Br. J. Pharmac. (1984), **83**, 603–605

6,9-deepoxy-6, 9-(phenylimino)-$\Delta^{6,8}$-Prostaglandin I_1, (U-60, 257) stimulates prostaglandin D_2 and inhibits thromboxane B_2 release from ionophore challenged human dispersed lung cells

S.T. Holgate & C. Robinson*

Medicine 1 and Clinical Pharmacology, Centre Block, Southampton General Hospital, Southampton SO9 4XY

6,9-deepoxy- 6,9- (phenylimino)-$\Delta^{6,8}$-Prostaglandin I_1 (U-60,257), a prostaglandin analogue known to inhibit leukotriene formation in a number of cell systems, potentiates mast cell release of prostaglandin D_2 from human dispersed lung cells activated with ionophore A23187. Over the same concentration range of 30–300 μM there was a related inhibition of ionophore-induced generation of thromboxane B_2 ($r=0.93$, $P<0.01$). As both prostaglandin D_2 and thromboxane A_2 are potent bronchoconstrictors, these observations may be relevant to the potential of this drug in the treatment of asthma.

Introduction The prostaglandin analogue, 6,9-deepoxy- 6,9- (phenylimino)-$\Delta^{6,8}$ prostaglandin I_1 (U-60,257) is a novel inhibitor of leukotriene formation in a variety of cell types (Bach *et al.*, 1982; Sun & McGuire, 1983) including human lung fragments (Dahlén *et al.*, 1983), which is undergoing evaluation in man. Its sites of action on the metabolism of arachidonic acid includes 5-lipoxygenase (Sun & McGuire, 1983) and a leukotriene A_4 glutathione-S transferase (Bach *et al.*, 1983). We now describe some unexpected actions of U-60,257 on ionophore-induced generation of the bronchoconstrictor agents prostaglandin D_2 (PGD_2) (Hardy *et al.*, 1984) and thromboxane A_2 (Hamberg *et al.*, 1976) in human dispersed lung cells (HDLC).

Methods Fresh human lung tissue was dissected free of major bronchi and blood vessels and chopped finely with scissors. The tissue was filtered over 60 μm gauze and the fragments obtained subjected to three sequential 30 min digestions with pronase (Sigma type XIV, 0.5–1.0 mg g^{-1} lung) and papaya latex chymopapain (Sigma, 0.13–0.25 mg g^{-1} lung) at 37°C. Digestions were performed in modified Tyrode solution (composition, mM: NaCl 137, glucose 5.5, NaH_2PO_4 0.4, KCl 2.7, $MgCl_2$ 0.5 and $CaCl_2$ 2.5) buffered with 20 mM N-2-hydroxyethylpiperazine-N' -2-ethanesulphonic acid (HEPES) containing 0.3% human serum albumin (HSA). Dispersed cells from each incubation were combined in the proportions 0.2:1:1, washed and resuspended with 10 mM HEPES-Tyrode solution containing 0.1% gelatin and 0.02 mg ml^{-1} deoxyribonuclease (Sigma) to minimize cell aggregation. After incubation at room temperature for 60 min the cells were washed, resuspended in albumin-free Tyrode solution and aliquots placed in Eppendorf tubes to give a final concentration of 10^7 nucleated cells ml^{-1}. Cells, prewarmed to 37°C for 5 min, were then incubated for 15 min with U-60,257 (Upjohn Co) dissolved in 10 μl ethanol. This vehicle had no effect on cell viability or mediator release, as measured by comparison with ethanol-free controls. The cells were challenged with A23187 at a final concentration of 2.5 μM which we have previously shown elicits maximum mast cell histamine release in HDLC (Holgate *et al.*, 1984). Reactions were terminated after 20 min by centrifugation at 10,000 *g* for 30 s. Supernatant fractions were frozen immediately and stored at −20°C until radioimmunoassayed for PGD_2, $PGF_{2\alpha}$ and TXB_2 as described (Holgate *et al.*, 1984). Thromboxane A_2 was measured as its hydrolysis product thromboxane B_2. Cross-reactivity of U-60,257 with the PGD_2 and $PGF_{2\alpha}$ antibodies was not detectable and was <0.3% for anti-TXB_2. In view of the fact that the large majority of PGD_2 originates from mast cells (Holgate *et al.*, 1984) we have normalized prostanoid release for mast cell content. Results, expressed as mean ± s.e.mean, were corrected for spontaneous release except where stated. Statistical comparisons were made by Student's *t* test for paired samples.

Results Metachromatic staining of wet preparations using Kimura's stain showed that HDLC consisted of 5.1 ± 0.9% mast cells. Spontaneous release of prostanoids was as follows (ng 10^{-6} mast cells):

*To whom correspondence should be addressed: Room CF104, Centre Block, Southampton General Hospital, Southampton, SO9 4XY, Hampshire.

PGD_2 2.51 ± 0.19; TXB_2 2.14 ± 0.38 and $PGF_{2\alpha}$ 1.29 ± 0.19 ($n = 6$ lungs). Calcium-dependent activation of the cells with A23187 resulted in increased release of PGD_2, $PGF_{2\alpha}$ and TXB_2 to 19.08 ± 4.87, 4.14 ± 1.07 and 19.48 ± 6.11 ng10^{-6} mast cells respectively ($P < 0.05$–0.001). Table 1 shows the net release of these mediators after correction for spontaneous release.

At low concentrations (1–10 μM) at which U-60,257 inhibits leukotriene formation (Bach *et al.*, 1982; Sun & McGuire, 1983; Robinson & Holgate unpublished data), there was no significant effect on the release of PGD_2, $PGF_{2\alpha}$ or TXB_2. However, at higher concentrations (30–300 μM) U-60,257 caused a concentration-related 50–130% enhancement of PGD_2 release ($P < 0.05$), with a corresponding 65–100% inhibition of TXB_2 generation ($P < 0.05$) (Table 1). There was a significant inverse correlation ($r = 0.93$, $P < 0.01$) between the release of PGD_2 and TXB_2 in the presence of increasing concentrations of U-60,257. Generation of $PGF_{2\alpha}$ was not significantly altered by U-60,257 at the concentrations used.

Discussion We have shown previously that proteolytic treatment of lung-tissue disperses a cell population relatively enriched in tissue mast cells (Holgate *et al.*, 1984) which release substantial amounts of PGD_2 and TXB_2 with ionophore or immunological challenge. The majority of this PGD_2 is mast cell-derived, whilst TXB_2 probably originates from activated macrophages (Holgate *et al.*, 1984). The present study demonstrates that U-60,257 modulates PGD_2 and TXB_2 formation in HDLC at concentrations which could be achieved locally in the lung after inhaled administration. These observations are unexpected as U-60,257 is not a thromboxane synthetase inhibitor and lacks prostaglandin-like activity (M.K. Bach, personal communication). Furthermore, U-60,257 has no general effect on cyclo-oxygenase activity since $PGF_{2\alpha}$ release was unaffected at concentrations where actions against PGD_2 and TXB_2 were noted.

Although we have not established the mechanism of action of this drug on arachidonic acid metabolism, these observations are relevant to the pharmacology of U-60,257 in man and may be pertinent to its potential as an anti-asthma drug. The correlation between the inhibition of TXB_2 formation and PGD_2 release suggests that these two events are related and further emphasizes the likely complex interrelationships between individual mediators when inflammatory cells are activated for mediator secretion.

References

BACH, M.K., BRASHLER, J.R., FITZPATRICK, F.A., GRIFFIN, R.L., IDEN, S.S., JOHNSON, H.G., McNEE, M.L., McGUIRE, J.C., SMITH, H.W., SMITH, R.J., SUN, F.F. & WASSERMAN, M.A. (1983). In vivo and in vitro actions of a new selective inhibitor of leukotriene C and D synthesis. In *Advances in Prostaglandin, Thromboxane and Leukotriene Research.* ed. Samuelsson, B., Ramwell, P.W. & Paoletti, R. Vol. 11, pp. 39–44. New York: Raven Press.

BACH, M.K., BRASHLER, J.R., SMITH, H.W., FITZPATRICK, F.A., SUN, F.F. & McGUIRE, J.C. (1982). 6,9-deepoxy-6,9- (phenylimino)-$\Delta^{6,8}$-prostaglandin I_1 (U-60, 257), a new inhibitor of leukotriene C and D synthesis: in in vitro studies. *Prostaglandins*, **23**, 759–771.

DAHLÉN, S.-E., HANSSON, G., HEDQVIST, P., BJORCK, T., GRANSTROM, E. & DAHLÉN, B. (1983). Allergen challenge of lung tissue from asthmatics elicits bronchial contraction that correlates with release of leukotrienes

Table 1 The effect of U-60,257 on ionophore A23187-induced generation of prostaglandin D_2 (PGD_2) and thromboxane B_2(TXB_2) in human dispersed lung cells

U-60,257 concentration (μM)	*Net release* (ng 10^{-6} mast cells) TXB_2	PGD_2	$PGF_{2\alpha}$
0	17.34 ± 5.80	16.54 ± 4.84	2.86 ± 0.99
1	18.77 ± 7.41	16.97 ± 5.00	1.97 ± 0.91
3	10.14 ± 2.74	19.09 ± 5.47	2.59 ± 1.00
10	8.47 ± 2.20	23.46 ± 7.87	2.26 ± 1.05
30	5.81 ± 1.86*	24.53 ± 6.89*	1.26 ± 0.37
100	0 *	38.64 ± 11.02**	1.23 ± 0.37
300	0 *	34.91 ± 8.06*	1.23 ± 0.36

*$P < 0.05$; **$P < 0.02$ with respect to untreated controls (paired t test).
Results are mean ± s.e.mean in six lungs.

C_4, D_4 and E_4. *Proc. natn. Acad. Sci. U.S.A.*, **80,** 1712–1716.

HAMBERG, M., SVENSSON, J., HEDQVIST, P., STRANDBERG, K. & SAMUELSSON, B. (1976). Involvement of endoperoxides and thromboxanes in anaphylactic reactions. In *Advances in Prostaglandin and Thromboxane Research.* ed. Samuelsson, B. & Paoletti, R. Vol 1., pp. 95–501. New York: Raven Press.

HARDY, C.C., ROBINSON, C., TATTERSFIELD, A.E., & HOLGATE, S.T. (1984). The bronchoconstrictor effect of inhaled prostaglandin D_2 in normal and asthmatic man. *New Engl. J. Med.*, **311,** 209–213.

HOLGATE, S.T., BURNS, G.B., ROBINSON, C. & CHURCH, M.K. (1984). The release of prostaglandin D_2, thromboxane B_2 and other cyclooxygenase products by anaphylactic and ionophore-dependent challenge of human dispersed lung cells. *J. Immunol.*, (in press).

SUN, F.F. & McGUIRE, J.C. (1983). Inhibition of human neutrophil arachidonate 5-lipoxygenase by 6,9-deepoxy-6,9- (phenylimino)-$\Delta^{6,8}$- prostaglandin I_1 (U-60,257). *Prostaglandins*, **26,** 211–221.

(*Received July 25, 1984.*)