Improving Donor Livers by Inhibiting TNF-α Production

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ABSTRACT
Hepatic ischemia/reperfusion (I/R) injury has a significant influence on the outcome of liver transplants. Inhibiting certain enzymatic reactions that occur during I/R injury may have a protective effect on the liver during transplantation. After reviewing the biochemical pathways involved in hepatic I/R injury, we describe a pharmacologic line of defense against this injury by means of the enzyme tissue inhibitor of metalloproteinase 3 (TIMP-3). Current results suggest that TIMP-3 will play a clinically relevant role in improving outcomes of liver transplants by reducing I/R injury to the donor liver.

INTRODUCTION
Over the past 30 years, orthotopic liver transplantation (OLT) has become the treatment of choice for end-stage liver disease. The qualified success of OLT can be measured by its quantifiable need. The 16,000 potential recipients on the national waiting list far surpass the 6,000 organs available per year for transplantation. Approximately 17% of potential OLT recipients on the transplant list die while waiting for a transplant. 1-3

Options for increasing the donor pool have included the use of living donors, split livers, and “marginal” livers. Marginal livers include those from donors who are older, have fatty livers, or have anticipated longer ischemic (deprived of blood flow) times. These marginal livers are at increased risk for the development of primary nonfunction after liver transplantation, a common cause for retransplanta-

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ing enzyme (TACE) into its biologically active form, TNF-α. TACE has been shown to be involved in a variety of physiologic or pathophysiologic processes. With the increasing use of expanded donor allografts, interest in I/R research in transplantation has been renewed.

We hypothesize that the inhibition of TACE will reduce hepatic I/R injury during transplantation. Manipulation of this inhibition will enable the increased use of marginal livers that would otherwise be discarded, thereby increasing the donor pool of organs. The novel implementation of a pharmacologic line of defense against I/R injury is an innovation that renders nonviable livers into organs eligible for transplantation.

**EXPERIMENTAL DATA**

This review highlights the work done in the Transplantation Research Laboratory at the Ochsner Clinic Foundation. The focus of the laboratory has been to identify potential enzymatic targets in donor livers that could be pharmacologically treated to improve outcomes in liver transplantation.

**TACE Upregulation**

In a well-established rat model of partial warm hepatic I/R injury, we found that low levels of TACE were detected in normal liver tissue by both reverse transcriptase–polymerase chain reaction (RT-PCR) and Western blot. Ten minutes of warm ischemia resulted in a logarithmic rise in TACE messenger RNA (mRNA) levels, which peaked 6 hours after hepatic reperfusion. At 24 hours, TACE mRNA levels remained overexpressed, when compared to baseline, but had declined from the 6-hour peak. After 30 minutes of ischemia, hepatic TACE mRNA levels demonstrated a similar upregulated pattern of expression, although each time point had a 2-fold increase when compared to its 10-minute ischemia counterpart. Western blot analysis demonstrated a strong increase in TACE protein levels 6 hours after the ischemic injury. At 10
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and 30 minutes of ischemia followed by reperfusion, TNF-α and TNF-α receptor mRNA levels were upregulated in a pattern similar to TACE mRNA levels. Serum TNF-α and interleukin 6 (IL-6) levels correlated with the observed increases in mRNA levels in the liver. This was the first reported documentation of TACE in liver tissue; this finding demonstrates that TACE is an inducible enzyme that is upregulated by ischemia.

TACE Inhibition

To further confirm the involvement of TACE and its role in hepatic I/R injury, we designed experiments to dampen TACE activity during hepatic I/R injury. Tissue inhibitor of metalloproteinase 3 (TIMP-3), a naturally occurring inhibitor of TACE, was given intraperitoneally 1 hour before hepatic I/R injury. Results are shown in Table 1. High doses of TIMP-3 markedly reduced both serum TNF-α and alanine aminotransferase (ALT) levels and preserved the architectural integrity during hepatic I/R injury (Table 2 and Figure 3). On the basis of these studies, we can conclude that TACE does play an important role in hepatic I/R injury. TIMP-3, through highly selective inhibition of TACE activity, does reduce hepatic I/R injury at both the biochemical and histologic levels.

TNF-α Regulation

Further TACE inhibition studies progressed to a total warm ischemia model, which is more directly comparable to a potential transplant application; also, it eliminated other potential pitfalls of the partial warm ischemia model.

To illustrate TACE activity during total warm I/R injury, TIMP-3 was given 1 hour before surgery. At a dosage of 1,000 ng/kg body weight, TIMP-3 significantly decreased serum TNF-α levels at all 4 time points (6, 24, and 48 hours and 7 days) compared to controls. The control animals subjected to I/R injury had high levels of serum TNF-α up to 7 days after the injury (61.3–107.6 pg/mL). With TIMP-3 pretreatment, notable inhibition (62%–90%, P < .05) was present for the first 48 hours after injury (ranging from 11.6 pg/mL to 27.1 pg/mL). On day 7, although the pretreated animals recovered, with increased TNF-α levels, their levels were still 53% below the levels of the control animals, at only 30 pg/mL (Figure 4).

At 24 hours after reperfusion, both control and TIMP-3–treated liver samples showed mild edema. At 7 days after reperfusion, TIMP-3–treated liver samples continued to show only mild edema. Control liver samples showed more pronounced ischemic changes, with hepatocyte collapse, necrosis, and hemorrhage (Figure 5).

HUMAN STUDIES

In a pilot study, the presence of TACE in human donor liver tissues was assessed. During organ procurement, liver biopsies and plasma samples were collected from 16 deceased donors. Four of the donors had documented hepatic ischemia within 48 hours of procurement. TNF-α, TNF-α receptor 1, had high levels of serum TNF-α up to 7 days after the injury (61.3–107.6 pg/mL). With TIMP-3 pretreatment, notable inhibition (62%–90%, P < .05) was present for the first 48 hours after injury (ranging from 11.6 pg/mL to 27.1 pg/mL). On day 7, although the pretreated animals recovered, with increased TNF-α levels, their levels were still 53% below the levels of the control animals, at only 30 pg/mL (Figure 4).

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and IL-6 were measured by enzyme-linked immuno-
sorbent assay in donor plasma sampled at the time of
procurement. Hepatic TACE, TNF-α, and glyceralde-
hyde 3-phosphate dehydrogenase mRNAs were
detected by RT-PCR. Serum ALT, aspartate amino-
transferase, and bilirubin levels were obtained and
analyzed (Table 3).

This study is the first to document the existence of
TACE in human liver tissue: TACE appears to be
upregulated in donor livers in response to ischemia.
Further study is necessary to evaluate the value of
TACE as a potential target of inhibition within donor
livers. Extrapolating from our animal studies, TACE
inhibition may ameliorate human hepatic injury from
ischemia. If true, inhibiting TACE in organ donors
might protect donor livers from ischemic injury and
perhaps even allow the salvage of organs from donors
who have sustained severe ischemia. This could
potentially expand the donor liver pool for transplan-
tation.

**CURRENT RESEARCH**

Because of its role and its potential inhibition
during the transplant event, TACE appears to be a
clinically viable target for improving “marginal” livers.
Currently, the laboratory is examining the effects of
TACE inhibition on liver apoptosis (programmed cell
death) and liver regeneration. The degree of TACE
inhibition may alter the balance of apoptosis and
regeneration. Understanding this balance is the next
step to developing a clinically useful pharmacologic modifier to increase the potential pool of donor organs and thus reduce the deaths of patients on the waitlist.

REFERENCES


