Biochemical Pharmacology, Vol. 38, No. 2, pp. 373–374, 1989.
Printed in Great Britain.

0006–2952/89 $3.00 + 0.00

SHORT COMMUNICATIONS

Platelet activating factor antagonist L-652,731 inhibits thymidine transport

(*Received* 1 *June* 1988; *accepted* 30 *August* 1988)

The lignan analogue *trans*-2,5-bis-(3,4,5-trimethoxyphenyl)tetrahydrofuran (L-652,731) is a highly specific antagonist to platelet activating factor (PAF), binding to the PAF receptor and inhibiting various PAF-mediated cellular responses [1, 2]. Whilst examining the effect of this compound on the growth of two haemopoietic cell lines, we found that L-652,731 significantly reduced the incorporation of ^{3}H thymidine (TdR) into these cells, indicating an inhibition of DNA synthesis. Subsequent experiments, however, have shown that L-652,731 acts as a competitive inhibitor of thymidine transport and not as an inhibitor of DNA synthesis.

Materials and methods

The myelomonocytic leukaemic cell line WEHI-3B was maintained in RPMI medium plus 10% fetal calf serum. The multipotent, Interleukin-3 dependent cell line FDCP-mix A4 [3] was grown in Fishers medium plus 20% horse serum and approximately 100 units/ml Interleukin-3 [4]. For experiments on DNA synthesis, cells were suspended at about 2×10^5/ml in serum-free medium containing 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 tranferrin, 3 μg/ml sodium ascorbate, 5 μg/ml linoleic acid, 20 μm ethanolamine–HCl and 0.01 M HEPES pH 7.0 FDCP-mix A4 cells were further supplemented with Interleukin-3. L-652,731 was synthesised from 3,4,5-trimethoxyacetophenone according to Biftu *et al.* [2]. It was added to culture media from a 50 mM stock solution in dimethyl sulphoxide so that final concentrations of dimethyl sulphoxide were less than 0.2%.

For measurement of thymidine uptake, cells were suspended at approximately 2×10^5/ml in serum-free medium containing 1 μCi/ml ^{3}H TdR with appropriate concentrations of non-radioactive carrier thymidine and L-652,731 when required. The cells were incubated at 37°, and at intervals, 1 ml aliquots were taken and added to 5 ml cold 5% trichloroacetic acid (TCA). The precipitated material was filtered on glass fibre filters (Whatman GF/C, 2.5 cm), washed five times with cold TCA, twice with ethanol and counted in a scintillation counter.

For measurement of cellular ^{3}H thymidine triphosphate content, cells at 2×10^5/ml were incubated with and without L-652,731 in serum-containing medium with 2 μCi/ml ^{3}H TdR (specific activity 65 Ci/millimole). After 10 or 20 min at 37° the cells were rapidly filtered on Whatman GF/C filters and washed with 2×5 ml medium plus 10% serum. The filters were transferred to flat-bottomed tubes containing 5% TCA to extract acid-soluble nucleotides. After 10 min at 4° the extract was centrifuged to remove particulate matter, then extracted five times with 2 ml diethyl ether to remove TCA. The pH was adjusted to 7 by adding Tris base and the extract was then freeze dried. The residue was dissolved in 100 μl water containing 4 mg/ml TTP. Ten microliters of the solution was applied to polyethylene imine TLC plates (Merk, PEI cellulose F) and developed in 1 M acetic acid/0.3 M lithium chloride. The plate was dried and the TTP spot located and marked under UV light; it was then cut out and counted in a scintillation counter.

Results and discussion

The PAF antagonist L-652,731 had a cytostatic effect on WEHI-3B and FDCPmix A4 cell lines; cells remained viable but proliferation was progressively reduced in the presence of increasing concentrations of L-652,731 (Fig. 1).

On measuring DNA synthesis we found that 50 μM L-652,731 strongly inhibited incorporation of ^{3}H thymidine into DNA on both cell lines giving greater than 90% inhibition after incubation for one hour (Fig. 2).

Extraction of the acid-soluble pool of nucleotides and analysis by thin-layer chromatography showed that, in the presence of 30 μM L-652,731 ^{3}H thymidine triphosphate in the cells was reduced by 50% in FDCP-mix A4 cells (10 min plus L-652,731 at 37°) and by 80% in WEHI-3B cells (20 min plus L-652,731 at 37°). Thus there was direct evidence that L-652,731 inhibited uptake of ^{3}H thymidine. The possibility that DNA synthesis, as well as thymidine transport, was being inhibited was ruled out by the experiment shown in Fig. 3. In the presence of sufficiently high concentrations of thymidine, competition by L-652,731 could be reduced to a negligible amount. In our experiments DNA synthesis was not significantly inhibited by L-652,731 at concentrations up to 30 μM provided that the thymidine concentration was maintained at 6 μM or more, and even at 50 μM L-652,731 inhibition was only 25%. Thus it is primarily thymidine transport, and not DNA synthesis, which is inhibited by the lignan.

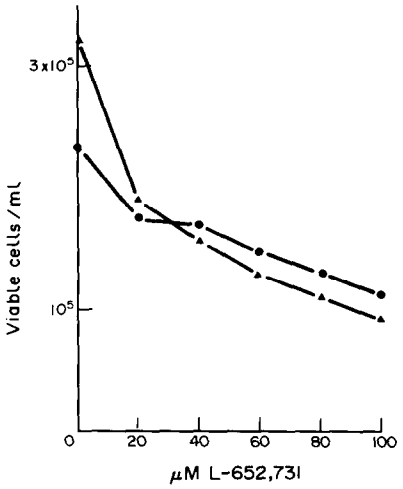

Fig. 1. Inhibition of proliferation by L-652,731. Viable cells were counted after 48 hr incubation with the indicated concentrations of L-652,731. Initial cell density was FDCP-mix A4: 1.0×10^5/ml WEHI-3B: 8.0×10^4/ml. FDCP-mix A4 was grown in Fishers medium/20% horse serum/Interleukin-3. WEHI-3B was grown in RPMI medium/10% fetal calf serum: ●—●, FDCP-mix A4; ▲—▲, WEHI-3B.

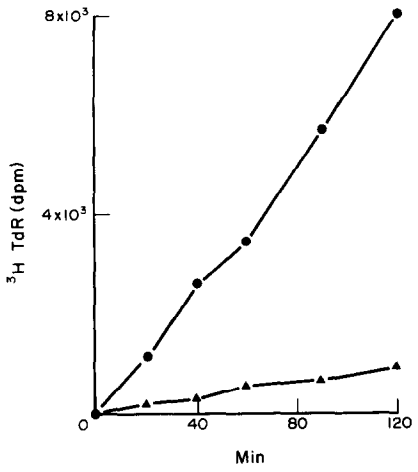

Fig. 2. Incorporation of ^{3}H thymidine into DNA by FDCP-mix A4 cells in the presence and absence of 50 μM L-652,731. Cells were incubated at 37° at a density of 2×10^5/ml in serum-free medium containing 1 μCi/ml ^{3}H TdR at specific activity of 65 Ci/millimole: ●—●, No L-652,731; ▲—▲, +50 μM L653,731.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

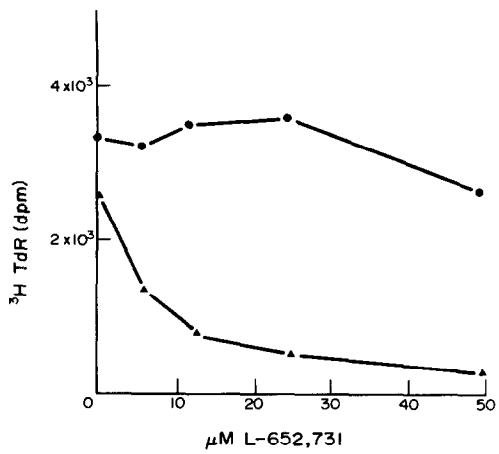

Fig. 3. High thymidine concentration reverses inhibition of ^{3}H TdR uptake by L-652,731. Incorporation of ^{3}H thymidine into DNA by FDCP-mix A4 cells incubated for 1 hr at 37° in a low or high concentration of thymidine (TdR). Cells at 2×10^5/ml were incubated in serum-free medium containing ^{3}H 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.

Acknowledgements—This work was supported by the Cancer Research Campaign and the Medical Research Council.

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Biochemical Pharmacology, Vol. 38, No. 2, pp. 374–377, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

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

(*Received* 1 *June* 1988; *accepted* 30 *August* 1988)

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