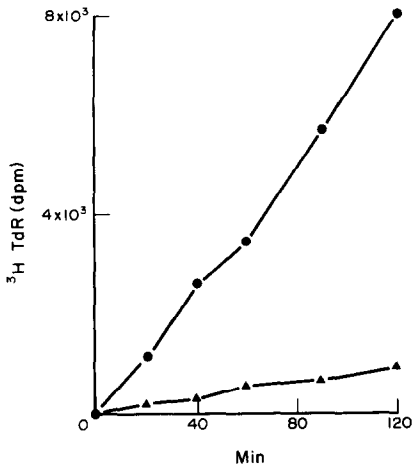

Fig. 2. Incorporation of ^{3}H thymidine into DNA by FDCP-mix A4 cells in the presence and absence of 50 μM L-652,731. Cells were incubated at 37° at a density of 2×10^5/ml in serum-free medium containing 1 μCi/ml ^{3}H TdR at specific activity of 65 Ci/millimole: ●—●, No L-652,731; ▲—▲, +50 μM L653,731. Similar results were obtained with WEHI-3B cells.

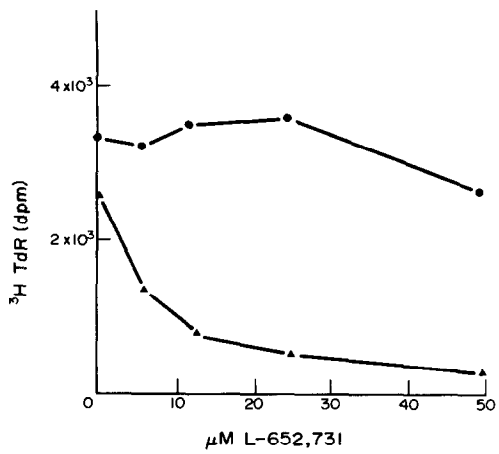

Fig. 3. High thymidine concentration reverses inhibition of ^{3}H TdR uptake by L-652,731. Incorporation of ^{3}H thymidine into DNA by FDCP-mix A4 cells incubated for 1 hr at 37° in a low or high concentration of thymidine (TdR). Cells at 2×10^5/ml were incubated in serum-free medium containing ^{3}H TdR at a constant specific activity of 2 μCi per nanomole, plus L-652,731 at 50 μM, 25 μM, 12.5 μM, 6.25 μM and 0 μM: ●—●, TdR concentration 6 μM; ▲—▲, TdR concentration 0.2 μM.

In a recent report [5] it was concluded that L-652,731 had an anti-proliferative effect on lymphocytes, inhibiting lectin-induced DNA synthesis. The present work shows that measurement of DNA synthesis by ^{3}H thymidine incorporation is valid only if the ratio of L-652,731 concentration to thymidine concentration is less than 4:1.

L-652,731 certainly has a cytostatic effect on the haemopoietic cells used in this study, even in the presence of 10 μM thymidine (data not shown). We do not yet know the reason for this, but one possibility—inhibition of DNA synthesis—can now be discounted.

In summary, we find that L-652,731 is a competitive inhibitor of thymidine transport and is not an inhibitor of DNA synthesis.

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An antagonist to platelet activating factor counteracts the tumouricidal action of alkyl lysophospholipids

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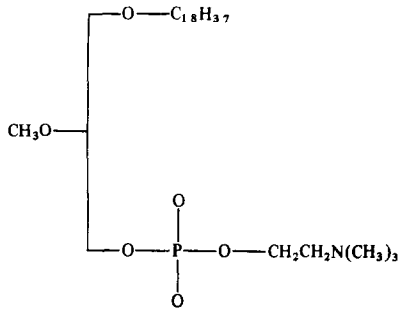
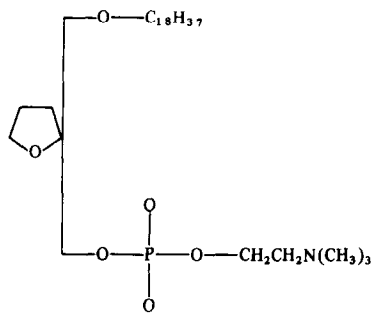
Analogues of platelet activating factor (PAF) in which a small, non-hydrolysable group such as —OCH_3 replaces acetyl in the sn2 position of PAF (Fig. 1a and 1b) kill some tumours at doses well below those tolerated by normal cells [1, 3, 4] (for reviews see Refs 2 and 5). This class of anti-tumour agent, often referred to loosely as alkyl lysophospholipids, has shown sufficient promise for clinical trials against a variety of human tumours to be undertaken



"$C_{18}OCH_3$—PAF"

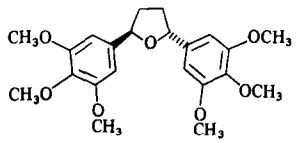
1 - 0 - octadecyl - 2 - 0 - methyl - *rac* - glycero - 3 - phosphocholine

(a)



SRI 62-834

(b)



L-652,731

(c)

Fig. 1.

[2]. They also have potential value as agents for purging bone marrow of leukaemic cells prior to autologous transplantation [6]. The basis for their selective cytotoxicity is therefore of considerable interest. Unlike most anti-tumour agents, alkyl lysophospholipids are not mutagens and do not interfere with DNA synthesis. It is generally agreed that part of their anti-tumour action, at least *in vivo*, is by activation of macrophages [5, 7]. They have also been shown, however, to exert a direct cytotoxic effect upon cells, the mechanism of which is more controversial. In the past decade, several explanations for the selective cytotoxicity of alkyl lysophospholipids against certain tumours have been advanced. It has been suggested that some tumour cells are deficient in an oxygenase necessary for the cleavage of —O-alkyl ethers, and that these compounds therefore accumulate in tumour cells, damaging cell membranes [3, 8]. Deleterious effects of $C_{18}OCH_3$-PAF on the synthesis and turnover of phosphatidyl choline have been reported [9], as also has inhibition of lysophosphatidyl choline acyl transferase [10]. These data suggest that alkyl lysophospholipids are capable of disrupting phospholipid metabolism in a number of ways, but it is not clear why tumour cells should be particularly sensitive to these perturbations.

In this note we report that WEH13B myelomonocytic leukaemic cells, which are killed by the PAF analogue $C_{18}OCH_3$-PAF, can be protected against the lethal effect of this compound by an antagonist of platelet activating factor which binds specifically to the PAF receptor (Fig. 1c). The simplest explanation of this observation is that the cytotoxicity of $C_{18}OCH_3$-PAF is mediated by a PAF receptor. This points to PAF receptor function as a potentially exploitable difference between normal haemopoietic cells and some types of leukaemic cell.

Materials and methods

$C_{18}OCH_3$-PAF was obtained from Sigma Chemical Co. (St. Louis, MO) and from Bachem (Switzerland). ^{3}H-$C_{18}OCH_3$-PAF was from Amersham International (U.K.), Compound SRI-62,834 was from Sandoz Research Institute.

Trans - 2,5-bis-(3,4,5-trimethoxyphenyl)tetrahydrofuran (L652,731) was synthesised from 3,4,5-trimethoxyacetophenone (Aldrich Chemical Co.) by the methods of Biftu *et al.* [11]. The *trans* isomer was separated from *cis* by repeated crystallisation from hexane 80%/ethyl acetate 20% NMR spectra and melting points of the product and intermediates in the synthesis agreed closely with those previously reported by Biftu *et al.* [11].

Addition of PAF analogues or antagonist to cell cultures was from solutions of these compounds in dimethyl sulphoxide at relatively high concentration (10 mM or 50 mM) so that the final concentration of dimethyl sulphoxide was less than 0.2%.

WEHI-3B myelomonocytic leukaemic cells were maintained in RPMI medium plus 10% fetal calf serum. FDCP-mix A4 cells (a mouse bone marrow cell line derived in this Institute which is non-leukaemic, growth factor dependent, and has many of the characteristics of normal haemopoietic stem cells [12]) were cultured in Fishers medium with 20% horse serum and approximately 100 units per ml of Interleukin-3 [13] as a growth stimulus. For experiments in serum-free medium, cells were suspended in MEM salts, MEM essential and non-essential amino acids, MEM vitamins, 1 mg/ml bovine serum albumin, 10 μg/ml insulin, 5 μg/ml iron-saturated transferrin, 5 μg/ml linoleic acid, 3 μg/ml sodium ascorbate and 20 μM ethanolamine-HCl. Cultures of FDCP-mix A4 cells were also supplemented with Interleukin-3.

Cell viability was usually measured by trypan blue exclusion. Cells were diluted 1:1 in a 0.5% solution of trypan blue in 0.85% saline solution and counted in a haemocytometer. Measurement of viability by clonogenic assays (see Ref. 12) in 0.33% agar/RPMI/20% fetal calf serum gave results similar to Trypan blue exclusion when viability was expressed as per cent control.

Uptake of $^3HC_{18}OCH_3$-PAF at 37° was determined in serum-free medium containing 10 μM $C_{18}OCH_3$-PAF plus 0.25 μCi/ml ^3H-$C_{18}OCH_3$-PAF. At appropriate intervals, 5 ml of cell suspension was filtered on glass fibre filters (Whatman GF/C, 2.5 cm dia.) and the filter was washed rapidly with 2 × 5 ml cold Fishers medium/20% horse serum. The filters were air dried and counted in a scintillation counter.

Results and discussion

Comparing the effects of PAF analogues on our two cell lines, we noted that after 48 hr incubation in serum-containing medium, about 50% of FDCP-mix A4 cells were killed by SRI 62,834 at a concentration of 100 μM, whilst 50% of WEHI-3B cells were killed at a concentration of 4 μM. In serum-free medium, sensitivity was greater: 50% of FDCP-mix A4 cells were killed at a concentration of 10 μM and 50% of WEHI-3B cells were killed at approximately 0.1 μM SRI-62,834. Corresponding concentrations for 50% kill by $C_{18}OCH_3$-PAF were: in medium and serum, FDCPmix A4 cells 90 μM, WEHI cells 6 μM; in serum-free medium FDCPmix A4 cells 10 μM, WEHI 0.6 μM. Considering the low concentrations required to kill the leukaemic cells, and considering also that anti-tumour effects are only shown by analogues which closely resemble PAF in their structure, it occurred to us that perhaps these analogues might act by interaction with a PAF receptor. In that case, it could be predicted that an efficient PAF antagonist, i.e. a molecule which would compete for binding to the receptor but not activate it, would protect tumour cells against the cytotoxic effects of $C_{18}OCH_3$-PAF, SRI 62,834 and similar analogues. A suitable antagonist has recently been described [11, 14]. A lignan analogue, L-652,731 (Fig. 1c) binds specifically to PAF receptors, displacing ^3H PAF. It inhibits platelet aggregation induced by PAF but not aggregation induced by other agents such as ADP or thrombin. It did not affect the binding of various other ligands to their receptors. Since this compound appeared to be a highly specific PAF antagonist, we carried out experiments to test whether or not it would protect the WEHI-3B tumour cells from $C_{18}OCH_3$-PAF or SRI 62,834. We found that as predicted on the hypothesis of receptor-mediated cytotoxicity, WEHI-3B cells could be protected from the lethal effects of $C_{18}OCH_3$-PAF by adding the antagonist (Fig. 2). Similar protection was also observed against SRI 62,834.

These experiments suggested that the observed differences in cytotoxicity of $C_{18}OCH_3$-PAF and SRI 62,834 to the leukaemic WEHI-3B cells and the non-leukaemic, multi-potent FDCP-mix A4 cells, may be due to differences in PAF-receptor mediated uptake by these cells. This was found to be the case: $^3HC_{18}OCH_3$-PAF was taken up by WEHI-3B cells at six times the rate that it was taken by FDPCmixA4 cells (Table 1). In both cell lines, the rate of uptake was reduced by the lignan L 652,731.

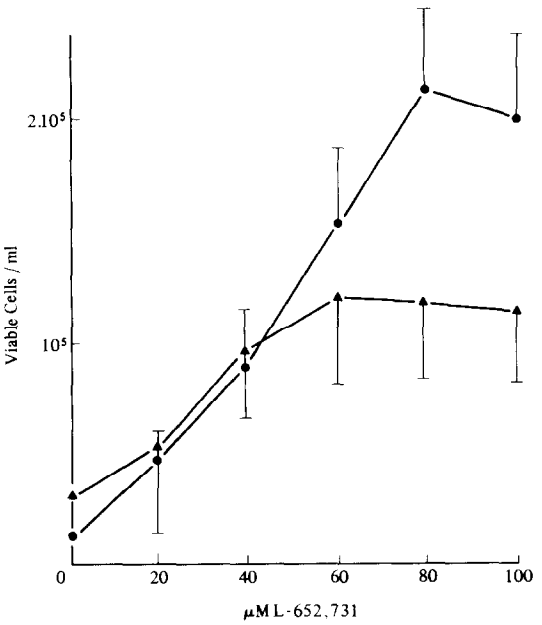


Fig. 2. Reversal of the cytotoxic effects of $C_{18}OCH_3$-PAF and SRI-62,834 by L-652,731. WEHI-3B cells were incubated for 48 hr at 37° in RPMI medium/10% fetal calf serum containing 10 μM $C_{18}OCH_3$-PAF or 10 μM SRI-62,834 plus the indicated concentrations of L-652,731. Initial cell density was 10^5/ml. Viable cells were counted by trypan blue exclusion after 48 hr: ●—●, 10 μM $C_{18}OCH_3$-PAF; ▲—▲, 10 μM SRI-62,834.

These results indicate that the cytotoxic effects of the tumouricidal alkyl lysophospholipids may be mediated in part via binding to a PAF receptor. However, the biochemical mechanisms underlying the cytotoxic activities are still uncertain. It is perhaps significant that the lignan L-652,731 (which binds to, but does not activate, the PAF receptor [11, 14] is itself growth inhibitory for both normal and tumour cells (Bazill and Dexter, submitted). This suggests that the PAF receptor binding and activation is an important component in proliferation. However, it has been clearly demonstrated that the normal, multi-potent FDCPmix A4 cells absolutely require a haemopoietic growth factor, Interleukin-3 for their survival and proliferation [12] and that Interleukin-3 is a sufficient stimulus for proliferation even under serum-free conditions (i.e. in the absence of exogenous PAF) (Cormier and Dexter, unpublished observations). Similarly, proliferation of WEHI 3B cells in similar culture conditions occurs via an autocrine loop involving the production of Interleukin-3 [15]. Thus if PAF and its receptor are involved in the proliferation events initiated by Interleukin-3, this must presumably occur as a consequence of auto-production of PAF following stimulation by the growth factor. Whilst

Table 1. Effect of 50 μM L-652,731 on uptake of ^3H-$C_{18}OCH_3$-PAF

	nmolcs bound/10^6 cells in 3 hr at 37°*
FDCPmix A4	0.224
FDCPmix A4 + 50 μM L-652,731	0.152
WEHI-3B	1.35
WEHI-3B + 50 μM L-652,731	0.61

* Uptake over the first 3 hr was approximately linear.

obviously speculative at present, the suggestion is open to experiment. If PAF receptor binding and activation is important for proliferation, it follows that the growth advantage of at least some tumours may be determined by an increase in the number or the affinity of PAF receptors. This would render them particularly sensitive to cytotoxic PAF analogues; and might explain, in part, why these compounds are selectively cytotoxic to certain tumour cells. Whatever the explanation, our results encourage an investigation of PAF receptor levels in a variety of tumour cell types which are known to be sensitive to the PAF analogues used in this study.

In summary, *trans*-2,5-bis-(3,4,5-trimethoxyphenyl)-tetrahydrofuran (L-652,731), an antagonist which binds to platelet activating factor receptors, protects WEHI-3B myelomonocytic leukaemic cells against the toxic effect of two alkyl lysophospholipids, $C_{18}OCH_3$-PAF and SRI 62,834. Uptake of $^3HC_{18}OCH_3$-PAF was reduced in the presence of the antagonist. These findings suggest that the selective anti-tumour action of alkyl lysophospholipids may be related to differences of PAF receptor function in normal and tumour cells.

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Cyclo-oxygenase inhibition does not unmask leukotriene release during ischaemia-reperfusion of the rat heart *in vitro*

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The calcium ionophore, A23187, stimulates leukotriene efflux from the rat isolated perfused heart [1, 2], probably reflecting calcium-induced activation of 5-lipoxygenase [3]. Reperfusion, following global ischaemia *in vitro*, promotes calcium accumulation by the myocardium (e.g. [4]), but does not induce leukotriene release [1, 2]. This stimulus does, however, promote prostaglandin synthesis, especially prostacyclin [2, 5]. Karmazyn [6] has recently reported that prostacyclin inhibits cardiac leukotriene release following calcium accumulation induced by the "calcium-paradox" in rat and guinea-pig, isolated perfused hearts. We have, therefore, determined the effect of cyclo-oxygenase inhibition on leukotriene release from the rat isolated perfused heart subjected to global ischaemia, followed by reperfusion.

Materials and methods

Male Wistar–Sprague–Dawley cross-bred rats (Glaxo Group Research Ltd., Ware, U.K.) were treated with aspirin (15 mg/kg i.v.) or vehicle (1% bicarbonate in 0.9% saline) at least 1 hr before sacrifice. Hearts were subsequently removed and perfused by the Langendorff tech-