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Role of leukotrienes B4 and C4 in liver allograft rejection

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Abstract. Previous studies have shown that eicosanoids may act in vitro as immunoregulatory substances. In this study, the concentrations of leukotriene B4 (LTB4) and leukotriene C4 (LTC4) were measured in a model of allograft rejection. Six orthotopic allotransplants of the liver were performed in dogs without the administration of immunosuppressives. LTB4 levels showed an increase coinciding with the start of rejection, significant differences being present between the basal levels and those measured 24 h post-revascularization ($P < 0.05$), and every day from the 3rd postoperative day ($P < 0.01$). LTB4 rose before the parameters generally used in evaluating rejection. LTC4 levels increased significantly ($P < 0.001$) in the first 24 h, and experienced no further variations. LTB4 may play an important role in the mechanisms which bring about the response to the allograft. This substance could be a specific and early marker for rejection.

Key words: Leukotrienes – Leukotriene B4 – Leukotriene C4 – Rejection – Liver rejection – Liver transplantation

Leukotrienes (LTS) are a group of compounds derived from the metabolism of arachidonic acid which, together with the prostaglandins and the tromboxanes, are given the collective name of eicosanoids. It has previously been shown that some of these metabolites, originating via the cycloxygenase or lipoxygenase routes, potentially act as immunoregulators [1], but experience in their clinical application is very limited [2]. Various in vitro experiments [3, 4] have demonstrated that leukotriene B4 (LTB4), as well as sharing the pro-inflammatory actions of the cysteinyl leukotrienes (LTC4, LTD4, LTE4), participates in the mechanisms producing tissue lesions in response to the allograft by means of an increase in leucocyte aggregation, in the proliferation of T lymphocytes, in the secretion of interleucine 1 and 2, and in the development of "natural killer" cell subpopulations. In this study we investigated the behaviour of LTB4 and LTC4 in the rejection of a hepatic allograft.

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Materials and methods

We performed orthotopic liver transplants on six mongrel dogs weighing between 20 and 30 kgs. We used the technique described by Starzl [5–7], with some modifications. For preservation, we used, Eurocollins at 4 °C, the cold ischemia time being 85 min ± 26 min. During the anhepatic phase, a femoro-porto-jugular bypass was performed with spontaneous flow. Afterwards, the venous anastomoses were performed in the following order: suprahepatic cava, infrahepatic cava, porta. After the portal venous system had been revascularized, an end to end arterial anastomosis was performed between the celiac axis of the donor and recipient. In two out of six animals the anastomosis was performed end to side between the hepatic artery of the donor and the origin of the celiac axis in the recipient. Biliary reconstruction was carried out by means of cholecystoduodenostomy. In all cases, the animals were given an autotransfusion of blood extracted a week before the operation. None of the animals was given immunosuppressives. Percutaneous liver biopsy was performed systematically on the 3rd and 5th postoperative days.

Blood samples were taken at the following stages during the study: 1 week before surgery (basal), prior to laparotomy, 8 h after revascularization of the graft through the portal vein, and every day during the postoperative period. At each stage the levels of the following were noted: aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, prothombin time (PT), LTC4 and LTB4. Levels of LTB4 and LTC4 were calculated by radioimmu-

Table 1.

	Basal ($n = 6$)	Day 1 ($n = 6$)	Day 3 ($n = 6$)	Day 5 ($n = 5$)	Day 7 ($n = 4$)
AST	14 ± 5	801 ± 181	170 ± 56	190 ± 83	493 ± 199
ALP	55 ± 7	223 ± 80	525 ± 267	2285 ± 414	6691 ± 392
PT	7.5 ± 0.5	11.4 ± 5.7	8.1 ± 0.6	10.4 ± 2.2	13.1 ± 3.4
Bil	0.22 ± 0.08	0.32 ± 0.16	0.61 ± 0.24	1.44 ± 1.17	2.24 ± 1.32

AST = Aspartate aminotransferase; ALP = alkaline phosphatase; PT = prothombin time; Bil = bilirubin

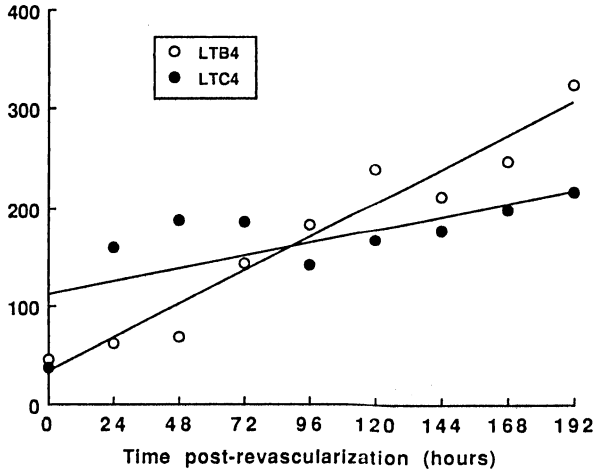

Fig. 1. Mean levels of LTB4 and LTC4

noassay. The antibody, anti-LTC4 was provided by Merck Frosst (Montreal, Canada). The antibody, anti-LTB4 was obtained from Advanced Magnetic Inc. (Cambridge, Mass. USA).

The results were expressed as mean ± SEM. The mean of the six basal dogs were used as control parameters. Observations were compared using Student's *t*-test for paired samples. The level of significance was set up at $P < 0.05$.

Results

The mean survival time of the six animals was 6.5 days (range 4–8 days). In all cases, pathology studies conducted after the animal's death demonstrated the presence of severe acute rejection. In two animals there were moderate pathological signs of rejection on the 3rd postoperative day. Both animals died before the 6th day.

LTB4 levels in the six dogs showed a variable development pattern. None the less, our findings showed a tendency for the mean levels of this parameter to increase as the postoperative period progressed (Fig. 1). A significant difference existed between the base level and the reading at 24 h ($P < 0.05$), and every day from the 3rd postoperative day ($P < 0.01$).

Figure 1 shows LTC4 levels rising significantly with respect to basal levels ($P < 0.001$) in the first 24 h, and then stabilizing for the rest of the postoperative period. The other parameters determined are shown in Table 1.

Discussion

This study analyzes the behaviour of LTB4 and LTC4 in a model of a hepatic allograft rejection without immunosuppression. Our results confirmed the findings of Post et al. [8], who have observed, in a similar model of allotransplant without immunosuppression, an increase in the activity of the enzymes of the 5-lipoxygenase route ocurring in the first 24 h after revascularization. These enzymes regulate the synthesis of LTB4 and LTC4.

The observation of the progressive rise in LTB4 levels as rejection becomes established constitutes a step forward in our understanding of the intimate mechanisms of the tissue lesion in response to allografts. The LTC4 levels rose in the first 24 h and showed no variations for the rest of the postoperative period. This initial increase has been reported as a parameter of good early functioning of the graft [9]. Further research is necessary in order to clarify its role in the pathology of rejection.

LTB4 levels rose earlier than the other parameters generally used to evaluate rejection (Table 1), which means that it could be used as a specific and early marker for rejection. The inhibition of the synthesis of this compound by blocking the metabolism of arachidonic acid via 5-lipoxygenase has been the subject of previous studies [10–12], and may well offer new therapeutic alternatives in the prevention and treatment of rejection.

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