BRIEF COMMUNICATION

NITRIC OXIDE: A USEFUL INDICATOR IN ACUTE ALLOGRAFT REJECTION AFTER LIVER TRANSPLANTATION

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ABSTRACT

The aim of this study was to monitor serum nitric oxide levels at various times, intraoperatively and following liver transplantation in humans. Nitric oxide production was assessed by cadmium reduction method in 15 patients undergoing liver transplantation. We observed strong correlation between nitric oxide production and allograft rejection. Sustained rise in nitric oxide level after transplantation might provide an indication of acute allograft rejection. Therefore measurement of nitric oxide, a stable end product appears to be an excellent marker that can help in the development of strategies intended to improve graft viability and patient's survival.

KEY WORDS

Ischemia/reperfusion injury, Nitric oxide, Allograft rejection, Nitric oxide synthase.

INTRODUCTION

Ischemia-reperfusion (IR) injury is one of the major causes of poor graft function early, after liver transplantation. This adversely influences patient survival after transplantation (1). The hemodynamic alterations occurring after graft reperfusion and the subsequent complications may result from the changes in biotic activator such as nitric oxide (2). Nitric oxide (NO), being an immunomodulatory molecule has been proposed as a mediator of acute cellular rejection in the hepatic allograft of humans (3). Increased serum NO levels as well as allograft expression of nitric oxide synthase (NOS) have been correlated with rejection of liver transplant (4, 5). In the post-operative period, an increased NO production could be expected either in response to rejection or infection. Whereas the elevated NO levels found 4 to 6 days after surgery in the absence of overt complication have been related to subclinical rejection (6).

Thus, an enhanced release of NO after liver transplantation might provide an indication of functional status of the liver. Therefore, in the present study, we have investigated the concentrations of NO generation in serum and their relationship with allograft cellular response in liver transplant recipients.

MATERIALS AND METHODS

The present study was carried out in the Department of Biochemistry, Global Hospitals, Hyderabad and Dr. V.M. Govt. Medical College, Solapur. Fifteen recipients with end-stage cirrhosis undergoing liver transplantation were enrolled in this prospective study. The blood samples were collected at different stages of transplantation such as pre-reperfusion, reperfusion and 2nd, 4th and 6th day after transplantation in non-heparinised vacutainer. Nitric oxide level was determined with cadmium reduction method by Wakid and Cortas (7) and obtained results were compared with 15 healthy age and sex matched control subjects.

Statistical analysis was carried out using student's paired and unpaired 't' test. P value less than 0.05 (P<0.05) was considered as significant.

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PR: pre-reperfusion, R: reperfusion; & ✕ Rejection cases.

RESULTS AND DISCUSSION

It has been well established that, low amounts of nitric oxide (NO) are typically needed in the liver to maintain homeostasis with its functional counterparts, such as endothelins and platelet-activating factor (8). Several reports have suggested that changes in this basal NO production may sustain a wide spectrum of biological functions during liver transplantation in animal models (8, 9). To our knowledge, few data have been published on NO production during liver transplantation in humans and available results are mainly derived from either NO level or there biological effects after inhibitory stimuli. In the present study NO production was assessed by cadmium reduction method in human subjects undergoing liver transplantation. Nitric oxide, itself is very unstable, reacting with a variety of molecule to form NO_2 , NO_3 and other nitrosocompounds, either directly or after decay of intermediates.

Table 1: Shows serum Nitric oxide levels at different stages of liver transplantations and in healthy control subjects.

Sr. No.	Groups	Serum Nitric Oxide ($\mu\text{mol/L}$)
01	Controls (n=15)	32.12 $\pm$ 3.21
2a	Pre-transplant (n=15)	47.48 $\pm$ 6*
2b	Reperfusion (n=15)	61.17 $\pm$ 2.7**
2c	6 th day of transplant (n=15)	33.9 $\pm$ 2.3*

n= number of subjects studied; Values are expressed as Mean $\pm$ SD

** P<0.001 as compared to controls; *P<0.001 group 2b versus 2a & 2c.

A first peak in NO concentration was demonstrated in the samples collected during reperfusion as compared to that of pre-transplant samples (Table 1) (P<0.001). Increased levels of NO were noted up to 2nd day of post transplantation as compared to that of controls. In further study, values were found to decline and reach within normal limits on 6th day of post-transplantation in total 13/15 survived patients. Where as controversial results were obtained in two cases, where sustained rise in NO level was noted even after 4th day of transplantation. In those patients unknown complications occurred and acute graft rejection took place (Figure1).

Sustained rise in NO levels found during reperfusion may be due to a hyperactivity of inducible NOS in different cell types, such as hepatocytes, endothelial cells, blood mononuclear cells and kupffer cells. Among possible triggering factors, hypoxia, endotoxins and cytokines as well as vascular adaptive changes, all of which may stimulate NO production under these conditions (10).

In this study, we have shown a strong correlation between NO production and rejection. Nitric oxide may contribute to the pathogenesis of rejection following activation of immune system as a cytotoxic molecule. The source of this increased production is likely to be the graft-infiltrating cells and/ or the cells of hepatic vasculature and hepatic parenchyma cells, following induction of NOS by products of the infiltrating cells. The elevated local concentrations of NO in the allograft are likely to be directly injurious to target cells in rejection, mediating damage in liver rejection to vascular endothelium and inhibiting hepatocytes protein synthesis. (11,12).

Immunological monitoring of allograft recipients is rarely used clinically because of the expense and complexity of assay systems involving lymphocytes and cytokine preparations. Sustained rise in NO level after transplantation might provide an indication of acute allograft rejection. Therefore measurement of NO, a stable end product appears to be an excellent marker that can help in the development of strategies intended to improve graft viability and patient's survival.

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