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Studies on the mechanism by which indomethacin increases the anticancer effect of methotrexate

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1 The effect of indomethacin on the response of the NC carcinoma to methotrexate has been examined *in vivo* and *in vitro*.

2 Survival was prolonged in mice treated with indomethacin 1.25 mg kg^{-1} twice daily plus methotrexate 4 mg kg^{-1} daily, compared to mice given either drug alone or controls.

3 Indomethacin 1 $\mu g\ ml^{-1}$ increased the killing of cultured NC cells by methotrexate. This was not due to displacement of methotrexate from binding sites on the serum proteins. Nor was it due (entirely) to inhibition of prostaglandin synthesis, since flurbiprofen did not mimic the effect. Inhibition of cyclic AMP phosphodiesterase seems unlikely to explain the effect of indomethacin since theophylline had little or no effect on NC cell killing by methotrexate.

4 Indomethacin 1 $\mu g\ ml^{-1}$ increased the accumulation of tritium in NC cells incubated with [^{3}H]-methotrexate.

5 In contrast, with normal epithelial cells from human embryonic intestine, indomethacin 1 $\mu g\ ml^{-1}$ did not alter the cytotoxicity of methotrexate or the accumulation of tritium during incubation with [^{3}H]-methotrexate.

6 The beneficial interaction between indomethacin and methotrexate may have therapeutic potential in man.

Introduction

As a result of our experiments on prostaglandins and human mammary cancer (Bennett *et al.*, 1977), we studied the effects of prostaglandin synthesis inhibitors on NC carcinomas in mice, usually following excision of the transplanted tumour after it had metastasized. The experiments showed that flurbiprofen or indomethacin prolonged the host survival (Bennett *et al.*, 1979; 1982), presumably because prostaglandin synthesis was inhibited (Bennett *et al.*, 1985). We also examined the effect of the prostaglandin synthesis inhibitors on the response of mice to cytotoxic chemotherapy (a mixture of methotrexate and melphalan which was currently being used in a trial on breast cancer patients, Murray *et al.*, 1978). The survival of mice with NC carcinoma was increased by the cytotoxic drugs. An even greater increase occurred when flurbiprofen or indomethacin was also given (Berstock *et al.*, 1979; Bennett *et al.*, 1982); this was probably due to more than just an addition of the separate effects of the chemotherapeutic drugs and the prostaglandin synthesis inhibitors, since the response of the cytotoxic drugs plus flurbiprofen tended to be greater than could be achieved with the highest tolerated doses of the cytotoxic drugs alone (Berstock *et al.*, 1982).

All the previously reported *in vivo* studies have been with the mixture of the cytotoxic drugs, so it was not possible to determine whether a beneficial interaction with indomethacin occurs with just one of the cytotoxic drugs. The present experiments demonstrate that the increase in survival with indomethacin plus methotrexate is greater than can be achieved with the highest tolerated dose of methotrexate alone. This effect also occurs *in vitro*, and we have examined its mechanism by studying the cytotoxicity to NC cells in culture, methotrexate uptake, and protein binding. In contrast to NC cells, the effect of methotrexate on epithelial cells from human normal embryonic intest-

ine was not increased by indomethacin

Methods

In vivo *studies*

The NC carcinoma arose spontaneously in the mammary region of a WHT/Ht mouse and has been passaged serially in this strain (Hewitt *et al.*, 1976). This tumour commonly gives rise to local lymphatic spread, metastases to the lungs and mediastinum, and recurrence in the excision scar. WHT/Ht male mice aged 2–4 months and weighing 25–38 g at the start of the experiments were fed SDS No. 1 modified diet, and had free access to water. They were weighed twice weekly from 2 weeks before the start of the experiment.

On Day 0 the mice were injected s.c. into the left flank with approximately 10^6 NC carcinoma cells prepared as described by Bennett *et al.* (1979, 1982). Indomethacin and methotrexate (MTX) were given by mouth in 0.1 ml 50% syrup. There were 4 separate experiments each with 6 groups (controls, indomethacin, MTX at 2 dose levels, and indomethacin + MTX), resulting in 21–30 mice per group in the combined experiments. A seventh group was given MTX in the high dose of 8 mg kg^{-1}, but this was used only in the first experiment because 6/8 mice died during the second period of dosing.

The mice were given indomethacin 1.25 mg kg^{-1} twice daily (5 days/week) from the day before tumour excision until death or termination of the experiment on Day 121. MTX 2 or 4 mg kg^{-1} was given to some control and indomethacin-treated groups on Days 15–17, 22–24, and 29–31. On Day 14 the tumours were excised under ether anaesthesia and weighed. Mouse survival time was measured from the day of tumour transplantation until death, and was analysed statistically by the method of Lee & Desu (1972). Mice with advanced carcinomatosis were killed humanely to prevent suffering (Bennett *et al.*, 1982), or at the end of the experiment on Day 121. Post mortem data, including incidences of scar recurrence, lymph node involvement, and distant metastases, were analysed by Fisher's exact test.

In vitro *studies*

Primary cultures of NC carcinoma cells were obtained from a WHT/Ht mouse with peritoneal metastases following i.p. injection. Cells in a sample of peritoneal fluid were maintained as a suspension in modified Eagle's medium containing 10% newborn bovine serum, 50 units ml^{-1} each of penicillin and streptomycin, and 1% nonessential amino acids prepared as directed by Flow Laboratories. After an initial lag phase the cells grew rapidly, the numbers increasing approximately 3 fold in 4 days. Aliquots were routinely subcultured for up to 4 months and formed malignant tumours after s.c. injection into WHT/Ht mice.

Microturbidimetric assay for measuring cell growth

NC cells grown in suspension were pelletted by centrifugation, resuspended in 1 ml trypsin/EDTA solution (0.05%:0.02%) and incubated for 30 s at 37°C. After rapidly adding 9 ml medium, the cells were gently disaggregated by repeated passage into and out of a pipette. The cell numbers were determined in a Coulter counter and adjusted to give 25,000 in 50 µl. To each of the 96 wells of a micro test plate were added 150 µl of modified Eagle's medium containing newborn bovine serum and drugs or vehicle at 1.33 times the desired final concentration, followed by 50 µl of the cell suspension or medium alone. The plates were incubated at 37°C, 5% CO_2 in air, 100% humidity, and after four days the absorbance of 600 nm light in each well was determined with a Dynatech microplate reader (Gaffen *et al.*, 1985).

Concentration-growth curves were obtained with MTX 2–40 or 4–20 ng ml^{-1} alone or with either indomethacin or flurbiprofen 1 µg ml^{-1} respectively. In addition, the growth of NC cells was examined after exposure to either indomethacin 1 µg ml^{-1} or theophylline 9 µg ml^{-1} alone or combined with MTX 8 ng ml^{-1}. This concentration of theophylline is equipotent to indomethacin 1 µg ml^{-1} as an inhibitor of cyclic AMP phosphodiesterase in toad bladder (Flores & Sharp, 1972).

Cloning assays of normal cells

Epithelium-like cells from human embryonic intestine (No. 407, Flow Laboratories) were grown as monolayers in Eagle's basal medium supplemented with 15% newborn bovine serum and 50 units ml^{-1} of penicillin and streptomycin. Their growth was assessed by clonogenic assay in liquid medium. Single cells in suspension were seeded into 5 cm tissue culture dishes (200 cells/dish) containing 5 ml of medium and added drugs or vehicle(s) only. The plates were incubated at 37°C in an atmosphere of 5% CO_2 in air, 100% humidity, for 9 days. After discarding the medium, the cells were fixed with 10% formalin in 154 mM NaCl for 15 min at room temperature. The fixative was removed, and the cells were stained with 1% methylene blue. Colonies with 50 or more cells were counted, to indicate the number of cells remaining fully viable after treatment. Concentration-growth curves were constructed for methotrexate 5–10 ng ml^{-1} ± indomethacin 1 µg ml^{-1}.

Methotrexate uptake studies

'MTX uptake' into the NC cells was studied by a modification of a method from Henderson *et al.* (1978). Bulk cultures of NC cells were generated by inoculating 5×10^6 disaggregated cells into 120 cm^2 glass culture flasks containing 100–120 ml of medium. After 4–5 days at 37°C, the cells were harvested by centrifugation, disaggregated, counted, and resuspended in glass centrifuge tubes (2×10^6 cells in 1.8 ml medium). The tubes were then preincubated for 15 min at 37°C under the conditions described previously, in the presence of indomethacin or flurbiprofen 1 $\mu g\,ml^{-1}$ or vehicle only. Radiolabelled MTX (0.2 ml of 20 μM [3′,5′,7′^{3}H]-methotrexate sodium salt, 250 mCi $mmol^{-1}$, Amersham) in PBS was then added to each tube and the cells incubated at 37°C for 1 h. Control samples were treated identically except that the tubes were placed in an ice/water bath 5 min before and during incubation with the isotope. The cell suspensions were washed twice with ice-cold PBS (8 ml and then 10 ml), and were centrifuged at 700 *g* and 1900 *g* respectively for 5 min at 4°C after each wash. The cell pellets were suspended in 1 ml distilled water, and transferred to glass counting vials with 2 aliquots of scintillation fluid (final volume 10 ml). Active accumulation of label was taken as the difference in the radioactivity between the experimental and control samples.

For 'MTX uptake' into the human normal epithelial cells, monolayers approaching confluency were detached from the flasks, disaggregated and counted: 2×10^6 cells per 5 ml medium were seeded into 5 cm Petri dishes and left to plate out for 15 h. The old medium was replaced with fresh medium containing indomethacin or flurbiprofen 1 $\mu g\,ml^{-1}$ or vehicle only (1.8 ml per plate), and the cells pre-incubated for 15 min at 37°C. After adding 0.2 ml of 20 μM [^{3}H]-MTX in PBS, the cells were incubated for a further 1 h. The medium was removed, and the cell monolayers washed twice with ice-cold PBS. Trypsin/EDTA solution (1 ml, 0.05%:0.02%) was added to each plate, and incubation continued at 37°C until the cells had just detached. Soybean trypsin inhibitor (0.2 ml of 10 $\mu g\,ml^{-1}$) was mixed with the cells which were then transferred to prelabelled, precooled tubes in an ice/water bath. Any cells remaining on the plates were washed off with 2 aliquots of ice-cold PBS, transferred to the appropriate tubes, and the final volume adjusted to 10 ml with PBS. After immediate centrifugation (700 *g* for 5 min at 4°C) the cell pellet was suspended in 1 ml of distilled water, and transferred to counting vials with aliquots of scintillation fluid (final volume 10 ml). Control plates were treated identically except that they were incubated at 4°C for 15 min before and during incubation with the isotope.

Determination of binding to serum protein

The ultrafiltration method of Dixon & Adamson (1965) was used to study the binding of [^{3}H]-MTX to newborn bovine serum alone or in the presence of indomethacin or sodium salicylate 1 $\mu g\,ml^{-1}$. In these studies, 0.4 ml of 20 μM [^{3}H]-MTX was added to 3.6 ml of either newborn bovine serum or Eagle's medium + 10% serum, together with drugs or vehicle: 2.5 ml of each solution was transferred to dialysis tubing suspended in 15 ml centrifuge tubes. Centrifugation at 20°C for 6 h at 300 *g* yielded 0.3–0.5 ml of ultrafiltrate. The amounts of MTX in the starting solution and in the ultrafiltrate were measured by liquid scintillation counting, and after subtracting the amount of membrane-bound MTX the percentage of protein-bound MTX was determined.

Drugs and solvents

The culture medium was prepared from powder (Flow Laboratories). Indomethacin and flurbiprofen were dissolved in water containing sodium bicarbonate (final pH 7.8 and 7.7 respectively). Both theophylline and MTX were made up in 154 mM NaCl; the methotrexate solution was adjusted to pH 8.4 with 0.1 M NaOH. Prostaglandin E_2 (PGE_2) was diluted as necessary in phosphate buffered saline (PBS) pH 7.4 from an ethanolic stock solution. All the water was double-distilled in glass, and the solutions were sterilized by filtration.

Results

Mouse tumour weights

The median weights of tumours were similar in all groups (170–210 mg). This was expected since treatment was started only the day before tumour excision.

Mouse survival

Treated mice survived longer than controls ($P = 0.05$ for indomethacin, and $P = 0.04$ and 0.08 respectively for 2 and 4 $mg\,kg^{-1}$ MTX; Table 1). A dose of 4 $mg\,kg^{-1}$ seems to be close to the maximum tolerated, since the median survival time was fractionally shorter than with 2 $mg\,kg^{-1}$, and most mice died with 8 $mg\,kg^{-1}$. The median survival of mice treated with MTX 2 $mg\,kg^{-1}$ + indomethacin was 10% longer than with either dose of methotrexate alone ($P < 0.1$). Mice given MTX 4 $mg\,kg^{-1}$ + indomethacin survived longest of all (25% longer than with MTX 4 $mg\,kg^{-1}$ alone, $P = 0.04$). In most groups there were 2–3 disease-free mice at Day 121, but in the MTX 4 mg kg^{-1} + indomethacin group 6 mice (22%) survived

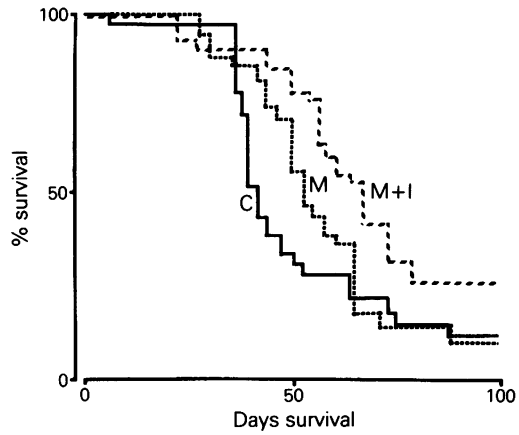

Figure 1 Methotrexate 4 mg kg^{-1} (dotted line, M) tended to increase the survival of mice with excised NC carcinomas, compared with controls (solid line, C; $P = 0.08$); the median survival was fractionally longer than this with methotrexate 2 mg kg^{-1} ($P < 0.04$ compared to controls), and the mice could not tolerate 8 mg kg^{-1} (data not shown). Thus 4 mg kg^{-1} was close to the maximum tolerated dose of methotrexate alone. The survival (days from tumour transplantation) was greater when methotrexate 4 mg kg^{-1} was given together with indomethacin 1.25 mg kg^{-1} twice daily (dashed line, M + I; $P < 0.04$ compared to methotrexate alone, $P < 0.003$ compared to controls, $n = 26-30$ per group).

disease-free to Day 121 (Figure 1 and Table 1).

Most of the post mortem findings (Table 2) show little difference between the treatments, but it must be remembered that mice which lived longer had more time for metastases to develop. Nevertheless, the spread to lymph nodes was lower in the MTX 4 mg kg^{-1} + indomethacin group compared to MTX 4 mg kg^{-1} or controls (both $P = 0.008$), and the incidence of local recurrence at the excision site was lower in mice given methotrexate 4 mg kg^{-1} with or without indomethacin ($P = 0.04$ compared to controls). A similar weak trend occurred with the other treatments, but lung metastasis at post mortem was unaffected (Table 2).

In vitro *results*

NC tumour cells As we previously reported (Gaffen *et al.*, 1985) MTX 2–40 ng ml^{-1} reduced the growth of NC cells as measured by microturbidimetry in a concentration-related fashion. Indomethacin 1 μg ml^{-1} alone had little or no effect on cell growth, but it increased the cytotoxicity of MTX. We now show that indomethacin increased the accumulation of ^{3}H when the NC cells were incubated with [^{3}H]-MTX (Figure 2).

However, the cyclo-oxygenase inhibitor flurbiprofen 1 μg ml^{-1} *decreased* the cytotoxicity of MTX, as judged by microturbidimetry measurements (Figure 3). This may have been at least partly because flurbiprofen alone appeared to increase cell growth slightly, since light absorption by the cells was increased by 10 ± 3% ($P = 0.01$). Furthermore, there was acting by inhibiting cyclic AMP phosphodiesterase. Indomethacin 1 μg ml^{-1} or theophylline were incubated with [^{3}H]-MTX ($P < 0.3$) (Figure 2).

We used the phosphodiesterase inhibitor theophylline to examine the possibility that indomethacin was acting by inhibiting cyclic AMP phosphodiesterase. Indomethacin 1 μg ml^{-1} or theophylline 9 μg ml^{-1} had little or no effect on the growth of the NC cells. However, indomethacin but not theophylline increased the killing of these cells by MTX 8 ng ml^{-1} (Figure 4).

Table 1 Mouse survival with indomethacin (Indo) and methotrexate (MTX) (median values and semiquartile ranges)

Treatment	n	*Survival*	*Disease-free survivors* (to Day 121)
Syrup control	30	41 (38–56)	2
Indo	22	49 (45–56)[a]	3
MTX 2 mg kg^{-1}	21	52 (44–57)[a]	2
Indo + MTX 2 mg kg^{-1}	21	57 (52–83)[bd]	3
MTX 4	26	51 (44–63)[c]	2
Indo + MTX 4 mg kg^{-1}	27	64 (54–91)[be]	6
MTX 8 mg kg^{-1}	8	27 (21–28)	0

Indomethacin or methotrexate 2 or 4 mg kg^{-1} increased mouse survival. However 6/8 mice given MTX 8 mg kg^{-1} died of drug toxicity in the 2nd course of treatment and this dose was not used again. Survival was longest in mice given Indo ± MTX. *P* values compared to controls: [a]< 0.05; controls; [b]< 0.005. [c] $= 0.08$ compared to methotrexate 2 mg kg^{-1} alone [d] $= 0.09$. *P* compared to methotrexate 4 mg kg^{-1} alone, [e] $= 0.04$.

Table 2 Post mortem data

Treatment	*Spread to lymph nodes*	*Scar recurrence*	*Lung metastases*
Syrup control	18/30	10/30	24/30
Indo	15/22	6/22	16/22
MTX 2 mg kg^{-1}	9/21	5/21	14/21
Indo + MTX 2 mg kg^{-1}	9/21	4/21	13/21
MTX 4	16/21	2/26[b]	19/26
Indo + MTX 4 mg kg^{-1}	6/27[a]	3/27[c]	16/27

The results are the numbers of mice within each group with cancer spread or recurrence. The only clear differences were the lower spread to the lymph nodes in the indomethacin (Indo)/methotrexate (MTX) 4 mg kg^{-1} group, and the lower incidences of scar recurrence in the groups given MTX 4 mg kg^{-1} alone or with Indo. However, the treated groups lived longer so that recurrence and spread had a longer time to develop.
P values compared to methotrexate 4 mg or syrup controls: [a]0.008; *P* compared to syrup controls: [b]0.04; [c]0.09. All other comparisons $P > 0.1$.

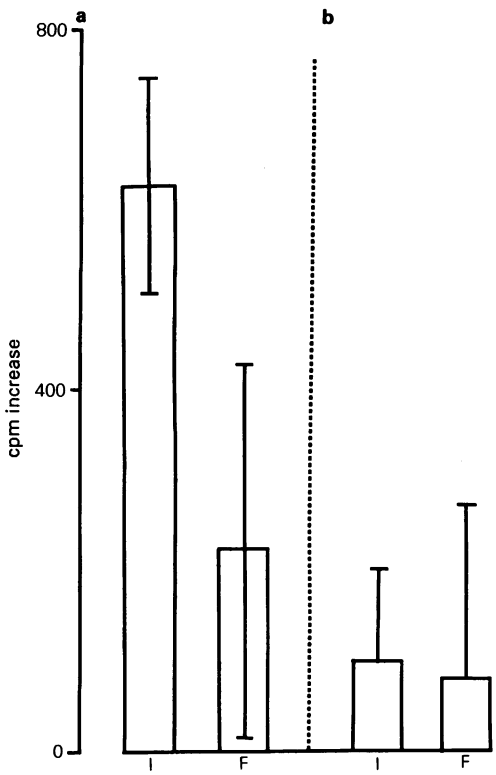

Figure 2 (a) In the NC carcinoma cells indomethacin 1 μg ml^{-1} (I) increased the accumulation of ^{3}H after 60 min incubation with [^{3}H]-methotrexate ($P < 0.001$; *t* test for paired data); flurbiprofen 1 μg ml^{-1} (F) showed at most a weak tendency to do this. (b) In the normal intestinal cells incubated with [^{3}H]-methotrexate, I or F (both 1 μg ml^{-1}) had little or no effect on the accumulation of label ($P < 0.4$ and < 0.7 respectively). The vertical bars are s.e.mean. $n = 5$ or 6 experiments for the NC cells, each with 3–5 replicates, and 4 or 5 experiments for the intestinal cells each with 3 or 4 replicates.

Epithelial cells from human normal embryonic intestine
The clonal growth of the normal epithelium-like intestinal cells was inhibited by MTX 5–10 ng ml^{-1} in a concentration-dependent fashion. Indomethacin 1 μg ml^{-1} alone had little or no effect on cell growth, and unlike the NC cells it did not alter the cytotoxity of MTX. Furthermore, neither indomethacin nor flurbiprofen affected the accumulation of label when the normal cells were incubated with [^{3}H]-MTX (Figure 2).

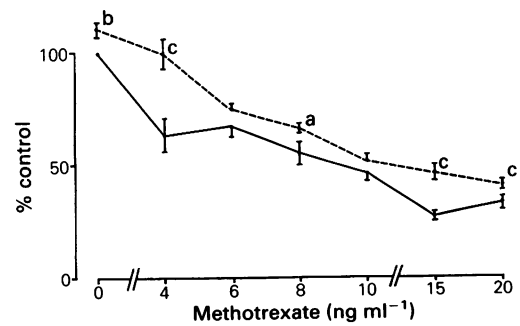

Figure 3 In contrast to indomethacin, flurbiprofen 1 μg ml^{-1} appeared to decrease the cytotoxicity of methotrexate in NC cells, as judged by microturbidimetry measurements. This may have been due (mainly) to the stimulation of cell growth by flurbiprofen. Solid line, methotrexate; dashed line methotrexate + flurbiprofen 1 μg ml^{-1}. Each point is the mean ± s.e.mean of the separate means from 8 or 9 experiments, with 8–16 replicates per experiment. *P* values: [a]< 0.05; [b]< 0.01; [c]< 0.005, Student's *t* test for paired data on the raw results.

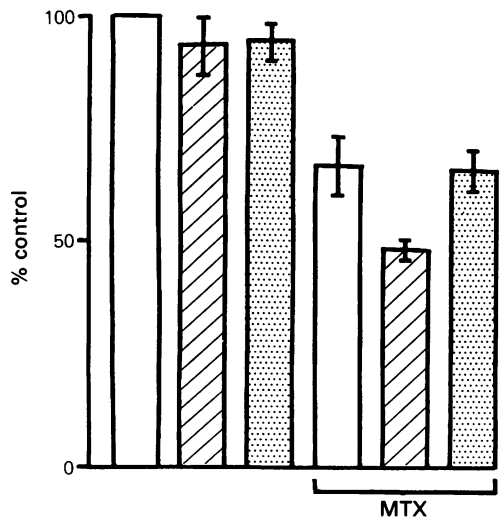

Figure 4 Indomethacin 1 $\mu g\,ml^{-1}$ (hatched columns) or theophylline 9 $\mu g\,ml^{-1}$ (stippled columns) had little or no effect on the growth of NC cells in culture; controls (open columns). Whereas indomethacin increased the killing of these cells by methotrexate 8 $ng\,ml^{-1}$ (MTX, $P < 0.02$), theophylline had no effect. The results are means ± s.e.mean of the separate means from 5 experiments, with 8–16 replicates/experiment.

[^{3}H]-methotrexate binding to serum proteins In newborn bovine serum containing 2 μM MTX, indomethacin or sodium salicylate 1 $\mu g\,ml^{-1}$ had little or no effect on the binding to serum protein. The respective binding to 10% NBS was: vehicle 35 ± 7%, indomethacin 34 ± 7% ($n = 7$); vehicle 33 ± 6%, salicylate 28 ± 8% ($n = 6$). Binding to 100% NBS was: vehicle 51 ± 3%, indomethacin 51 ± 3% ($n = 7$); vehicle 51 ± 3%, salicylate 50 ± 2% ($n = 6$).

Discussion

Our results indicate that the beneficial interaction between the cytotoxic drugs methotrexate/melphalan and indomethacin on the survival of mice with NC tumours may be explained by the interaction between methotrexate and indomethacin. However, it is not known whether there is a contributory interaction with melphalan. It seems that the effect of indomethacin + MTX is greater than can be achieved with higher doses of MTX alone, presumably because indomethacin either potentiates the effect and/or reduces the toxicity of MTX. Survival with MTX 4 $mg\,kg^{-1}$ was almost identical to that with 2 $mg\,kg^{-1}$, and 8 $mg\,kg^{-1}$ killed most of the mice, so that 4 $mg\,kg^{-1}$ seems to be near the maximum tolerated dose. The possibility of a pharmacokinetic interaction is suggested by the increased toxicity in man with aspirin (British National Formulary, 1986), but our previous (Bennett *et al.*, 1982; 1986) and present findings argue against the possibility that indomethacin acts by displacing MTX from binding to serum proteins. Displacement *in vivo* could theoretically increase the anticancer effects of MTX, but it would be expected that toxicity would increase simultaneously unless indomethacin also protects against MTX-induced damage. The latter possibility has not been examined with MTX, but indomethacin reduced the toxicity of melphalan or radiotherapy on gut and bone marrow respectively (Powles *et al.*, 1978).

Evidence that indomethacin may potentiate the effect of MTX by increasing its uptake comes from our work with the labelled drug. The increased accumulation of label is consistent with the greater killing of NC carcinoma cells when indomethacin, in a concentration which has little or no effect on its own, is given together with various concentrations of MTX.

We do not know if the increased accumulation of label represents increased uptake of methotrexate or a degradation product, or decreased release of methotrexate or its products such as polyglutamates (Jolivet *et al.*, 1983). Nor is it clear to what extent the effect of indomethacin involves inhibition of prostaglandin synthesis. Henderson *et al.* (1978) found that PGE_2 inhibited the accumulation of label when L1210 cells were incubated with [^{3}H]-MTX, possibly because the PGE_2 stimulated adenylate cyclase. It would therefore follow logically that indomethacin could increase MTX accumulation by inhibiting PGE_2 formation. However, NC tumour cells in culture have a low ability to produce prostaglandins. After incubating 1–2 × 10^6 NC cells for 1 h at 37°C, 5% CO_2 in air, either alone or in the presence of either bradykinin 5 $\mu g\,ml^{-1}$ or the calcium ionophore A23187 1 $\mu g\,ml^{-1}$, no PGE_2 or thromboxane B_2 (TXB_2) was detected by radioimmunoassay (RIA, limits of detection 300 and 100 $pg\,ml^{-1}$ respectively). Nevertheless, cells incubated with [^{14}C]-arachidonic produced small amounts of substances chromatographing (Tavares *et al.*, 1985) with cyclo-oxygenase and lipoxygenase products (mean percentage incorporations into prostaglandins and LTB_4 1.3 ± 0.07% and 1.04 ± 0.07% respectively, unpublished). Another question about the importance of prostaglandin synthesis inhibition is raised by the finding that although the mean accumulation of radiolabel was somewhat higher with flurbiprofen, this may well have arisen by chance ($P < 0.3$). Furthermore, if anything, flurbiprofen reduced rather than increased the cytotoxicity of MTX, although this effect may have been due in part to a stimulation cell growth by flurbiprofen. Differences in the extents to which indomethacin and flur-

biprofen inhibit prostaglandin synthesis or divert substrate metabolism into lipoxygenase products might affect the response. We found that the formation of lipoxygenase products from [^{14}C]-arachidonic acid by rat peritoneal leucocytes differed when indomethacin, piroxicam or isoxicam were used (Tavares *et al.*, 1985).

The possibility that the indomethacin/MTX interaction is due to inhibition of cyclic AMP phosphodiesterase (Flores & Sharp, 1972) seems unlikely; theophylline, at a concentration equipotent to indomethacin on this enzyme in toad bladder (Flores & Sharp, 1972), did not affect cell killing with MTX (8 ng ml^{-1}). We therefore cannot identify any single property of indomethacin which accounts for the interaction with MTX. However, we have not excluded the possibility that two or more pathways must be altered simultaneously, and that indomethacin may be able to do this (e.g. cyclo-oxygenase; phosphodiesterase; calcium binding; Northover, 1973), unlike flurbiprofen which does not inhibit phosphodiesterase.

The ability of indomethacin to increase the uptake of radiolabelled MTX by NC cells, and to increase their sensitivity to the cytotoxic drug has obvious therapeutic potential. This is particularly so since the normal epithelial cells from human intestine were resistant to the potentiation of MTX by indomethacin. An obvious possibility is the use of indomethacin with MTX in the treatment of human gastrointestinal cancers, since chemotherapy is currently of little value in these diseases (Wrigley & Slevin, 1981). In addition, we have preliminary data that indomethacin increases the cytotoxicity of methotrexate to human breast cancer cells in culture (unpublished).

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