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SHORT COMMUNICATIONS

Selective effect of pulmonary oedema on prostaglandin E_2 pharmacokinetics in rat lung

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Pulmonary oedema, induced *in vivo* by injection of α-naphthylthiourea (ANTU) in rats, affected the pharmacokinetics of exogenous prostaglandin E_2 (PGE_2) in isolated, perfused lungs [1]. One of the variables measured in these experiments was the $T_{\frac{1}{2}}$ value which is related to the transit time of the radioactive substrate and metabolites through the pulmonary circulation. This transit time is in turn related to the permeability of the vasculature and the extravascular volume available to the substrate and to the metabolism of the substrate. We report here experiments in the same model of pulmonary oedema designed to compare the effects of oedema on $T_{\frac{1}{2}}$ values for substrates other than PGE_2.

Materials and methods

Briefly, male rats (200–280 g) were injected i.p. with 10 mg/kg body weight of ANTU suspended in olive oil (4 mg/ml). At the stated times after injection, the rats were anaesthetized with pentobarbitone and lungs removed either for weighing or for perfusion with Krebs solution at 8 ml/min. The lungs were weighed immediately after removal and then again after drying to constant weight. From these measurements, lung wet weight : body weight and lung dry : wet weight ratios were calculated.

Perfused lungs [2] were used in the measurement of $T_{\frac{1}{2}}$; 0.1 ml bolus injections of radioactive substrates (^{3}H-labelled PGE_2, TxB_2, ^{14}C-labelled PGE_2 or sucrose) either together or separately were given into the perfusate flow entering the lung. The combined substrates used were ^{3}H-PGE_2 (352 ng, 50 nCi) and ^{14}C-sucrose (342 ng, 10 nCi) or ^{14}C-PGE_2 (352 ng, 10 nCi) and ^{3}H-TxB_2 (124 pg, 42 nCi). In some experiments, ^{3}H-TxB_2 at the same dose was injected alone.

The effluent perfusate was collected in 4 drop fractions (*ca* 3 sec) immediately before, during and after the injection for a total of 2 min. Radioactivity in each fraction was measured by liquid scintillation methods. The time taken for 50% of the injected radioactivity to emerge from the lung was used as the $T_{\frac{1}{2}}$ value for that substrate.

To measure metabolism of TxB_2, lung effluent was collected in a single fraction for 5 min after the injection of ^{3}H-TxB_2. The TxB_2 and metabolites in the effluent were adsorbed on a Sep-Pak cartridge (C_{18}, Waters). The cartridge was washed with methanol (5 ml) and water (5 ml) and then the effluent (40 ml) was applied. After a further water wash (10 ml), the TxB_2 plus metabolites were eluted in methanol (4 ml). The methanolic eluate was evaporated to dryness, redissolved in methanol (0.1 ml) and analysed by thin-layer chromatography as described earlier [1]. The developed chromatogram was cut into 1 cm strips and the radioactivity in each strip measured by liquid scintillation [1].

ANTU was obtained from Eastman Kodak and sucrose (Analar grade) from BDH Chemicals Ltd. Radioactive (1-^{14}C)-PGE_2, 58 mCi/mmole, (U-^{14}C)-sucrose, 555 mCi/mmole, and (5,6,8,11,12,14,15, (*n*)-^{3}H)-PGE_2, 160 Ci/mmole, were obtained from the Radiochemical Centre (Amersham U.K.), (5,6,8,9,11,12,14,15, (*n*)-^{3}H)-TxB_2, 125Ci/mmole, was from New England Nuclear (Boston, MA).

Results are expressed as mean values (± S.E. mean) from N experiments (lungs). Differences between means were tested for significance using the unpaired *t*-test and values of $P < 0.05$ taken as significant.

Results

By 2 hr following the single injection of ANTU, lung wet weight : body weight ratios increased above the value for untreated rats (7.1 ± 0.29 vs 5.6 ± 0.09 g/kg respectively; means ± S.E. from 4–6 rats; $P < 0.05$), and remained elevated until 50 hr. The lung dry : wet weight ratio, more usually accepted as a physical sign of oedema, was below control (21 ± 0.7%) only at 4 hr (16.5 ± 0.7%) and 6 hr (16.8 ± 0.5%) following ANTU (see Fig. 1).

In untreated rats, $T_{\frac{1}{2}}$ values for sucrose and PGE_2 measured in the same lungs with combined substrates were different (16 ± 2s vs 41 ± 3s respectively). After treatment with ANTU, $T_{\frac{1}{2}}$ values for both substrates increased but the magnitude of the effect on PGE_2-$T_{\frac{1}{2}}$ was very much greater. Thus at 28 hr, the PGE_2-$T_{\frac{1}{2}}$ value was 107 ± 4s, an increase of 150% over that in lungs from control animals whereas the maximal effect on sucrose-$T_{\frac{1}{2}}$ at the same point, was a 50% increase giving a value of 25 ± 2s. By 50 hr after ANTU injection, $T_{\frac{1}{2}}$ values for either substrate had recovered to normal levels.

In other experiments, the $T_{\frac{1}{2}}$ values for TxB_2 and PGE_2 were compared. In lungs from untreated rats, the $T_{\frac{1}{2}}$ values for these two substrates, measured simultaneously, were very close. At 6 hr and at 28 hr after ANTU treatment, the $T_{\frac{1}{2}}$ for TxB_2 was unchanged whereas that for PGE_2 was increased as observed in the earlier experiments (Fig. 1). There is evidence that TxB_2 competes with PGE_2 for uptake in lung [3–5] and as this competition might have interfered with an effect of ANTU on TxB_2 pharmacokinetics, we re-estimated TxB_2-$T_{\frac{1}{2}}$ values using ^{3}H-TxB_2 alone. The results, shown in Fig. 2, are essentially the same as in the combined substrate experiments. There was no change in $T_{\frac{1}{2}}$ values at 6 hr or 28 hr after ANTU compared with the values from untreated rats.

Lung effluent after ^{3}H-TxB_2 alone was analysed for TxB_2 metabolite and these results are also shown in Fig. 2. The proportion of radioactivity associated with the TxB_2-metabolite decreased progressively from about 30% in untreated lungs to less than 5% in lungs from rats 28 hr after ANTU. Recovery of ^{3}H in effluent collected for analysis was 70 ± 8% in untreated lungs and was not significantly different 6 hr or 28 hr after ANTU (86 ± 2%, 83 ± 5% respectively; N = 3 at each time).

Discussion

We have compared the effects of pulmonary oedema on the pharmacokinetics of PGE_2 and two other substrates, sucrose and TxB_2. In this report, we have chosen to emphasise the assessment of pharmacokinetics by the $T_{\frac{1}{2}}$ value (time for 50% of the administered radioactivity to appear in the lung effluent) because it provides a quick and easy measure which could be used as a biochemical index of pulmonary oedema. Sucrose was selected as a substrate because it is often used as a marker for the extracellular space and it has a molecular weight (MW) of 342 daltons

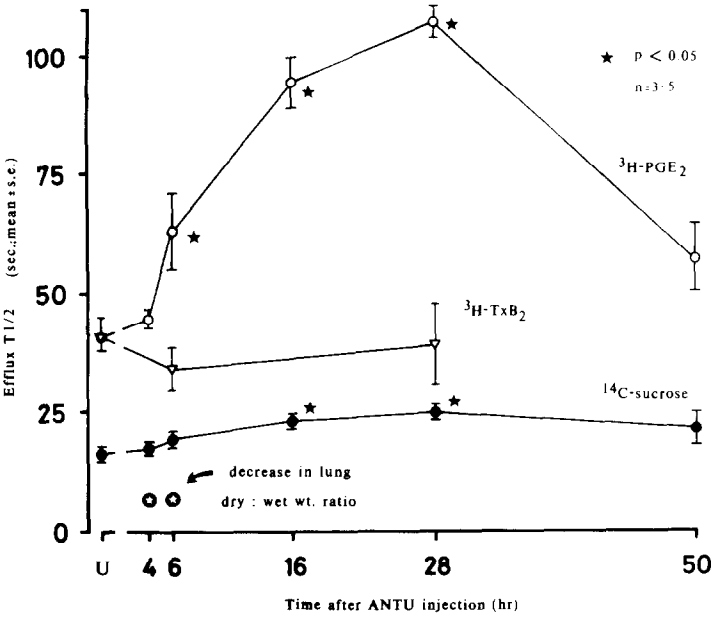

Fig. 1. Time course of the effects of ANTU treatment on pharmacokinetics of PGE_2, sucrose and TxB_2 in rat isolated lung. The $T\frac{1}{2}$ values shown are the mean (± S.E. mean) value of results from 3–5 lungs at each time. The values at the zero time-point represent $T\frac{1}{2}$ measured in lungs from untreated animals. Note that the oedema as measured by dry:wet weight ratios was present at only 4 hr and 6 hr after ANTU treatment (as marked on the figure). However, both PGE_2 and sucrose showed a slower efflux, i.e. $T\frac{1}{2}$ values increased, at 16 hr and 28 hr well after the time of maximum oedema. By comparison, $T\frac{1}{2}$ values for TxB_2 were unchanged at either 6 hr, at the peak of oedema, or at 28 hr, the time of peak effect for the other two substrates.

very close to that of PGE_2, 352 daltons. If neither substrate entered lung cells, their rates of efflux from the lung and their $T\frac{1}{2}$ values should be the same. In normal lungs, PGE_2 had a $T\frac{1}{2}$ value more than twice that of sucrose, because PGE_2, in contrast to sucrose, is taken up by the cells and metabolised.

In oedematous lungs, there is an increase in extravascular volume and this increased volume should be accessible equally to sucrose and PGE_2 if they were both inert because their MWs are so close. The increased volume in oedematous lungs produced a slower efflux for sucrose, increasing $T\frac{1}{2}$ by about 50% at the maximum. However the increase in $T\frac{1}{2}$ for PGE_2 was much greater, absolutely or relatively, suggesting that the effects on PGE_2 kinetics were not simply due to an increased extracellular volume in the lung. The increased $T\frac{1}{2}$ for PGE_2 in lungs after ANTU treatment measured either radiochemically or biologically has been shown to be accompanied by decreased metabolism [1]. Since PGE_2 is a substrate for the PG transport system and the 15-oxo metabolites are not [6], an increase in unchanged PGE_2 in the lung will lead to a relative retention of radioactivity in the lung and hence to a slower efflux. It appears therefore that the increase in PGE_2-$T\frac{1}{2}$ values does not simply reflect an increase in extracellular volume, but is related to the disturbed biochemical state.

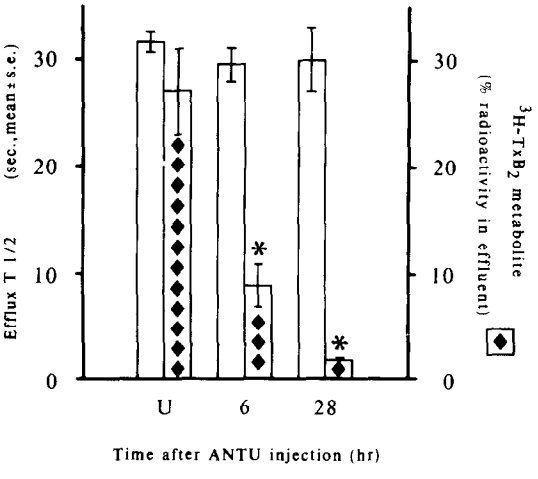

Fig. 2. Effect of treatment with ANTU on $T\frac{1}{2}$ values and metabolism of TxB_2 in rat isolated lung. The height of the bars represents mean values (with one S.E. shown) from 4–5 lungs ($T\frac{1}{2}$) or 3 lungs (metabolism). The $T\frac{1}{2}$ values did not change from the value observed in untreated lungs (U), at either 6 hr or at 28 hr after ANTU. However, the TxB_2-metabolite fell progressively over the same period.

The second substrate chosen for comparison was TxB_2, because this eicosanoid is metabolised in perfused lung via oxidation of the 15-OH group [7] and its uptake appears to be related to that of PGE_2 [3–5]. In our present experiments, ANTU treatment decreased TxB_2 metabolism, as previously found with PGE_2 [1], a result compatible with the general similarity in the disposition of these substrates in the lung we have pointed out above. However, the $T\frac{1}{2}$ value for ^{3}H efflux from lung was not changed over the same period (up to 28 hr) when ^{3}H-TxB_2 was given either together with ^{14}C-PGE_2 or alone as in the metabolism studies. In the latter set of experiments, the variation in results was such that we would have been able to detect a 50% increase in $T\frac{1}{2}$, a change comparable to that observed for sucrose.

One possible explanation for the different effects of ANTU-treatment on $T\frac{1}{2}$ for PGE_2 and TxB_2, even though metabolism is decreased for both substrates, could lie in the relatively greater metabolism of PGE_2 (approximately

90%, ref. 1) than that of TxB_2 (approx. 30%, this paper). Thus a decrease in PGE_2 metabolism to 75% would lead to a 2.5-fold increase in PGE_2 survival from 10% to 25% whereas for TxB_2, a similar decrease in metabolism would increase survival by a factor of 1.3 (from 70% to 95%). Our method of collecting 3 sec fractions may not be of sufficient resolution to detect the smaller change in TxB_2 efflux kinetics.

In other studies with ANTU-treated lung, the metabolism of 5-hydroxytryptamine [8] and that of AMP was decreased [9] but no efflux measurements were reported in either study. In the absence of any direct estimates we would speculate that the pharmacokinetics of $PGF_{2\alpha}$, a substrate metabolised as extensively as PGE_2 in rat lung [10], would respond as do those of PGE_2 to ANTU treatment.

In summary, the pharmacokinetics of PGE_2 and sucrose in lungs taken from rats were altered by the oedema induced *in vivo* by ANTU, but the magnitude of the effect on PGE_2 kinetics was much greater than that on sucrose. As sucrose is a marker for extracellular volume, this result showed that the slower efflux of PGE_2 was not merely due to an increase in extracellular volume in oedematous lung. Furthermore since the efflux of radioactivity derived from TxB_2 was not affected by ANTU treatment, although its metabolism was inhibited, PGE_2 appears to be the best substrate so far tested to demonstrate the pharmacokinetic effects of this type of pulmonary oedema.

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REFERENCES

1. Y. S. Bakhle, *Biochem. Pharmac.* **31**, 3395 (1982).
2. Y. S. Bakhle, A. M. Reynard and J. R. Vane, *Nature* **222**, 956 (1969).
3. A. L. A. Boura and R. D. Murphy, *Clin. exp. Pharmac. Physiol.* **5**, 387 (1978).
4. T. M. Fitzpatrick, L. S. Friedman, P. A. Kot and P. W. Ramwell, *Br. J. Pharmac.* **70**, 295 (1980).
5. K. Patel and Y. S. Bakhle, *Prostaglandins, Leukotrienes and Medicine* **10**, 221 (1983).
6. T. E. Eling, H. J. Hawkins and M. W. Anderson, *Prostaglandins* **14**, 51 (1977).
7. C. Robinson, S. H. Peers, K. A. Waddell, I. A. Blair and J. R. S. Hoult, *Biochim. biophys. Acta* **712**, 315 (1982).
8. E. R. Block and F. J. Schoen, *Am. Rev. resp. Dis.* **123**, 69 (1981).
9. Y. S. Bakhle and C. J. Grantham, *J. Physiol.* **365**, 106P (1985).
10. M. W. Anderson and T. E. Eling, *Prostaglandins* **11**, 645 (1976).

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Marked inhibition of histamine formation in transplantable histamine-producing gastric carcinoid of *Mastomys natalensis* by (*S*)-α-fluoromethylhistidine and its potent antiulcer effect on tumor-bearing hosts

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(*S*)-α-Fluoromethylhistidine (FMH) is a potent and specific inhibitor of L-histidine decarboxylase (HDC, L-histidine carboxy-lyase, EC 4.1.1.22) and strongly inhibits histamine formation *in vitro* and *in vivo* [1–3].

Matsomys natalensis, an African rodent ranging in size between a mouse and a rat, is the only mammal other than humans to develop gastric carcinoid at a high incidence [4, 5]. Our previous studies showed that gastric carcinoids, either primary or transplantable, contained large amounts of histamine and revealed appreciable HDC activity [6–8]. The most conspicuous effect of gastric carcinoid on the host is the development of severe duodenal ulcer(s) due to the hypersecretion of gastric acid evoked by histamine released from a growing tumor [9].

It seems important to elucidate the *in vivo* effect of FMH on histamine formation in a growing transplanted tumor and the ulceration in the duodenum of the host.

Materials and methods

Mastomys used, tumor transplanation, urine collection and determinations of histamine in urine and tumor tissues have been described in detail in previous reports [6–9]. The transplantable tumor strain producing large amounts of histamine belongs to strain B as described in a previous study [9], and six male *Mastomys*, each bearing a growing transplant in the 12th generation, were used. Three animals from one litter were untreated, and three from another litter were treated with FMH. FMH was donated by Dr. J. Kollonitsch of Merck Sharp & Dohme Research Laboratories, Rahway, NJ, U.S.A. It was administered by either daily intraperitoneal (i.p) injection (100 mg/kg) or continuous subcutaneous (s.c.) infusion (100 mg/kg/24 hr) through an Alzet osmotic minipump (model 2001, Alza Corp., Palo Alto, CA, U.S.A.), as used in a previous experiment [10].

Results

In the present experiment, the transplanted tumors were palpable 4 months after transplantation. The urinary histamine levels (normal level: 0.56 ± 0.15 μg/24 hr [8]) in the untreated group paralleled the tumor growth (Fig. 1A). When the animals became sluggish (sign preceding perforating duodenal ulcer), they were killed, their duodena were inspected, and tumor histamine concentrations were measured. The duodena were enormously distended and were accompanied by multiple ulcers (Fig. 2A). The histamine concentration in the tumor tissues of three animals (mean ± S.E.) was 98.4 ± 10.2 μg/g wet tissue (range, 75.8 to 118.7 μg).

On the other hand, when the tumor-bearing *Mastomys* were given FMH by daily i.p. injections, their urinary histamine levels fell 20% below the preinjection level (Fig. 1B). Three days after termination of FMH injections, i.e. day 13 of observation, the tumor-bearing *Mastomys* excreted about three times more histamine in urine than on day 1 of observation. On the next day, the osmotic minipumps filled with FMH were implanted subcutaneously in the dorsal region of the same animals, and continuous infusion of FMH was initiated. The histamine levels in urine were more drastically lowered to 6, 8 and 8% of