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# Transient activation of topoisomerase I in leukotriene $D_4$ signal transduction in human cells

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U937 human monoblast cells incubated with leukotriene $D_4$ ($LTD_4$) rapidly released arachidonic acid metabolites into the culture medium. Release was suppressed by the high-affinity $LTD_4$ receptor antagonist SK&F 104353. Arachidonic acid release induced by $LTD_4$ has been linked to a rapid induction of gene expression, and the propagation of the receptor binding signal is probably associated with enzymes that regulate gene expression. We have studied the participation of DNA topoisomerase I in $LTD_4$ signal transduction. $LTD_4$-specific release of arachidonic acid metabolites was inhibited (60–80%) by the topoisomerase I inhibitor camptothecin. $LTD_4$ increased protein-linked DNA strand breakage induced by camptothecin in U937 cells; this enhancement was prevented by coincubation of the cells with $LTD_4$ plus the receptor antagonist SK&F 104353. In addition, $LTD_4$ produced a rapid transient increase in extractable topoisomerase I activity, which was maximum within the first 10 min after addition of $LTD_4$ to the culture medium. Incubation of cultures for $>10$ min with $LTD_4$ before the addition of camptothecin resulted in no enhancement of camptothecin-induced DNA strand breakage, consistent with a reversal of topoisomerase I activation. Staurosporine, an inhibitor of protein kinase C, blocked $LTD_4$-induced arachidonic acid release and attenuated the effect of $LTD_4$ on camptothecin-induced DNA strand breakage. These results are consistent with the view that the regulation of topoisomerase I activity is involved in the propagation of $LTD_4$-mediated signals in U937 cells.

## INTRODUCTION

Recent studies have identified and characterized the receptors and signal-transduction processes for the peptidyl-leukotriene $D_4$ (peptidyl-$LTD_4$), a physiologically important metabolite of the arachidonyl-5-lipoxygenase pathway [1]. $LTD_4$ receptors are located in the plasma membrane and coupled via at least two guanine-nucleotide-binding (G-) proteins to a phosphoinositol bisphosphate ($PIP_2$)-specific phospholipase C [2,3]. Interaction of agonists with the $LTD_4$ receptor results in an extremely rapid release of diacylglycerol and inositol phosphates and mobilization of $Ca^{2+}$ from internal stores as well as an influx of extracellular $Ca^{2+}$ [3–5]. Furthermore, $LTD_4$-receptor activation results in transient activation of protein kinase C (PKC) [6]. A potent $LTD_4$ receptor antagonist, namely SK&F 104353, has been synthesized [7].

In many cell types, subsequent to the rapid metabolism of inositol phosphates, $Ca^{2+}$ mobilization and activation of protein kinase C, agonist interactions at the $LTD_4$ receptor induce an increase in the release of arachidonic acid (AA) or its metabolites [2,8]. This increase, observed within minutes of receptor–ligand binding [2,8], is dependent on the induction of transcription and translation [8]. The induced protein selectively increases the apparent $V_{max.}$ of intracellular phospholipase $A_2$ ($PLA_2$) [8]. Purified $PLA_2$ is similarly activated by an isolated protein of $M_w$ (weight-average $M_r$) 28000 [9], which has been named 'PLAP' (phospholipase A-activating protein). PLAP has recently been shown to be induced in CPAE (bovine endothelial) cells by tumour-necrosis factor (TNF), a cytokine which, like $LTD_4$, binds to cell receptors, is coupled to a G-protein and induces AA release within 5–10 min [10]. The released AA is metabolized in various ways, the principal end products depending on cell type [11]. AA metabolites released by $LTD_4$ mediate the cyclo-oxygenase-dependent effects induced by this ligand [1,11,12]. Thus activation of $LTD_4$ receptors in the plasma membrane is associated with enhanced transcription of at least one gene (that of PLAP).

It is not known how the $LTD_4$ binding signal results in specific gene expression. The purpose of the present study was to determine whether topoisomerases participate in the mechanism of the leukotriene-receptor-mediated signal transduction and AA release that we have observed. Topoisomerases I and II are classes of enzymes which catalyse interconversions, via transient single- or double-strand breaks, among various topological states of DNA [13,14], thus regulating a number of forms of DNA metabolism, including transcription [15–18]. Topoisomerase activity itself may be governed by post-translational modification, such as phosphorylation [19]. Leukotriene receptors do not enter the nucleus, but, via a $Ca^{2+}$/diacylglycerol signal, activate protein kinase C, which can phosphorylate a number of proteins, including topoisomerases [20]. Thus we hypothesized that: $LTD_4$ binding leads to a change in the state of topo-

Abbreviations used: $LTD_4$, leukotriene $D_4$; $PIP_2$, phosphatidylinositol bisphosphate; AA, arachidonic acid; $PLA_2$, phospholipase $A_2$; PLAP, phospholipase $A_2$-activating protein; TNF, tumour-necrosis-factor; PKC, protein kinase C; PBS, phosphate-buffered saline (8 mM-$Na_2HPO_4$/1 mM-$KH_2PO_4$/0.13 M-NaCl, pH 7.3); G-protein, guanine-nucleotide-binding protein.

isomerase activity, perhaps involving PKC phosphorylation and that the activated topoisomerase participates in the induced gene expression. As a first test of this hypothesis, we sought to establish whether $LTD_4$ binding was linked to changes in intracellular topoisomerase I or II activity and whether known topoisomerase inhibitors had any effect on the release of metabolites of [$^3$H]AA, a metabolic indicator of $PLA_2$ activation and, hence, receptor-mediated gene expression [8]. In addition, we studied the effects of staurosporine, a potent inhibitor of PKC [21], on both $LTD_4$-stimulated AA release and $LTD_4$-stimulated potentiation of DNA strand breakage induced by camptothecin.

## MATERIALS AND METHODS

### Materials

$LTD_4$ was prepared by total synthesis in the Department of Medicinal Chemistry of these Laboratories. The $LTD_4$ receptor antagonist SK&F 104353 was kindly provided by Dr. J. Gleason of these Laboratories. Camptothecin (NSC 94600) and teniposide (VM-26; NSC 122819) were obtained from the National Cancer Institute, Bethesda, MD, U.S.A. [5,6,8,9,11,12,14,15-$^3$H(n)]AA (60–100 Ci/mmol), [$^3$H]methylthymidine (83 Ci/mmol) and 2-[$^{14}$C]thymidine (50 mCi/mmol) were purchased from New England Nuclear Corp. Staurosporine was a gift of Dr. R. Johnson and Dr. J. Westley of these Laboratories. It had originally been obtained from Dr. S. Omura, The Kitasato Institute, Kitasato, Japan.

### Cell culture

U937 human monoblast cells were obtained from Dr. J. Lee of these Laboratories. They were maintained at 37 °C in suspension culture in humidified air/$CO_2$ (19:1) incubators. Growth medium was RPMI 1640 supplemented with 10% (v/v) heat-inactivated fetal-bovine serum and the antibiotics penicillin and streptomycin.

### Release of AA metabolites

A modification of the method of Clark *et al.* [2] was used. U937 cells were incubated at 37 °C overnight (14 h) with [$^3$H]AA (10 $\mu$Ci/ml) in growth medium. The cell density was $\sim 5 \times 10^5$ cells/ml. All subsequent operations were at room temperature. The AA-charged cells were prepared for leukotriene treatment and release assay as follows. Aliquots (1 ml) of charged cells were pipetted into 15 ml conical centrifuge tubes (one for each treatment). The cells were centrifuged at low speed for 5 min and washed twice with PBS. A 1.0 ml portion of PBS was added to each sample, and the cells were incubated for 10 min, centrifuged, and washed twice in PBS. After the last wash, the cells were resuspended gently in 2.0 ml of PBS. Appropriate amounts of $LTD_4$ [dissolved in 50% (v/v) methanol] $\pm$ SK&F 104353 ($LTD_4$ receptor antagonist dissolved in carbonate buffer) were added. After gentle rapid mixing, triplicate aliquots (100 $\mu$l each) were removed ('zero-time' samples) and centrifuged in a table-top microcentrifuge to pellet the cells. Supernatants were removed, and their $^3$H radioactivity was determined by liquid-scintillation spectrometry. Cell pellets were solubilized with 0.10 M-NaOH, neutralized, and their $^3$H radioactivity was determined. The percentage of total AA metabolites released from the cells of each aliquot was calculated by dividing the supernatant radioactivity by the total radioactivity. The remainder of the sample ($\sim$1.7 ml) was incubated for 8 or 10 min, during which period triplicate 100 $\mu$l aliquots were removed at various times for determination of $^3$H release as described above. Before the removal of aliquots, the cell suspensions were agitated gently to ensure homogeneity. The assay does not discriminate among free AA or the various metabolites of the cyclo-oxygenase or lipoxygenase pathways.

In those experiments measuring the effect of camptothecin or staurosporine upon $LTD_4$-stimulated AA release [$^3$H]AA-charged cells were incubated at 37 °C for 60 or 30 min respectively in PBS containing various concentrations of camptothecin or staurosporine, after which $LTD_4$ was added (final concn. 25 or 100 nM) and the release of AA metabolites was assayed as described above. In these experiments zero-time release included that occurring during camptothecin or staurosporine treatment; data were corrected by subtracting the zero-time values from the values obtained at each time point.

### Alkaline-elution assay for topoisomerase-associated DNA strand breaks

The alkaline-elution method of Kohn *et al.* [22] was used to assay topoisomerase I-associated DNA strand breaks induced by camptothecin [23–25]. Details of the elution procedure have been published [24]. U937 cells were radiolabelled overnight with [$^{14}$C]thymidine for the elution assay. They were treated for 10 min at 37 °C with 25 nM-$LTD_4 \pm 500$ nM-SK&F 104353, after which topoisomerase-active drugs were added to appropriate final concentrations and incubation was continued at 37 °C for an additional 60 min to permit topoisomerase–DNA lesions to be trapped by the drugs. Cells were then centrifuged, resuspended in PBS, and deposited on to filters for determination of DNA strand breakage by alkaline elution. Experiments with camptothecin and staurosporine were performed similarly; staurosporine alone, $LTD_4$ alone or staurosporine plus $LTD_4$ were incubated with cells for 10 min before the addition of camptothecin; incubation with camptothecin was for an additional 60 min.

### Determination of topoisomerase I activity in $LTD_4$-treated U937 cells

U937 cells in complete growth medium were incubated at 37 °C with 25 nM-$LTD_4$ for 0, 1, 5 or 10 min. Subsequent procedures were performed at an ice temperature. Cells were centrifuged (200 *g*, 10 min), washed with PBS and resuspended in 1 ml of hypo-osmotic buffer [5 mM-potassium phoshate (pH 7.0)/2 mM-$CaCl_2$/1 mM-phenylmethane sulphonyl fluoride/1 mM-2-merceptoethanol/0.1 mM-EDTA], in which they were incubated for 30 min. The swollen cells were disrupted by 30 strokes of a Dounce homogenizer. Nuclei were collected by centrifugation (200 *g*/10 min.) and resuspended at $3 \times 10^7$ per ml in iso-osmotic buffer [5 mM-potassium phosphate (pH 7.5)/100 mM-NaCl/10 mM-2-mercaptoethanol/0.5 mM-phenylmethanesulphonyl fluoride). NaCl (5 M) was added to a final concentration of 0.35 M and the nuclei were vortex-mixed gently. Nuclear suspensions were incubated for 30 min and then the nuclei were sedimented by centrifugation (1000 *g* for 10 min).

Supernatants (the 0.35 M-NaCl extracts) were assayed for topoisomerase I activity. Supercoiled pBR322 DNA (Bethesda Research Laboratories) was suspended at 20 ng/$\mu$l in TE buffer (10 mM-Tris/1 mM-EDTA, pH 7.5). Relaxation of the plasmid DNA was performed essentially as described by Hsiang *et al.* [23]. Nuclear extracts were diluted serially (1:100, 1:200... 1:1600) in 2-fold-concentrated reaction buffer [100 mM-Tris/HCl (pH 7.5)/200 mM-KCl/1 mM-EDTA/20 mM-$MgCl_2$/bovine serum albumin (60 $\mu$g/ml)/10 mM-2-mercaptoethanol]. Portions (5 $\mu$l) of diluted extract were mixed with 5 $\mu$l of pBR322 DNA (100 ng). The reactions were allowed to proceed for 30 min at 37 °C and then stopped by the addition of 2 $\mu$l of 15% (w/v) Ficoll/5% (w/v) SDS/1% (w/v) xylene cyanol. The DNA was electrophoresed in 1% agarose gels in Tris/borate (TBE) buffer (89 mM-Tris/borate/89 mM-boric acid/2 mM-EDTA, pH 8.0) and revealed by ethidium bromide staining. Topoisomerase I activity was detected as the disappearance of the rapidly migrating band of supercoiled DNA and appearance of slower-migrating topoisomers and completely relaxed DNA.

### Kinetics of enhancement of camptothecin-induced DNA breakage by $LTD_4$

U937 cells were incubated with 25 nM-$LTD_4$ for 0, 1, 5, 10, 15, 20, 30 or 60 min under conditions identical with those used to assay topoisomerase I activity (see above), after which camptothecin was added to 2 $\mu$M and incubation was continued for 60 min. Cells were then processed for alkaline elution and determination of DNA strand breakage, as described above. Control cells were treated with camptothecin for 60 min. Data were expressed as ratios of numbers of strand breaks for each $LTD_4$ incubation time divided by number of breaks in the control cells.

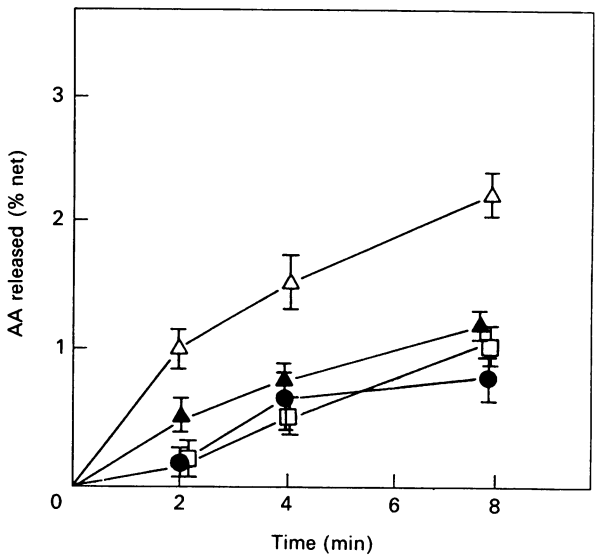


**Fig. 1. Release of [$^3$H]AA from human U937 cells treated with $LTD_4$**

U937 cells were incubated at 37 °C for 12–14 h with [$^3$H]AA (New England Nuclear; 94.5 Ci/mmol). They were then washed twice with PBS and incubated at room temperature with 25 nM-$LTD_4$ (△), 500 nM-$LTD_4$-receptor antagonist SK&F 104353 (□), 25 nM-$LTD_4$ plus 500 nM-SK&F 10353 (▲) or solvent only (0.01% methanol; ●). AA release at various times after the addition of $LTD_4$ was determined as described by Clark *et al.* [2]; the percentage of total AA released was calculated from radioactivity contained in the cell supernatants and lysed-cell pellets. Data are expressed as the means ± S.E.M. of triplicate from a representative experiment.

## RESULTS

### Release of AA metabolites

U937 cells released AA metabolites within 2 min of exposure to ⩾10 nM-$LTD_4$ (Fig. 1). The amount released specifically by 25 nM-$LTD_4$, the concentration chosen for most experiments, varied between ~0.25 and ~2% of total cellular AA (or ~50–170 pmol of AA/$10^5$ cells) and, in each experiment, was equal to two or three times the background release. AA release was rapid and transient (specific release 2–8 min after $LTD_4$ treatment) and was prevented by SK&F 104353, a selective high-affinity $LTD_4$-receptor antagonist [7] (Fig. 1) which, in five independent experiments, induced no AA release above background when incubated by itself with U937 cells. Thus $LTD_4$-specific AA release from U937 cells was likely mediated by the binding of $LTD_4$ to membrane-localized receptors in a process similar to that previously reported in studies with other cell lines [2,8].

### Assays for topoisomerase-linked DNA strand breakage

Topoisomerase activity *in vivo* was inferred by the use of inhibitors specific for either topoisomerase I or topoisomerase II. Camptothecin inhibits topoisomerase I activity by stabilizing the covalent enzyme–DNA intermediate with DNA in a single-strand-cleaved state [21]. Treatment of this complex with SDS results in the recovery of topoisomerase I covalently linked to the 3′-terminus of the nicked DNA. Removal of the protein with proteinase K results in DNA that is nicked at the site of topoisomerase I activity [17]. *In vivo*, camptothecin produces DNA strand breaks that are detectable upon treatment of the SDS-lysed cells with proteinase K [24], and they have been shown by immunoblot analysis to be linked covalently to topoisomerase I [25]. VM-26 (teniposide) is an inhibitor of topoisomerase II (but not topoisomerase I). This compound stabilizes the covalent topoisomerase II–DNA intermediate with DNA in a double-strand-cleaved state. SDS treatment yields topoisomerase II covalently linked to the 5′-termini of the nicked DNA strands, and proteinase K treatment yields double-strand-cleaved DNA [26]. *In vivo*, VM-26 produces DNA breaks that are linked covalently to topoisomerase II [27]. 25 nM-$LTD_4$, incubated with U937 cells for 10 min before the addition of camptothecin, increased the yield of camptothecin-induced lesions (Fig. 2). Potentiation of camptothecin-induced breakage has a slight leukotriene-concentration-dependence, average potentiation being 20–25% at 10 nM and 40–50% at 25 or 100 nM (results not shown). $LTD_4$ alone produced no strand breakage, and thus its synergistic effect on camptothecin-induced strand breakage was related to topoisomerase I (results not shown). SK&F 104353 had no effect on the number of camptothecin-induced breaks, but prevented further breakage upon subsequent $LTD_4$ treatment (Fig. 2). In similar experiments, VM-26-induced strand breakage was unaffected by prior treatment with $LTD_4$ (results not shown).

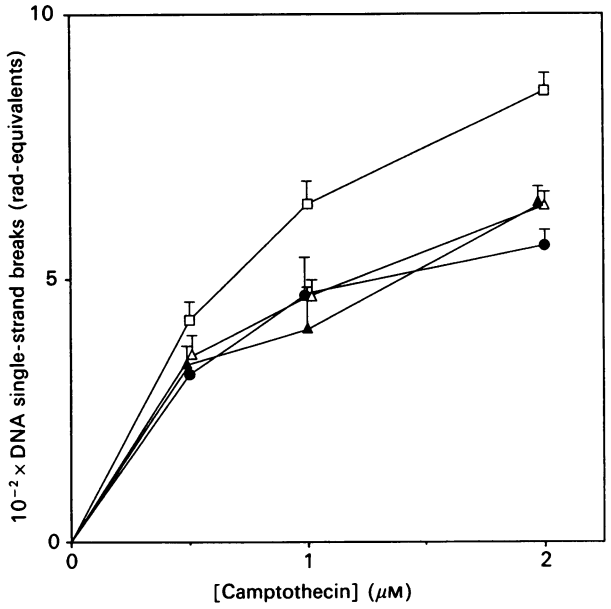


**Fig. 2. Effect of $LTD_4$ and antagonist on camptothecin-induced DNA strand breakage**

U937 cells were treated for 10 min at room temperature with 25 nM-$LTD_4$ (□), 500 nM-SK&F 104353 (▲), 25 nM-$LTD_4$ plus 500 nM-SK&F 104353 (△) or growth medium alone (control) (●). Camptothecin was added at the indicated concentrations to the cultures, which were incubated for an additional 60 min at 37 °C. DNA single-strand breaks were assayed by alkaline elution. Data are mean values + S.E.M. from three independent experiments.

### Activation of extractable topoisomerase I activity by $LTD_4$

25 nM-$LTD_4$ incubated for 1 or 5 min with U937 cells increased the activity of topoisomerase I extracted from nuclei in 0.35 M-NaCl (Fig. 3). The activation was transient as well as rapid, since cells incubated with $LTD_4$ for 10 min had extractable topoisomerase I activity equal to that at zero time. In similar experiments, activation of topoisomerase II was not observed (results not shown). The transient nature of $LTD_4$-induced topoisomerase I activation was reflected in a disappearance of the enhancement of camptothecin-induced DNA strand breakage in cells treated for > 10 min with $LTD_4$ and *then* for 60 min with camptothecin (Fig. 4). Within a single experiment, variation in the alkaline-elution assay is 10–15%; thus enhancement was significant only at 2, 5 and 10 min.

### Inhibition of $LTD_4$-induced AA release by camptothecin

Camptothecin blocked the release of AA metabolites normally induced by $LTD_4$ (Fig. 5). A 60 min treatment with 200 nM-camptothecin had no effect by itself on non-specific release of AA metabolites, but blocked $LTD_4$-specific release by 60–80%.

### Effect of staurosporine on $LTD_4$-induced AA release and topoisomerase I-linked DNA strand breakage

Staurosporine, a microbial alkaloid, has been demonstrated to be a potent inhibitor of partially purified protein kinase C [$IC_{50}$ (concentration causing 50% inhibition) = 3 nM] [28]. We found that prior treatment with 10 nM-staurosporine inhibited the $LTD_4$-specific release of AA metabolites (Fig. 6) and decreased the ability of $LTD_4$ to potentiate camptothecin-induced DNA strand breakage (Fig. 7).

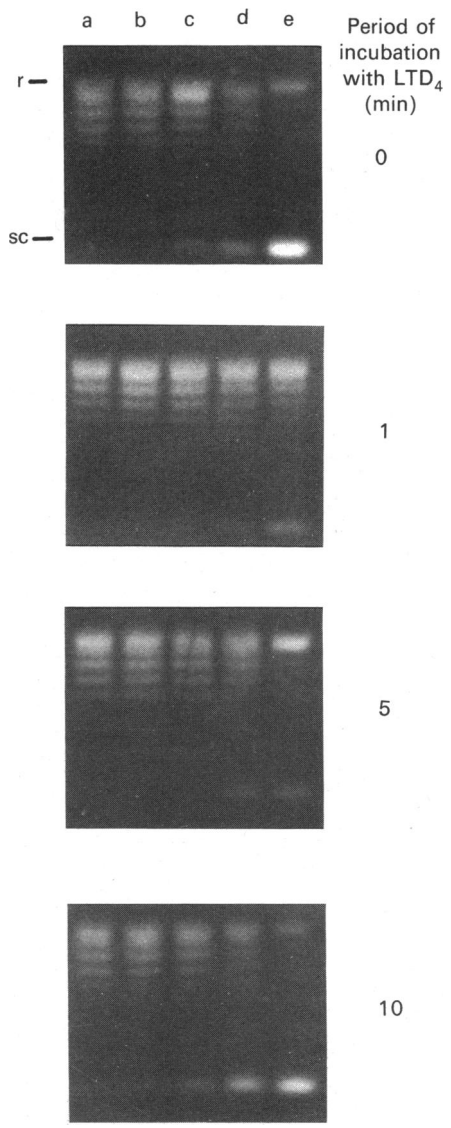


**Fig. 3. Changes in the topoisomerase I catalytic activity of 0.35 M-NaCl extracts of cells incubated for various times with $LTD_4$**

U937 cells were treated for 0, 1, 5 or 10 min with 25 nM-$LTD_4$. Nuclei were isolated and 0.35 M-NaCl extracts prepared as described in the text. Topoisomerase I catalytic activity contained in 1:100 (a), 1:200 (b), 1:400 (c), 1:800 (d) or 1:1600 (e) dilutions of nuclear extracts of these cultures was estimated from the ability of the extracts to increase the linking number of supercoiled plasmid pBR322 DNA (migrating to the position indicated by 'sc' in the first gel), producing relaxed DNA (migrating to 'r' in the first gel) in the absence of ATP. Those dilutions which left some of the pBR322 DNA supercoiled were deemed to contain partial topoisomerase I activity. The protein content of the extracts was 600 ± 80 μg/ml.

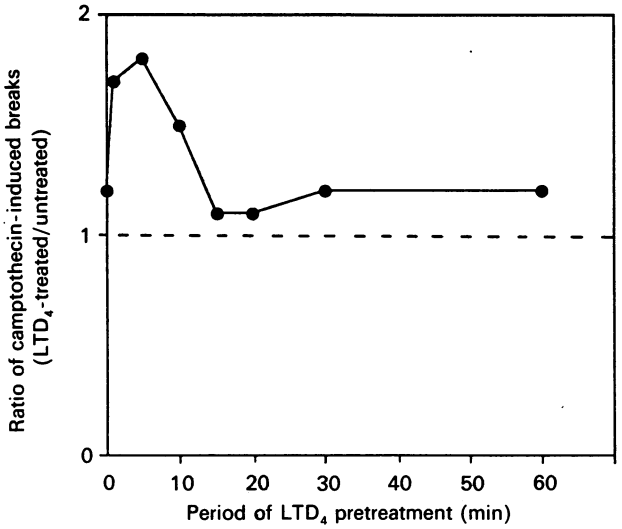


**Fig. 4. Enhanced camptothecin-induced DNA strand breakage in U937 cells as a function of pretreatment with $LTD_4$**

U937 cells were treated for 0–60 min with 25 nM-$LTD_4$ and then for an additional 60 min at 37 °C with 2 $\mu$M-camptothecin. DNA single-strand breakage was assayed quantitatively by alkaline elution. Results are expressed as ratios of breaks produced by camptothecin in the cells treated with $LTD_4$ to breaks produced in cells treated only with camptothecin.

## DISCUSSION

Human U937 cells released AA metabolites into the culture medium within a few minutes of treatment with $LTD_4$. Release was attenuated by the $LTD_4$-receptor antagonist and thus was receptor-mediated. Rapid release of AA metabolites (by $LTD_4$) is characteristic of a number of diverse cells and tissues including guinea-pig lung [4], bovine endothelial cells [2], and murine smooth-muscle cells [8]. In experiments with bovine endothelial cells or murine smooth-muscle cells, AA metabolites were observed to be released 4–8 min after $LTD_4$ treatment [8]. In the latter, this release was preceded by an increase in $PLA_2$ activity (observed 1–2 min after $LTD_4$ treatment) that was dependent upon RNA and protein synthesis [8]. Thus $LTD_4$-specific release of AA metabolites followed the rapid activation of a gene (or genes) whose product(s) enhanced the activity of $PLA_2$.

The $LTD_4$ signal-transduction scheme can be divided

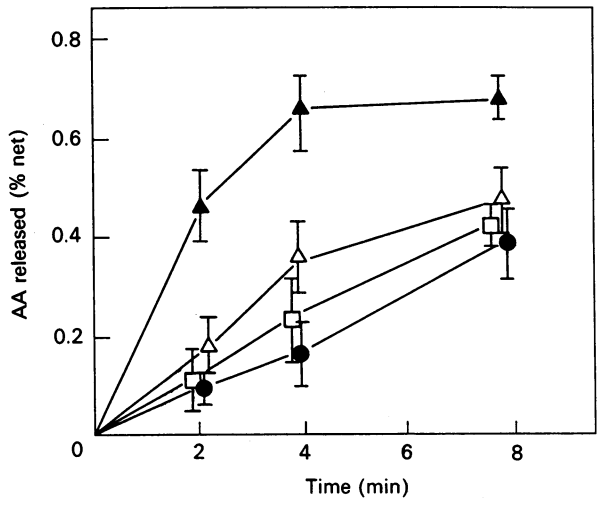


**Fig. 5. Inhibition of $LTD_4$-induced AA release by camptothecin**

U937 cells charged with [$^3$H]AA were treated for 60 min at 37 °C with or without camptothecin and then for an additional 2–8 min at room temperature with solvent (0.01% methanol) or with 100 nM-$LTD_4$. ▲, No camptothecin, 100 nM-$LTD_4$; □, 200 nM-camptothecin, no $LTD_4$; △, 200 nM-camptothecin, 100 nM-$LTD_4$; ●, no camptothecin, no $LTD_4$. Release of AA metabolites was calculated as described in the legend to Fig. 1.

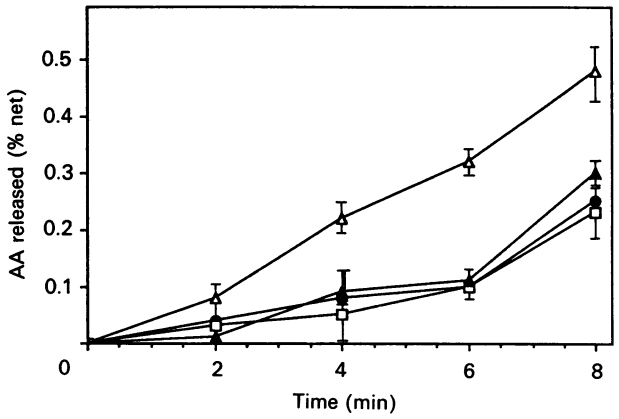


**Fig. 6. Inhibition of $LTD_4$-induced AA release by staurosporine**

U937 cells charged with [$^3$H]AA were treated for 30 min at 37 °C with or without staurosporine and then for an additional 2 or 6 min at room temperature with 0.01% methanol or with 25 nM-$LTD_4$. △, No staurosporine, 25 nM-$LTD_4$; □, 10 nM-staurosporine, no $LTD_4$; ▲, 10 nM-staurosporine, 25 nM-$LTD_4$; ●, no staurosporine, no $LTD_4$. Release of AA metabolites was calculated as described in the legend to Fig. 1.

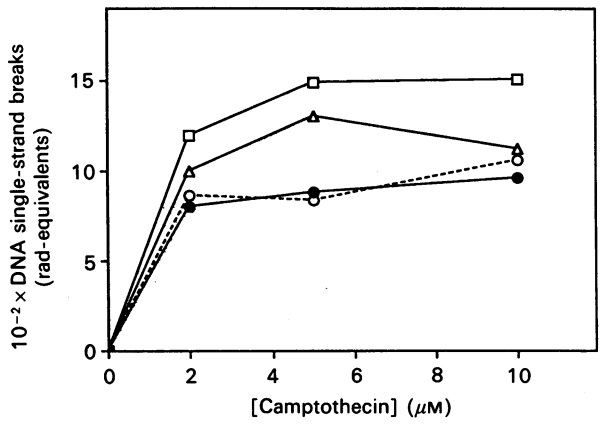


**Fig. 7. Effect of $LTD_4$ and staurosporine on camptothecin-induced DNA single-strand breaks**

U937 cells were left untreated (●) or treated with 25 nM-$LTD_4$ (□), 10 nM-staurosporine (○) or $LTD_4$ plus staurosporine (△). Camptothecin was added at the indicated concentrations, and DNA single-strand breakage was assayed by alkaline elution. Within a single experiment the variation in alkaline elution was 10–15%.

temporally into two phases. In the first, the binding of $LTD_4$ to receptor is coupled, via guanine-nucleotide-binding proteins, to $PIP_2$–phospholipase C, resulting in diacylglycerol production and a $Ca^{2+}$ transient. Subsequently, protein kinase C is activated [6]. The second phase of the signal-transduction process begins with the activation of genes (e.g. the gene for PLAP [8,9,10]), whose protein products enhance the activity of phospholipase $A_2$ ($PLA_2$), increasing the release of AA, which is metabolized to products that induce a variety of later effects in cells. The 'elbow' in the signal-transduction pathway is the activation of transcription of the gene for PLAP.

To demonstrate that this link is provided by PKC-mediated topoisomerase activation, it is necessary first to determine whether topoisomerase activity is part of the sequence of events initiated by leukotriene binding and ending with the release of AA metabolites. Our data are consistent with the notion that topoisomerase I is involved in this sequence. Camptothecin-induced DNA strand breakage was enhanced by $LTD_4$. This enhancement was inhibited by the receptor antagonist SK&F 104353 (Fig. 2). Altered transport or metabolism of camptothecin cannot be ruled out as causes of our observing increased breakage upon $LTD_4$ treatment. This explanation is extremely unlikely, however. First, it would require that transport alteration be both receptor-mediated (since a receptor antagonist blocks the increase) and able to discriminate between camptothecin and VM-26, which does not increase its DNA-breaking activity upon $LTD_4$ binding. Secondly, the possibility that a change in intracellular metabolism of camptothecin derives from $LTD_4$ receptor binding seems remote.

Direct measurements of extractable topoisomerase I activity support our conclusions concerning the ability of $LTD_4$ to enhance camptothecin-induced DNA strand breakage. In addition, we found that extractable topoisomerase I was activated transiently. Kinetics experiments employing enzyme assays alone cannot distinguish between a true activation and de-activation of the enzyme and changes in extractability. The finding that cells incubated for longer than 10 min with $LTD_4$ did not sustain greater numbers of camptothecin-induced strand breaks than controls strongly suggests that $LTD_4$ rapidly activates topoisomerase I and that the enzyme is de-activated shortly thereafter. Thus an early step in $LTD_4$ signal transduction appears to be a regulation of topoisomerase I activity.

Staurosporine would be expected to exert a pharmacological effect on steps of the $LTD_4$ cellular signalling pathway that occur subsequent to PKC action. Our data (Figs. 6 and 7) strongly suggest that, in $LTD_4$-treated U937 cells, PKC performs a function related to signal propagation; its action leads to the release of AA metabolites. PKC appears to have a regulatory function as well, i.e. to potentiate topoisomerase I activity. That these two functions are part of a single pathway is suggested by the result that inhibiting topoisomerase I with camptothecin blocks AA release (Fig. 5). Thus topoisomerase I is activated sometime between PKC activation and the release of AA metabolites [8]. Extractable topoisomerase I activity is clearly enhanced within 1 min of $LTD_4$ treatment. This activation may be the result of phosphorylation of topoisomerase I directly by PKC or, alternatively, be mediated by a third protein that was itself activated by PKC.

Early speculation regarding the cellular functions of topoisomerase I was centred around problems encountered by the transcription complex [29]. Since then, a good deal of evidence has linked topoisomerase I with active transcription (reviewed in [13,14]). Experiments with camptothecin have provided evidence that topoisomerase I is active at transcribed regions of induced (*Drosophila* heat-shock) genes [17]. Topoisomerase I was identified by immunoprecipitation as an enzyme that was cross-linked to heat-shock genomic sites, i.e. to active genes. Thus topoisomerase I likely functions at sites of active transcription to relieve torsional strain. Eukaryotic topoisomerase I, which can relax both (+)- and (−)-supercoils, could function at each transcription site. The dependence of the completion of the $LTD_4$ signal (AA release) upon a camptothecin-sensitive target suggests that topoisomerase I is required to express PLAP.

The increase in camptothecin-induced DNA strand breakage by $LTD_4$ over the 70 min treatment time was approx. 150 rad-equivalents. Assuming $0.9 \times 10^{-9}$ strand breaks/nucleotide per rad-equivalent [30], it is estimated that the number of camptothecin-induced strand breaks was increased on average by 450/cell as the result of $LTD_4$-receptor binding. Thus the binding of $LTD_4$ to its receptor appears to have a profound effect on DNA metabolism. Our data suggest that, if the increase in camptothecin-induced DNA strand breakage represents primarily the action of topoisomerase I in transcription, then the transcription of several genes is affected by $LTD_4$-receptor binding. Other receptor-active molecules, e.g. growth factors, rapidly induce the expression of more than one gene [31].

Additional support for the notion that membrane-localized receptors that activate $PIP_2$–phospholipase C may induce rapid gene activation has recently been reported. Angiotensin II-receptor activation induces the transcription of an oncogene, c-*fos* [32]. In addition, in our laboratory, vasopressin stimulation of $V_1$ receptors has been shown to induce c-*fos* production [33]. In both cases PKC activation has been postulated to play a key role. Thus additional precedents for gene activation secondary to $PIP_2$–phospholipase C activation are reported. These studies are the first of which we are aware to show the induction of gene transcription of a specific product as integral in a signal-transduction process. Given these findings and those obtained with TNF [10], it will be of interest to determine whether inositol phosphate-linked cellular-signalling pathways in general depend upon PKC-mediated activation of topoisomerase I for effects that may require gene activation.

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