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Effects of lipoxygenase inhibitors in arachidonate-induced sudden death†

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Both BW 755c, a cyclo-oxygenase and lipoxygenase inhibitor, and nordihydroguaiaretic acid (NDGA), a selective lipoxygenase inhibitor, were tested for their protection against arachidonate-induced sudden death in rabbits. 100% survival was seen with BW 755c (1 mg kg^{-1}), while NDGA showed 0 and 17% survival (2 mg kg^{-1} and 4 mg kg^{-1}). BW 755c prevented 12-fold increases in plasma thromboxane B_2 concentrations and the formation of pulmonary artery thrombi normally seen with arachidonate-induced sudden death, while NDGA showed no such protective effect. Radioimmunoassay of rabbit plasma for leukotrienes (LTC_4, LTD_4 and LTE_4) indicated that they do not accumulate in blood in the model and BW 755c had no effect, suggesting that the deleterious effects seen are caused by cyclo-oxygenase pathway metabolites such as thromboxane A_2, but not by lipoxygenase pathway products such as leukotrienes.

Intravenous administration of sodium arachidonate is a potent and reproducible method of invoking sudden death in rabbits (Silver et al 1974; Araki et al 1981; Lefer et al 1981; Okamatsu et al 1981). The combined effects of intravascular platelet aggregation in the pulmonary circulation, arterial vasoconstriction, and bronchoconstriction, are thought to be the causative factors. One metabolite of the cyclo-oxygenase pathway of the arachidonic acid cascade thought to play a key role in the development of sudden death is thromboxane A_2, a known potent vascular constrictor and platelet aggregator (Smith et al 1980b). Carbocyclic thromboxane A_2 (CTA_2), a stable analogue of thromboxane A_2, causes coronary vasoconstriction (Smith et al 1981a) and can induce death in rabbits within 10 min (Lefer et al 1980). Specific thromboxane synthetase inhibitors protect against arachidonate-induced sudden death in rabbits (Puig-Perellada & Planos 1977; Lefer et al 1981).

The purpose of the present study was to assess the effects of lipoxygenase pathway inhibitors on sodium arachidonate-induced sudden death. We compared the protective capabilities of 3-amino-1-{m-(trifluoromethyl)-phenyl}-2-pyrazoline (BW 755c), a known inhibitor of both the cyclo-oxygenase and lipoxygenase pathways of arachidonate metabolism (Higgs et al 1979) and nordihydroguaiaretic acid (NDGA), a specific inhibitor of the lipoxygenase pathway (Tappel et al 1953).

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Methods

Twenty-five adult male New Zealand rabbits, 2·6–3·7 kg, were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.v.). A tracheal cannula was inserted and connected to a Statham pressure transducer to monitor airway pressure. Mean arterial blood pressure and central venous pressure were measured by introducing polyethylene catheters into the left carotid artery and the left femoral artery, respectively. A scalar electro-cardiogram in Lead III was recorded by placing needle electrodes under the skin of the three limbs. Sodium arachidonate infusion was carried out via a polyethylene catheter threaded into the inferior vena cava by way of the right femoral artery. Sodium arachidonate (Sigma Chemical Co., 95% pure) 2 mg kg^{-1}, was freshly diluted in 1 mM Na_2CO_3 just before use and injected over 30–40 s.

Intravenous injection of BW 755c (1 mg kg^{-1} in 0·9% NaCl) and NDGA (2 and 4 mg kg^{-1} in 95% ethanol) was made 15 min before arachidonate challenge.

Blood samples (3 ml) were drawn (i) just before drug injection, (ii) just before arachidonate injection and (iii) 3 min after injection of arachidonate (i.e. just before death or a time equivalent to sudden death in survivors). Blood was drawn in one-hundredth volume EDTA (450 mM) in the presence of 100 μM sodium meclofenamate to prevent spontaneous formation of thromboxane A_2 during collection, centrifuged at 2500*g* and 4 °C for 15 min and the plasma decanted and frozen. Plasma samples were later assayed for thromboxane B_2, the stable metabolite of thromboxane A_2, by radioimmunoassay according to Lewy et al (1980). Assays for 6-keto $PGF_{1\alpha}$, the stable breakdown product of prostacyclin, were also made on the blood samples (Smith et al 1980b). In additional rabbits, blood samples were also assayed for leukotrienes (LTC_4, LTD_4 and LTE_4) by a newly developed radio immunoassay procedure (Aharony et al 1984) with a sensitivity limit of 0·045 pmol LTD_4 at a final plasma dilution of 1 : 70. Displacement of 50% of bound [^{3}H]LTD_4 was obtained with 0·44 ± 0·03 pmol LTD_4. The antibody cross-reacts with LTC_4 and LTD_4, 100%, LTE_4 57%, LTB_4 <0·03% and 5-HETE, 15-HETE, arachidonic acid, thromboxane B_2, PGE_2 and 6-keto $PGF_{1\alpha}$ <0·03%.

Specimens of lung tissue were removed from several rabbits 5 to 10 min after arachidonate injection. The condition of pulmonary vessels was assessed by light

microscopy from 7 μm sections stained with haematoxylin and eosin.

Isolated tissue preparations were made to investigate the actions of BW 755c (20 μg ml^{-1}) on liver lysosomes and pancreatic homogenates. Isolated lysosomes of cat liver were assayed for the activity of the lysosomal hydrolases, cathepsin D and β-gluronidase, using the methods of Smith et al (1980a). Cat pancreatic homogenates were analysed for proteolytic effects of BW 755c according to Curtis & Lefer (1980).

Thromboxane B_2, 6-keto $PGF_{1\alpha}$ and leukotriene plasma concentration data were analysed by an unpaired Student's *t*-test.

Results

Intravenous injection of sodium arachidonate (2 mg kg^{-1}) results in a mortality rate of 90 to 100% in rabbits (Smith et al 1980b; Araki et al 1981; Okamatsu et al 1981). We found that, given alone, it resulted in 100% mortality, whereas after BW 755c (1 mg kg^{-1}) 15 min before arachidonate challenge, there was a 100% survival (Table 1). NDGA (2 and 4 mg kg^{-1}) 15 min before arachidonate challenge resulted in 0 and 17% survival, respectively. We did not observe any noticeable effects of either BW 755c or NDGA on heart rate, blood pressure or ecg. Rabbits died within 5 min of arachidonate infusion, most within 3 or 4 min. Rabbits alive 60 min after arachidonate challenge were considered survivors. These results (Table 1) indicate the beneficial effects of BW 755 compared with NDGA.

Pulmonary arteries were clear in the surviving rabbits biopsied, but rabbits that suffered sudden death exhibited prominent pulmonary artery thrombi that included platelet aggregates (Fig. 1).

Liver lysosomal suspensions incubated with 10 μg ml^{-1} BW 755c showed no significant change in the rate of release of β-glucuronidase (−0·28 ± 1·62, NS) or cathepsin D (−0·52 ± 1·9, NS). BW 755c also showed no significant change from control values for proteolysis in pancreatic homogenates (+0·30 ± 0·38% change, NS). These results suggest that BW 755c does not exert direct lysosomal stabilizing or anti-proteolytic effects, and this lack of action could not account for its protective effect in sudden death.

Initial thromboxane B_2 concentrations in all three groups were 0·6 to 0·8 pmol ml^{-1} and these rose to 18·1 ± 3·3 pmol ml^{-1} and 17·4 ± 1·9 pmol ml^{-1} in the vehicle and NDGA-treated group, respectively ($P <$ 0·001 from initial values) approximately 3 min after arachidonate injection whilst BW 755c prevented the increase, mean values being 2·1 ± 0·6 pmol ml^{-1} ($P <$ 0·001 from the test groups).

The results of the plasma 6-keto $PGF_{1\alpha}$ analyses showed that BW 755c blunted the rise in 6-keto $PGF_{1\alpha}$ values seen with the vehicle treated group (5·3 ± 0·3 pmol ml^{-1} compared with 18·0 ± 2·5 for vehicle, $P <$ 0·01); NDGA showed no significant inhibition (final values of 14·8 ± 3·3 pmol ml^{-1} compared with 18·2 ± 2·5 for vehicle, NS). These results indicate that BW 755c displayed marked inhibition of cyclo-oxygenase activity while NDGA showed no significant inhibition of the formation of cyclo-oxygenase products (i.e. thromboxane A_2 and PGI_2).

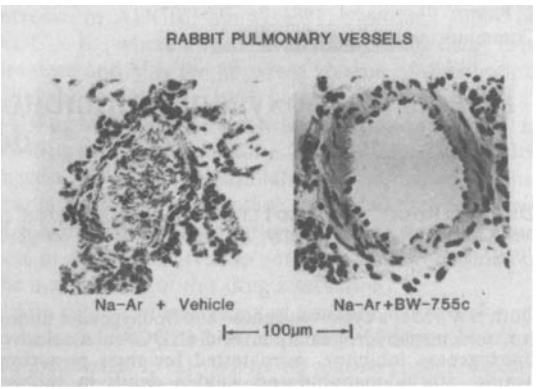

FIG. 1. Photomicrographs of two rabbit pulmonary vessels (450x). (a) Shows a thrombosed vessel from a rabbit given sodium arachidonate ± vehicle (i.e., 0·9% NaCl). (b) Exhibits a patent pulmonary vessel obtained from a rabbit given BW 755c (1 mg kg^{-1}) + sodium arachidonate.

Four rabbits given arachidonate alone, exhibited only a slight, not significant increase in leukotriene concentrations from 37 ± 6 before arachidonate injection to 48 ± 8 pmol ml^{-1} just before death. In five rabbits given BW 755c, leukotriene concentrations were 33 ± 5 before arachidonate and 37 ± 5 pmol ml^{-1} 3 min after its injection, so the results do not support a critical role for leukotrienes in arachidonate-induced sudden death.

Discussion

BW 755c is a potent inhibitor of lipoxygenase and cyclo-oxygenase activity in-vitro (IC 50 = 1·70 and 0·72 μg ml^{-1}, respectively) (Higgs et al 1979) and a potent anti-inflammatory and anti-chemotactic agent in-vivo (Eakins et al 1980). On the basis of our 6-keto $PGF_{1\alpha}$ and thromboxane B_2 measurements, along with the previously reported data, we assumed substantial inhibition of both cyclo-oxygenase and lipoxygenase activity with the dose of BW 755c we used (1 mg kg^{-1}).

NDGA (1 × 10^{-6} M) inhibits the formation of lipoxygenase pathway products in-vitro (Sircar et al 1983). Hammarström & Falardeau (1977) showed it to inhibit thromboxane B_2 formation in human platelets but at an IC 50 of 0·3 mM. Our choice of 2 and 4 mg kg^{-1} as experimental doses (approx. 6 × 10^{-5} and 1 × 10^{-4} M, respectively, based on equilibration in an intravascular compartment of 6 to 8% body weight) was made to maximize lipoxygenase inhibition while not inhibiting thromboxane synthesis. Our 6-keto $PGF_{1\alpha}$ and thromboxane B_2 data substantiate that at these doses NDGA displayed no significant cyclo-oxygenase inhibition.

Table 1. Arachidonate-induced sudden death in rabbits.

Agent	Dose (mg kg^{-1})	Total number	Survivors	Survival (%)
—	—	7	0	0
BW 755c	1	7	7	100
NDGA	2	5	0	0
	4	6	1	17

Our results show that BW 755c prevents arachidonate-induced sudden death in rabbits while NDGA displays no such protective effect, and coupled with the fact that cyclo-oxygenase inhibitors themselves protect against arachidonate-induced sudden death (Silver et al 1974; DiPasquale & Mellace 1977; Smith et al 1980b), seem to indicate that lipoxygenase pathway metabolites are not vital to the process involved in arachidonate-induced sudden death. The major reasons for this conclusion are: (i) thromboxane concentrations increased twelve-fold shortly after arachidonate injection, (ii) synthetic thromboxane analogues demonstrate coronary vasoconstriction (Smith et al 1981a), aggravate myocardial ischaemia (Smith et al 1981b), and induce sudden death (Lefer et al 1980), (iii), thromboxane synthetase inhibitors display clear-cut protection against arachidonate-induced sudden death (Smith et al 1980b), (iv) peptido-leukotriene concentrations do not increase significantly in arachidonate-induced sudden death (present study). It therefore seems that thromboxane A_2, but not leukotrienes LTC_4 and LTD_4, is a leading candidate as a mediator of sudden death induced by arachidonate injection.

Although the lipoxygenase pathway metabolites seem to be of little importance in the mechanism of arachidonate-induced sudden death, they may have a significant role in other physiological and pathophysiological states. It has been demonstrated that leukotriene B_4, a product of lipoxygenase activity from arachidonate acid, is a potent chemotactic agent for polymorphonuclear leukocytes (Jubiz 1983) and may play a significant role in the pathophysiology of endotoxic shock and acute myocardial ischaemia where pulmonary and cardiac trapping of leukocytes is known to occur. The full biological importance of the lipoxygenase pathway products, such as HPETE and the leukotrienes, is still unclear (Randall et al 1980), although leukotrienes have recently been shown to constrict coronary arteries (Letts & Piper 1982).

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