Meclomen was donated by the Warner Lambert/Park Davis Laboratory, Detroit, MI. Radioimmunoassay kits for prostaglandin measurements were purchased from Seragen, Inc., Boston, Ma. Human IFN-α, IFN-β, IFN-γ antibody were prepared and characterized at our Institute (Chadha and Sulkowski, 1984; Dembinski and Sulkowski, 1986). IFN-α antibody was obtained from the National Institutes of Health, Bethesda, MD.

Cells. Human foreskin fibroblasts cells (BG-9) were isolated and characterized at RPMI (Horoszewicz *et al.*, 1978). Bovine embryo kidney cells were purchased from American Type Culture Collection, Bethesda, MD. Human lung adenocarcinoma cell line (A-549) was obtained from Dr. Fogh of Sloan Kettering Institute for Cancer Research, Rye, N.Y. All cell lines were grown in MEM supplemented with 10% FCS and maintained at 37 °C in a humidified atmosphere with 5% CO_2.

Buffy coats. Lymphocytes employed in IFN studies were isolated from the buffy coats obtained from the donors at Buffalo Branch of the American Red Cross, Buffalo, N.Y.

Viruses. Vesicular stomatitis virus (VSV) utilized to assay IFN-α antiviral activity was produced and plaque assayed on African green monkey kidney (CV-1) cells in our laboratory. Sendai virus (Cantell's strain) utilized as an inducer to IFN-α was grown in 9-day-old embryonated eggs and assayed by haemagglutinin test utilizing chicken red blood cells.

Lymphocyte isolation. Buffy coats were processed within 2 hr after the tests for hepatitis B and human immunodeficiency virus were completed. Lymphocytes were isolated by the Ficoll-

-Hypaque sedimentation method (Boyum, 1968). The lymphocytes were harvested from the interface, and cells were washed twice with PBS and then counted. Cell concentration was adjusted to 5×10^6 cells/ml with RPMI-1640 media enriched with 10% FCS. The viability of leukocytes were measured by using trypan blue exclusion method.

Interferon production. The lymphocytes were exposed to Sendai virus (150 HAU/ml) for IFN-α synthesis. The meclomen was added at different times either prior to, during, or post Sendai virus induction as stated in the text. The IFN synthesis was allowed to proceed for 20 hr. At the end of the production period, cell suspension was centrifuged at $400 \times g$ for 10 min to get rid of the cells. The supernatant was adjusted to a pH 2.2 to inactivate the Sendai virus. After 24 hr, the pH was raised to 7.0 and the IFN preparation stored at -70 °C until antiviral assays were performed.

Interferon antiviral assay. Interferon levels were measured according to the dye uptake method (Finter, 1969). Two-fold serial dilutions of IFN preparations were added to the confluent monolayers of BG-9 cells for 18 hr and then challenged with VSV for 36 hr. The cytopathic effect was quantitated by the neutral red dye uptake method. Interferon titres were expressed as the reciprocal of the dilution that protected 50% of the cells against the viral cytopathic effect. International reference HuIFN-α supplied by MRC, England, was used in all assays and results are expressed as International Units (IU).

Antiproliferative activity assay. Human lung adenocarcinoma (A549) cells at a concentration of 2×10^4/ml were seeded in 24 well tissue culture trays in MEM containing 10% FCS. Cells were then exposed to meclomen and/or HuIFN-β at the indicated concentrations. Cells were incubated at 37 °C in an atmosphere of 5% CO_2. Forty-eight hr later, daily cell counts were performed using a Coulter counter for up to 7 days. The data are expressed as per cent inhibition of the cell growth in control wells.

Prostaglandin (PGE_2) assay. Intra-leukocytic levels of PGE_2 were measured during IFN induction both during the presence or absence of 10^{-5} mol/l meclomen. At indicated time intervals either prior to or after the beginning of IFN-α induction, samples were centrifuged ot $400 \times g$ for 10 min. The cell pellets were used to determine the intracellular PGE_2 levels by the radioimmunoassay using commercially available assay kits. Briefly, lymphocyte pellets were washed twice with PBS. Lymphocytes were resuspended in 0.5 ml of distilled water containing 4 mmol/l EDTA. After 30 min on ice, cells were sonicated for 10 seconds. The sonicated preparation was chilled and then acidified to pH 3.5. The protein contents were determined according to Bradford (1976). It was then extracted with ethylacetate. The aqueous phase containing prostaglandins was removed and dried under vacuum. The residue was reconstituted into 0.5 ml of BGG phosphate buffer. PGE contents were determined using radioimmunoassay kit (Seragen Inc., Boston, MA). The PGE_2 levels were expressed in terms of ng/mg of protein.

Results

Toxicity of meclomen to human fibroblasts and lymphocyte cells

Confluent monolayers of human foreskin fibroblast (BG-9) cells or freshly isolated lymphocytes from normal healthy donors were exposed to a range of meclomen concentrations: 0,10 $^{-4}$, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} mol/l. In the case of lymphocytes, cell viability was determined on day 1, 2, 3, and 4 after exposure to different concentrations of meclomen and for fibroblasts on day 1, 3, 5, and 7. The results are presented in Table 1. It can be concluded from these results that meclomen at concentrations up to 10^{-5} mol/l is nontoxic to both fibroblasts and lymphocytes. In all subsequent experiments, meclomen was used at a concentration of 10^{-5} mol/l or lower.

Effect of meclomen on synthesis of HuIFN-α

Lymphocytes were isolated from buffy coats of normal healthy individuals. Lymphocytes from each single donor were divided into five groups:

Table 1. Effect of meclomen on viability of lymphocyte and fibroblast cells

	Per cent cell viability (lymphocytes) at meclomen concentrations (mol/l)					
	0	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Day 1	95	82	95	96	95	94
2	94	60	94	94	92	94
3	89	50	88	89	90	88
4	88	27	91	93	90	90

	Per cent cell viability (fibroblasts) at meclomen concentrations (mol/l)					
	0	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Day 1	100	90	97	96	96	100
3	100	85	100	100	100	100
5	100	77	100	100	100	100
7	100	51	84	93	98	94

a) control; b) lymphocytes treated with 10^{-5} mol/l meclomen for 24 hr prior to Sendai virus induction; c) lymphocytes treated with meclomen at the same time when cells were induced with Sendai virus; d) lymphocytes treated for 24hr prior to Sendai virus and during Sendai virus induction; and (e) lymphocytes treated with meclomen 6 hr after the Sendai virus induction.

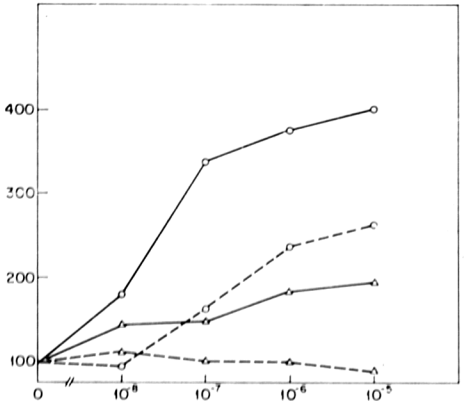

Fig. 1.

Lymphocytes were isolated from the normal healthy donors and induced with Sendai virus

IFN was harvested 20 hr post Sendai induction and assayed for antiviral activity on BG-9 cells. Average titre of control IFN was 12,500 IU. △----△ meclomen (10^{-5} mol/l) was added 6 hr after Sendai virus; △——△, meclomen and Sendai virus were added concurrently; ○----○, lymphocytes were treated with meclomen for 20 hr prior to Sendai virus, and ○——○, lymphocytes were treated with meclomen prior to Sendai virus and its presence continued during IFN production period. Results presented here are from donors belonging to the category of average HuIFN-α -producers. However, identical results were obtained in the case of low and high producers.

Abscissa: meclomen concentration mol/l; ordinate: human IFN-β synthesis (% of control).

Table 2. Effect of meclomen on intracellular level of PGE_2 and HuIFN-α production

Induction time (hr)	Control		Meclomen (10^{-5} mol/l)	
	PGE_2[1]	HuIFN-α (IU)	PGE_2[1]	HuIFN-α (IU)
−4	4.65	<5	4.55*	<5
−3	3.95	<5	0.14**	<5
0	4.11	<5	0.04	<5
1	3.34	5	0.85	40
2	5.29	150	0.94	1,100
4	5.33	540	1.07	1,300
8	10.70	6,600	2.37	12,500
20	4.1	11,500	1.40	26.000

[1] PGE_2 levels expressed as ng/mg protein. Each number is an average of four separate experiments

* Represents PGE_2 levels prior to their treatment with meclomen.

** Represents PGE_2 levels one hr after lymphocytes were treated with meclomen.

In each case, Sendai virus induction was allowed for 20 hr for maximal IFN-α synthesis. Interferon induction was terminated by lowering the pH of induction medium to 2.2 for 24 hr and subsequently raised to pH 7.0. Each sample was dialyzed against sterile PBS prior to assay for its antiviral activity on BG-9 cells. The results are presented in Fig. 1. An average of six donors from each category of low, average, and high IFN-α producers were examined. In nearly all cases, the yield of HuIFN-α from lymphocytes treated with meclomen was significantly increased as compared to the controls. The increase varied anywhere between 200% to 600% of the control (Fig. 1). However, no increase in HuIFN-α yield was seen where lymphocytes were treated with meclomen 6 hr after Sendai virus induction (Fig. 1).

Effect of meclomen intracellular levels of PGE_2 and its relationship to IFN-α production

Lymphocytes from an individual donor were divided into two groups. Lymphocytes in one group were treated with 10^{-5} mol/l meclomen for 4 hr prior to IFN-α induction by the Sendai virus. Lymphocytes in the second group were under identical conditions in the medium alone. At end of 4 hr time period, lymphocytes in both groups were infected with 150 HAU of Sendai virus for IFN-α synthesis. At the time intervals indicated in Table 2, samples of induced lymphocytes were taken and spun for 10 min at 400 × g. The supernatant in each case was used to determine antiviral activity and the cell pellets were used to determine intracellular levels of PGE_2 by the procedure described in Materials and Methods. In the case of lymphocytes that were not treated with meclomen, detectable levels of IFN-α were seen as early as 2 hr after Sendai virus induction. The maximal level of IFN-α was detected 20 hr post induction. The PGE_2 levels in these lymphocytes remained unaltered until 4 hr post Sendai induction. At 8 hr post Sendai induction, maximal level of PGE_2 (10.70 ng/mg protein) was observed. At

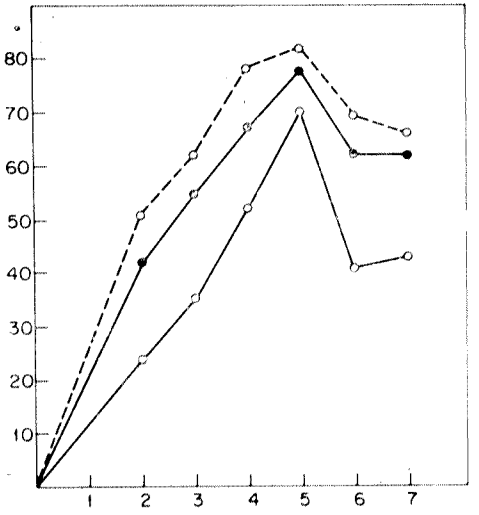

Fig. 2.

Human lung adenocarcinoma (A549) cells were seeded in 24 well tissue culture trays at a concentration of 4×10^4 cells per well

Cells were allowed to attach overnight and then treated with IFN (500 IU) and/or meclome (10^{-5} mol/l). Cell counts were made daily beginning on day 2. The experiment was terminated on day 7 of meclomen treatment. Results presented on average of three separate experiments. In each experiment, cell counts were made in quadruplicate. ○—○, HuIFN-α (500 IU); ○----○, HuIFN-α + meclomen (10^{-7} mol/l); ●——●, HuIFN-α + meclomen (10^{-5} mol/l). Abscissa: days; ordinate: growth inhibition (% of control).

the peak of accumulated IFN-α (20 hr post induction), the level of PGE_2 dropped essentially to the level (4.1 ng/mg protein) prior to IFN-α induction. In the case of lymphocytes that were treated with 10^{-5} mol/l of meclomen, a significant drop (97%) in the level of PGE_2 was seen within one hour (Table 2). When meclomen treated cells were induced with Sendai, no significant rise in PGE_2 level was seen until 8 hr post induction. At 8 hr post IFN induction, the PGE_2 level was 2.37 ng/mg protein which is approximately 22% of control lymphocytes. A significant drop in the level of intracellular PGE_2 (78%) seems to have no bearing on the ability of meclomen-treated lymphocytes to synthesize IFN-α. On the contrary, a 2—3 fold increase in IFN-α synthesis was seen in meclomen treated cells. It is also significant to note that release of IFN-α into the culture media was significantly greater in meclomen treated cells as compared to the control cells.

Effect of meclomen on antiproliferative activity of HuIFN-α

Human lung adenocarcinoma (A549) cells were seeded in 24 well tissue culture trays at a concentration of 2×10^4 cells/well in MEM containing 10% FCS. Cells were allowed to attach overnight. The following morning, the cells were exposed in quadruplicate sets to a range of HuIFN-α doses: 10 IU, 100 IU, 500 IU, and 1000 IU. A set of wells were retained as controls. The cell growth was measured by counting cells each day for a total of 7 days. A dose-dependent inhibition of cell growth was observed (data not shown). From these results, a dose of 500 IU that gave an average of 70% inhibition on day 5 was selected for studies involving meclomen and HuIFN-α. In a separate set of experiments, A549 cells were seeded in 24 well tissue culture trays at the rate of 2×10^4 cells/well and exposed to

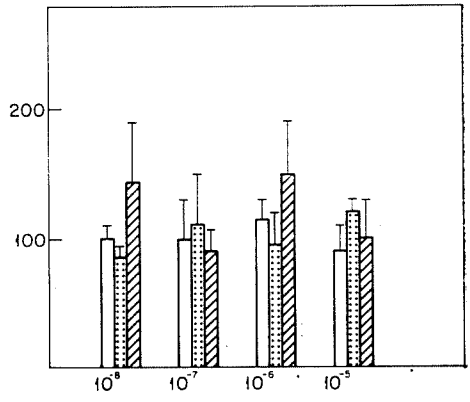

Fig. 3.
Influence of meclomen on antiviral activity of HuIFN-α
Confluent monolayers of BG-9 cells were treated with different concentrations of meclomen as indicated and at different times 1000 IU of HuIFN-α was titrated on these meclomen treated cells. □, monolayers were treated with meclomen for 24 hr prior to titration of HuIFN-α; ⁞⁞⁞, meclomen was added concurrently to HuIFN-α titration; and ////, meclomen was added to cells for 24 hr prior to and also during HuIFN-α titration.
Abscissa: meclomen concentration (mol/l); ordinate: antiviral activity (% of control).

HuIFN-α and meclomen as follows: a) medium control; b) 500 IU of HuIFN-α; c) 500 IU of HuIFN-α plus 10^{-5} mol/l meclomen; and d) 500 IU of HuIFN-α plus 10^{-7} mol/l meclomen. Forty-eight hr after exposure of cells to either reagents, daily cell counts were made up to day 7. Biologically active HuIFN-α was easily detectable in the supernatant at the end of the experiment. The results are presented in Fig. 2. In the case of HuIFN-α, antiproliferative activity was evident within the first 48 hr, and it reached its maximum (70%) by the day 5 and subsequently declined to about the 40% level. The decline in antiproliferative activity of HuIFN-α on day 7 may be related to decline in the titre of IFN-α, since it was added once only at the beginning of the experiment. However, in case when cells were exposed to both HuIFN-α and meclomen, a significantly greater level of antiproliferative activity was evident (40—50%) on day 2 as compared to HuIFN-α alone (approximately 20% inhibition). This synergistic response was maintained throughout the experimental period. Concentrations of meclomen 10^{-5} or 10^{-7} mol/l used were neither toxic to the cells nor had any antiproliferative activity against A549 cells (data not shown). It is interesting to note that lower concentrations of meclomen (10^{-7} mol/l) is marginally superior than higher concentrations (10^{-5} mol/l) as far as enhancement of HuIFN-α antiproliferative activity is concerned.

Effect of meclomen on antiviral activity of HuIFN-α

Confluent monolayers of BG-9 cells grown in 24 well tissue culture trays were exposed to a range of meclomen concentrations (10^{-5} to 10^{-8} mol/l) either a) prior to interferon treatment, b) simultaneous to IFN-α treatment or c) prior to and simultaneous to IFN-α treatment. Control monolayers were treated with the medium alone. Approximately 2000 IU were added to the cells. HuIFN-α used for these studies was produced and purified in our laboratory (Chadha and Sulkowski, 1984). Results representing an average of four separate experiments are shown in Fig. 3. It is obvious from

these data that treating fibroblast cells with meclomen either prior to or concurrent to the exposure of cells to IFN-α does not alter the antiviral activity of this IFN.

Discussion

The ability of the WBCs of normal, healthy individuals to synthesize IFN-α upon *in vitro* stimulation varies considerably (Chadha *et al.*, 1981). Such variability may be due to the suppressor cells or differences in the expression of feedback inhibition within the IFN-α system of each individual (Stewart, 1979). The variation could also have a genetic basis or be a result of undetermined chronic exogenous or endogenous stimuli. We have observed in our laboratory that patients with AIDS or advanced malignancies belong to the category of low IFN-α producers (Ikossi-O'Connor and Chadha, 1984). It is conceivable that normal, healthy individuals that are classified as low IFN-α producers might be at higher risk of contracting AIDS or other malignancies, provided other risk factors are available. Thus, attempts to modulate the low IFN-α production status of apparently healthy individuals may be of considerable significance.

In our studies, meclomen potentiated IFN-α synthesis in the case of all blood donors regardless of whether they were low or high producers. We have earlier reported that indomethacin similarly potentiates IFN-α synthesis in individuals that are low IFN-α producers only. In this matter, meclomen seems to be superior to indomethacin. On the other hand, meclomen has no detrimental effect on the antiviral activity of exogenously administered IFN-α. This suggests that meclomen and HuIFN-α can be given to patients without compromising the antiviral and immunomodulatory potential of IFN-α and at the same time avoiding undesirable effects of excessive PGE achieved due to interferon. There is controversy regarding the role of prostaglandins in the growth of tumour cells. There are several reports (Jaffe *et al.*, 1974, 1976; Jubiz *et al.*, 1979) that indicate that excessive PGE inhibits the growth of various tumour cells in tissue culture. However, others have reported (Hial *et al.*, 1977) that meclomen that significantly inhibits endogenous PG levels also inhibits the growth rate of tumour cells in tissue culture. We have observed that meclomen (10^{-5}—10^{-7} mol/l) potentiates the antiptoliferative effect of HuIFN-α on the A549 human cells. The major differences in cell growth inhibition were evident as early as 2 days after meclomen treatment. Cells exposed to IFN-α alone were inhibited by 20% on day 2 as compared to 50% inhibition of cells treated concurrently with the same dosage of IFN-α and 10^{-7} mol/l of meclomen. The differences in the rate of cell growth inhibition were maintained for the two groups throughout 7 days of the experiment. Neither concentrations of meclomen used alone had any effect on the growth of A549 cells. The enhancement of the antiproliferative activity of HuIFN-α by meclomen may be related to the inhibition of PGE levels in these treated cells.

Our data (Table 2) suggest that meclomen enhances the production of IFN-α in the treated lymphocytes induced with Sendai virus. The peak of

IFN-α production is around 20 hr post induction. Meclomen 10^{-5} mol/l significantly reduces PGE_2 levels (90%) and at the same time results in 200% increase in the IFN-α synthesis. This increase in IFN-α synthesis could be due to an increase in the level of IFN-α message in treated cells or due to its slower degradation. However, it is evident that inhibiting the level of endogenous PGE_2 levels has no detrimental effect on synthesis of IFN-α by the lymphocytes. It is also conceivable that enhanced synthesis and IFN-α is dependent upon changes in levels of c-AMP and leukotriens that are significantly influenced by the meclomen in treated cells.

In conclusion, meclomen at nontoxic levels significantly enhances IFN-α synthesis, has no detrimental effect on the antiviral activity of IFN-α and moderately enhances its antiproliferative activity. These effects might not be entirely related to modulation of prostaglandins by the meclomen and other mechanisms might be involved.

Acknowledgements. The authors wish to express special thanks to Mr. L. DiBerardino and Mrs. V. Belmister for their technical halp. Also, we express our appreciation to M. Held for her valuable help in preparing this manuscript.

References

Boyum, A. (1968): Separation of leukocytes from blood and bone marrow. *Scand. J. clin. Invest.* **21**, Suppl 97, p. 1.

Bradford, M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248.

Buctor, A. M., Eickholt, M. and Pugsley, T. A. (1986): Meclomenamate sodium is an inhibitor of both the 5-lipooxygenase and cyclooxygenase pathways of the arachidonic acid cascade *in vitro*. *Prostagl. Leukot. Med.* **23**, 229.

Burka, J. F., and Eyre, P. (1976): A pharmacological study of SRS-A on the bovine cutaneous vasculature. *Can. J. Physiol. Pharmacol.* **55**, 904.

Chadha, K. C., and Sulkowski, E. (1984): Method for the production and purification of human leukocyte interferon. *U.S. Patent* #4485,038.

Dembinski, W. E., and Sulkowski, E. (1986): Improved large scale purification procedure of natural human fibroblast interferon. *Prep. Biochem.* **16**, 175.

Eagle, H. (1955): Propagation in a fluid medium of a human epidermoid carcinoma, strain K. B. (21811). *Proc. Soc. exp. Biol. (NY)* **89**, 362.

Finter, N. B. (1969): Dye uptake methods for assessing viral cytopathogenicity and their application to interferon assays. *J. gen. Virol.* **5**, 419.

Flower, R. J. (1974): Drugs which inhibit prostaglandin biosynthesis. *Pharmacol. Rev.* **26**, 33.

Hial, V., DeMello, M. C. F., Horakoraz, and Beaven, M. A. (1977): Antiproliferative activity of anti-inflammatory drugs in two mammalian cell culture lines. *J. Pharmacol. exp. Therap.* **202**, 446.

Horoszewicz, J. S., and Leong, S. S. (1978): Human fibroblast interferon in human neoplasia: clinical and laboratory study. *Cancer Treat. Rep.* **62**, 1899.

Ikossi-O'Connor, M. G., Goldrosen, M., and Chadha, K. C. (1981): Identification of target cells responsible for the production of interferon. *Surg. For.* **32**, 74.

Ikossi-O'Connor, M. G., and Chadha, K. C. (1984): The effect of malignant epithelial tumours, surgical trauma and infection upon various parameters of interferon system. *J. Surg. Oncol.* **26**, 27.

Ikossi-O'Connor, M. G., Ambrus, J. L., and Chadha, K. C. (1986): Regulation of *in vitro* human leukocyte interferon production: effect of prostaglandin synthetase and phosphodiesterase inhibition. *Res. Commun. Chem. Path. Pharmacol.* **54**, 379.

Ikossi-O'Connor M. G., Ozer, H., Van Oss, C. J., and Chadha, K. C. (1986): Characterization of non-T effector cell subpopulations involved in production of human alpha interferon. *Immun. Invest.* **15**, 559.

Jubiz, W., Frailey, J., and Smith, J. B. (1979): Inhibitory effect of prostaglandin F2-α on the growth of a hormone dependent rat mammary tumour. *Cancer Res.* **39**, 998.

Lengyel, P. (1982): Biochemistry of interferons and their actions. *Ann. Rev. Biochem.* **51**, 251.

McLean, J. R., and Gluckman, M. D. (1983): On the mechanism of pharmacologic activity of meclofenamate sodium. *Arz.-Forsch.* (*Drug Res.*) **33**, 627.

Sannoro, M. G., Philpott, G. W., Jaffe, B. M. (1976): Inhibition of tumour growth *in vivo* and *in vitro* by prostaglandin E. *Nature* (*Lond.*) **263**, 777.

Stewart, W. E. II (1979): *The Interferon System*, Springer-Verlag, Heidelberg.

Thomas, R. D., Philpott, G. W., and Jaffe, B. M. (1974): The relationship between concentrations of prostaglandin E and rates of cell replication. *Exp. Cell Res.* **84**, 40.

Vermylin, J., and Deckman, H. (1983): Reorientation of prostaglandin endoperoxide metababolism by a thromboxane synthetase inhibitor *in vitro* and clinical observation. *Br. J. clin. Pharmacol.* **15**, 175.