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# RELEASE OF PEPTIDE LEUKOTRIENES FROM RAT KUPFFER CELLS

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SUMMARY : Kupffer cells isolated from the normal rat liver were incubated with calcium ionophore A23187, and the levels of peptide leukotrienes ($LTC_4$, $LTD_4$, and $LTE_4$) contained in the culture supernatant were determined by the combined technique of reverse-phase high-performance liquid chromatography and radioimmunoassay. In response to A23187, Kupffer cells released $LTC_4$, $LTD_4$, and $LTE_4$. After 10 min-preincubation of Kupffer cells with AA861, a 5-lipoxygenase inhibitor, the generation of $LTC_4$, $LTD_4$, and $LTE_4$ from A23187-stimulated Kupffer cells was significantly suppressed. Platelet activating factor (PAF), a phospholipid mediator, significantly enhanced the release of $LTC_4$, $LTD_4$, and $LTE_4$ from Kupffer cells stimulated with A23187. These results suggested that Kupffer cells may participate in inflammatory and immunologic events in the liver tissue by the release of peptide leukotrienes.


Kupffer cells, as resident macrophages of the liver, are part of the organism's mononuclear phagocyte system and show many typical functions common to macrophages of different provenance (1-3). It is also reported that Kupffer cells may have the capacity to influence immunologic and inflammatory events in chronic liver diseases (4-6).

Peptide leukotrienes ($LTC_4$, $LTD_4$, and $LTE_4$) are derived from arachidonic acid via the lipoxygenase pathway (7) and are potent mediators of inflammatory and allergic reactions. $LTC_4$, $LTD_4$, and $LTE_4$ may contribute significantly to the pathogenesis of various inflammatory and ischemic disorders (8). Leukotrienes are known to be produced from macrophages, polymorphonuclear leukocytes, and mast cells (9). Although Kupffer cells stimulated with certain substances release prostaglandin $E_2$, prostaglandin $I_2$, thromboxane $A_2$, and a variety of other inflammatory mediators (10), how

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$LTC_4$, $LTD_4$, and $LTE_4$ are formed by Kupffer cells is as yet unknown. In this study, we examined the production of $LTC_4$, $LTD_4$, and $LTE_4$ from rat Kupffer cells.

## MATERIALS AND METHODS

Chemicals: Calcium ionophore A23187, Hank's balanced salt solution, Tyrode's solution, medium RPMI 1640, pronase, collagenase, L-serine, and gelatin were obtained from Sigma, Tokyo, Japan. AA861 (2-[12-hydroxy-dodeca-5,10-diynyl]-3,5,6-trimethyl-1,4-benzoquinone) was a generous gift from Takeda Chemical Industries, Osaka, Japan. Platelet activating factor, synthetic $LTC_4$, $LTD_4$, and $LTE_4$ were kindly supplied by Ono Pharmaceuticals, Osaka, Japan. The radioimmunoassay kit for $LTC_4$, $LTD_4$, and $LTE_4$ was purchased from Amersham Japan, Osaka, Japan.

Preparation and incubation of rat Kupffer cells: Kupffer cells were isolated from the Wistar rat liver as previously reported (6). Kupffer cell suspension in Tyrode's solution containing 20mM L-serine and 1% gelatin was prepared. Based on histochemical staining, 95% of the adherent cells from the rat liver were found to be Kupffer cells. The Kupffer cell suspension ($5x10^5$ cells/ml) was incubated with calcium ionophore A23187 (2.5 $\mu$M) with or without $10^{-9}$M PAF. The Kupffer cell suspension was also incubated with $10^{-6}$M AA861, a specific inhibitor of 5-lipoxygenase (11), which was added to the cell suspensions 10 min before the addition of calcium ionophore. Immediately after the incubation of Kupffer cells, a four-fold amount of ice-cold ethanol was added, and the supernatant was obtained by centrifugation.

Purification and assay of $LTC_4$, $LTD_4$, and $LTE_4$: $LTC_4$, $LTD_4$, and $LTE_4$ in the culture supernatant were fractionated by reverse-phase high-performance liquid chromatography (HPLC) and determined by radioimmunoassay as follows. The ethanol-extracted leukotriene-rich supernatant was evaporated to dryness under nitrogen gas in a water bath at 37°C. This was then suspended in 1.5 ml of 40% methanol and used as the sample for the fractionation by HPLC. One milliliter of the above sample was injected into a pre-column (YMC-BBC-5 S-5 ODS; Yamamura Chemical Lab., Osaka, Japan), and this was washed with distilled water (flow rate: 1 ml/min) for 4 min. The flow was then changed by the column switching method using a system controller (SCL-6A; Shimazu, Osaka, Japan), and the mobile phase (acetonitrile/methanol/water/acetic acid : 1500/500/1500/1, adjusted to pH 5.6 with triethylamine) was injected from the opposite side of the pre-column at a flow rate of 1 ml/min and drawn into the main column. Develosil (ODS 5k; 4.6x150 mm; Nomura Chemicals, Aichi, Japan) was used as the main column, and the absorbance of elutes at 280 nm was measured using a spectrophotometer (SPD-6A; Shimazu) and monitored using Chromatopac (C-R4A; Shimazu). Each fraction corresponding to the standard preparation of $LTC_4$, $LTD_4$, and $LTE_4$ was collected by a fraction collector (FRAC-100; Pharmacia Japan, Osaka, Japan). These fractionated materials were evaporated to dryness and resuspended in Tyrode's solution. $LTC_4$, $LTD_4$, and $LTE_4$ in the HPLC-purified sample were measured by radioimmunoassay according to the method of Levi-Schaffer et al (12).

## RESULTS

When incubated with calcium ionophore A23187 (2.5 $\mu$M), Kupffer cells generated $LTC_4$, $LTD_4$, and $LTE_4$ (Fig. 1, Fig. 2, and Fig. 3). $LTC_4$ level in the culture supernatant of Kupffer cells reached the peak at 5 min after the addition of A23187 with a decline thereafter (Fig. 1). AA861, a specific 5-lipoxygenase inhibitor, had an inhibitory effect on leukotriene release

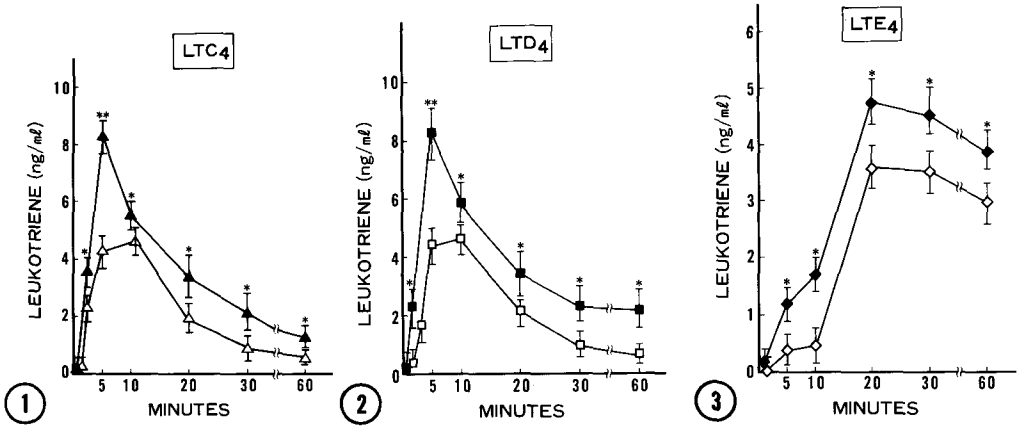


Fig. 1 Release of $LTC_4$ from rat Kupffer cells.
Kupffer cells were treated with 2.5 $\mu$M A23187 (▲) or with 2.5 $\mu$M A23187 and $10^{-6}$M AA861 (△). The values are means of five independent experiments, and bars indicate SD. At each time point, the difference between A23187-stimulated cells and A23187-stimulated cells pretreated with AA861 was significant (*p<0.01, **p<0.001).

Fig. 2 Release of $LTD_4$ from rat Kupffer cells.
Kupffer cells were treated with 2.5 $\mu$M A23187 (■) or with 2.5 $\mu$M A23187 and $10^{-6}$M AA861 (□). The values are means of five independent experiments, and bars indicate SD. At each time point, the difference between A23187-stimulated cells and A23187-stimulated cells pretreated with AA861 was significant (*p<0.01. **p<0.001).

Fig. 3 Release of $LTE_4$ from rat Kupffer cells.
Kupffer cells were treated with 2.5 $\mu$M A23187 (◆) or with 2.5 $\mu$M A23187 and $10^{-6}$M AA861 (◇). The values are means of five independent experiments, and bars indicate SD. At each time point, the difference between A23187-stimulated cells and A23187-stimulated cells pretreated with AA861 was significant (*p<0.01).

from rat Kupffer cells stimulated with calcium ionophore A23187. As shown in Fig. 1, pretreatment of Kupffer cells at 37°C for 10 min with $10^{-6}$M AA861 caused a significant inhibition of $LTC_4$ release. $LTD_4$ release by rat Kupffer cells was essentially similar to that noted in $LTC_4$ (Fig. 2). On the other hand, $LTE_4$ level in the culture supernatant of Kupffer cells reached the peak at 20 min after the addition of A23187 with a decline thereafter (Fig. 3). In addition, AA861 significantly inhibited the release of $LTE_4$ from A23187-stimulated Kupffer cells.

When Kupffer cells were incubated in the presence of both $10^{-9}$M PAF and 2.5 $\mu$M calcium ionophore A23187 at 37°C, PAF enhanced the production of peptide leukotrienes from Kupffer cells (Table 1). No leukotriene was detectable in the culture supernatant of Kupffer cells not stimulated with A23187 or PAF.

Table 1 Enhancement of the release of peptide leukotrienes from rat Kupffer cells by PAF

| A23187 ($\mu$M) | PAF (M) | $LTC_4$[a] | $LTD_4$[a] | $LTE_4$[b] |
|---|---|---|---|---|
| | | (ng/5x$10^5$cells) | | |
| 0 | 0 | <0.01 | <0.01 | <0.01 |
| 0 | $10^{-9}$ | <0.01 | <0.01 | <0.01 |
| 2.5 | 0 | 8.21±0.42[c] | 8.12±0.64[e] | 4.76±0.45[g] |
| 2.5 | $10^{-9}$ | 10.56±0.51[d] | 11.4±0.78[f] | 6.72±0.66[h] |

Incubation time : (a) 5 min, (b) 20 min. Significant difference between (c) and (d) at $p<0.01$, (e) and (f) at $p<0.005$, (g) and (h) at $p<0.01$.

## DISCUSSION

It has been suggested that leukotrienes may be the key mediators in inflammatory liver diseases (9). Since Kupffer cells produce various chemical mediators (10) and have the capacity to influence immunologic events (6), they are thought to be closely related to inflammatory and immune responses in the liver.

Since $LTC_4$ is converted to $LTD_4$ and then to $LTE_4$, there is a prolongation of the biological effects of the peptide leukotrienes as they undergo metabolism. In many systems, $LTD_4$ is more biologically active than $LTC_4$, so that, at least initially, the metabolism of $LTC_4$ enhances the effects of peptide leukotrienes. However, $LTE_4$ is usually less active than $LTD_4$, although it still has appreciable biological activity (13). Moreover, since $LTE_4$ accumulates during leukotriene metabolism in Kupffer cells, $LTE_4$ may play a more important role in the pathogenesis of liver diseases than was previously thought. Our results show the possibility that Kupffer cells may affect inflammatory cell infiltration and the immune response in the liver through the production of $LTC_4$, $LTD_4$, and $LTE_4$.

PAF is a phospholipid mediator produced from macrophages and polymorphonuclear leukocytes, and is involved in anaphylaxie shock and various inflammatory responses (14). PAF is known to increase the production of prostaglandin $E_2$ from polymorphonuclear leukocytes (15). In this study,

the production of $LTC_4$, $LTD_4$, and $LTE_4$ from Kupffer cells was enhanced by PAF, suggesting that PAF may also be involved in inflammatory liver diseases by causing the increase in $LTC_4$, $LTD_4$, and $LTE_4$ production.

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