

## Endogenous Endotoxemia During Orthotopic Liver Transplantation in Dogs

T. Miyata, S. Todo, O. Inventarza, Y. Ueda, H. Furukawa, and T.E. Starzl

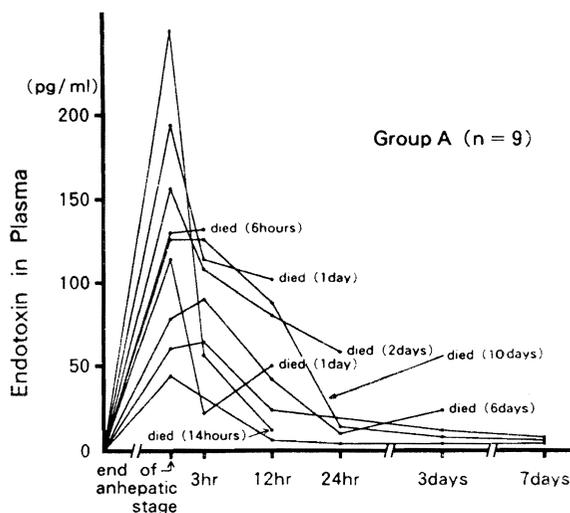
**A**LTHOUGH the 1-year survival rate after clinical orthotopic liver transplantation (OLT) has been improved to about 70%,<sup>1</sup> 15% of the patients still die within the first month as a result of complications, such as shock, multiple organ failure, and bleeding. Although various factors have been found to be involved in these postoperative complications, the overall mechanism has not been well elucidated.

The liver normally contributes more than 80% of the blood cleaning functions of the reticuloendothelial system.<sup>2</sup> During OLT, this hepatic contribution temporarily is lost (anhepatic phase), and toxic substances including endotoxin can flood the circulation. To our knowledge, there has been no report concerning endotoxemia during OLT.

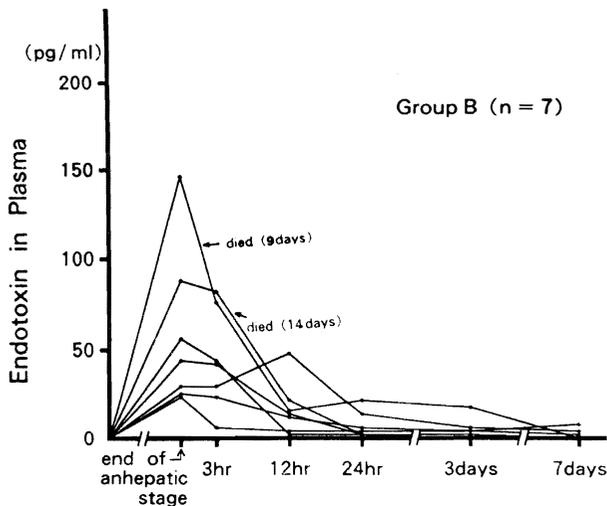
Endotoxin is a cell wall component of gram-negative bacteria and has lipid A as the central component in its structure. Endotoxin triggers shock,<sup>3</sup> pulmonary failure,<sup>4</sup> and disseminated intravascular coagulation.<sup>5</sup> It is absorbed from the intestine even under conditions of health.<sup>6</sup> Recently, a highly sensitive quantitative method has been developed for plasma endotoxin determination using chromogenic substrate and perchloric acid.<sup>7</sup>

### MATERIALS AND METHODS

We used a modification of this method<sup>8</sup> to determine whether endotoxemia occurred at the time of OLT in dogs. Briefly, dogs' platelet poor plasma was added to perchloric acid, and the neutralized supernatant from this mixture was incubated with Japanese horseshoe crab *Tachypleustridentatus* and a chromogenic substrate (Seikagaku Kogyo, Tokyo). The absorbance was measured at 545 nm after diazotization. All procedures before diazotization were



**Fig 1.** Change of peripheral blood endotoxin levels in dogs not given neomycin preoperatively (group A).



**Fig 2.** Change of peripheral blood endotoxin levels in neomycin-treated dogs (group B).

carried out in a pyrogen-free manner. As a reference, endotoxin 0111 B4 was used. Student's *t*-test was used for the comparison of endotoxin values, and the  $\chi^2$  test was employed for the comparison of mortality.

Sixteen healthy beagle female recipients weighing 10-15 kg underwent OLT from mongrel donors of either sex. The experiments were divided into two groups. In group A ( $n = 9$ ), no antibiotic treatment was given before the OLT, whereas in group B ( $n = 7$ ), 750 mg neomycin was given by mouth 3 times/day for 3 days. All the operations were performed by the same team, using a standard technique.<sup>9</sup> The livers were infused and preserved with chilled lactated Ringer's solution and had cold ischemia times of 25-40 minutes before portal revascularization. Beginning on the morning after transplantation, the dogs were treated orally with 20 mg/kg/day CyA, which was dissolved in ethanol and in olive oil to a concentration of 100 mg/ml of solution.

Samples for endotoxin measurement were drawn from a peripheral vein, collecting 3 ml in a pyrogen-free disposable syringe. The blood was anticoagulated with 10 units heparin/ml sample. Sampling was immediately before liver grafting, at the end of the anhepatic phase, 3, 12, and 24 hours after the end of the anhepatic phase, and 3 and 7 days after the operation if the dog lived this long.

From the Department of Surgery, University of Pittsburgh School of Medicine, and the Veterans Administration Medical Center, Pittsburgh, Pennsylvania.

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Address reprint requests to Thomas E. Starzl, MD, PhD, Falk Clinic, 3601 Fifth Avenue, Pittsburgh, PA 15213.

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The sample was immediately centrifuged at 3000 rpm for 10 minutes and stored at  $-80^{\circ}\text{C}$  until the analysis.

## RESULTS

Before OLTX, the endotoxin levels in the peripheral blood ( $<5$  pg/ml) did not differ between group A and group B. During the anhepatic phase, the maximum endotoxin levels in group A increased to  $128.1 \pm 20.6$  pg/ml (Fig 1) and in group B to  $59.0 \pm 15.5$  pg/ml (Fig 2). The difference between groups was significant ( $p < 0.01$ ). This endotoxemia lasted for more than 12 hours after the end of the anhepatic phase. Seven of the 9 dogs not given neomycin (group A) died within 14 days of pulmonary failure (5), bleeding (4), and lymphatic ascites (1); 3 of these animals had more than one principal cause of death. The perioperative endotoxemia tended to be greater in the animals that did not survive through the 2-week observation period (Figs 1, 2). Two of the 7 dogs given neomycin (group B) died within 14 days, both from intestinal intussusception. The difference between the groups in 2-week mortality was significant ( $p < 0.05$ ).

## CONCLUSIONS

These data suggest that endogenous endotoxemia occurring during OLTX may play an important role in the development of complications after OLTX. Endotoxin currently is being measured during and after OLTX in humans.

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