Possible therapeutic effect of lipid supplementation on neurotoxicity in liver transplant recipients

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Running title: A novel therapeutic approach for neurotoxicity

Abstract

Neurotoxicity represents a serious complication following liver transplantation and may be evoked by various perioperative factors or develop due to toxicity of immunosuppresion. The symptoms of neurotoxicity associated with calcineurin inhibitors (CNIs) may be reversed by discontinuing or reducing the dose of CNIs; however, this may increase the risk of rejection. To address this issue, we examined 45 adult living donor liver transplant recipients. At the time of neurotoxicity diagnosis, serum cholesterol values were significantly lower in the patients with neurotoxicity than in those without neurotoxicity. Based on our hypothesis that lipid supplementation prevents lipophilic CNIs from crossing the blood-brain barrier, soybean oil was administered to 5 patients with neurotoxicity. In all these patients, the neurological symptoms improved without discontinuing or reducing the dose of CNIs. This use of this therapy may help in avoiding insufficient immunosuppressive therapy because neither dose reduction nor substitution of CNIs would be then required.

Key words: cholesterol, living donor liver transplantation, neurotoxicity, soybean oil

Along with advances in surgical techniques, the application of calcineurin inhibitors (CNIs) has significantly improved the outcome of organ and tissue transplantation (*1*, *2*). However, these potent immunosuppressive drugs have the potential to cause toxic side effects. Toxicity in the central nervous system presents as a wide spectrum of mild to severe neurological and psychiatric disorders that have been observed in 6%–47% of liver transplant recipients treated with CNIs (*3*-*6*). The wide range of disorders might be attributed to the difficulties in differentiating between psychiatric disturbances, peripheral and autonomic neuropathy associated with diabetes, and neural deficits resulting from the metabolic consequences of hepatic failure.

It has been reported that the risk factors for CNI-induced neurotoxicity probably include hypoglycemia, hypomagnesemia, hypocholesterolemia, elevated CsA and Tacrolimus blood levels, hypertension, acute renal failure, preoperative hepatic encephalopathy, decreased liver function, and electrolyte disturbances such as hyper- or hyponatremia (*7*-*10*). Generally, the symptoms of neurotoxicity can be reversed by discontinuing or reducing the dose of CNIs (*5*-*11*). However, these strategies may in turn increase the risk of acute cellular rejection (ACR).

In the present study, to assess the factors that promote the development of post-transplant neurotoxicity, we retrospectively evaluated 45 patients who underwent living donor liver transplantations (LDLTs) at Hiroshima University Hospital between June 2000 and March 2006 who had not suffered from pre-transplant neurotoxicity. The 45 patients included 27 males and 18 females; their ages ranged from 20 to 66 years (mean age ± SD, 50.9 ± 9.4 years). The primary diseases included hepatitis B/C virus-related cirrhosis in 11 patients (associated with hepatocellular carcinoma in 19 patients), primary biliary cirrhosis in 5, autoimmune hepatitis in 4, alcoholic cirrhosis in 2 and other diseases in 4 patients. The graft donors included 30 offsprings, 9 spouses, 3 siblings and, 2 parents; their ages ranged from 18 to 61 years (mean age, 32.5 ± 12.1 years). The graft weight and graft-to-recipient body weight ratio (GRWR) ranged from 262 to 896 g (mean weight, 593.2 ± 150.4 g) and 0.49% to 1.92% (mean ratio, 0.97% ± 0.27%), respectively. The basic immunosuppressive regimen after LDLT comprised Tacrolimus/CsA and methylprednisolone with/without basiliximab. Of the 45 patients, 11 received CsA-based immunosuppressive therapy, while the remaining 34 received Tacrolimus-based immunosuppressive therapy. The choice between CsA and FK-506 was left to the surgeon. The target blood levels of CNIs were achieved in all the 45 recipients in this

series. The trough whole blood levels of Tacrolimus were maintained between 8 and 15 ng/ml in the first few postoperative weeks and between 5 and 10 ng/ml thereafter; those of CsA were maintained between 150 and 250 ng/ml in the first few postoperative weeks and between 100 and 150 ng/ml thereafter.

Of the 45 patients, 10 presented with a wide range of neurological and psychiatric disorders (neurotoxicity group). The remaining 35 patients did not show any symptoms of CNI-induced neurotoxicity (no neurotoxicity group). The characteristics of these patients are listed in Table 1. No significant differences were observed in age, sex, operation time, time to extubation, GRWR, and type of CNIs used for immunosuppression between the neurotoxicity group and the no neurotoxicity group. The mean onset of symptoms was 6.9 ± 4.3 postoperative days.

At the time of neurotoxicity diagnosis, no significant difference was observed in the levels of neurotoxic substances such as bilirubin or ammonia between the neurotoxicity group and the no neurotoxicity group (Table 2). A clear relationship was not observed between the blood levels of CNIs and the incidence of neurotoxicity. No significant difference was observed in pre-transplant serum cholesterol values between the neurotoxicity group and

the no neurotoxicity group (145.1 ± 56.6 and 135.3 ± 52.1 mg/dl, respectively). Notably, at the time that neurotoxicity developed, serum cholesterol values were significantly lower in the patients with neurotoxicity than in those without neurotoxicity (Table 2).

In the patients suffering from neurotoxicity (Table 3), the first 2 patients (cases 1 and 2) recovered after the dose of CNIs was reduced. The next 3 patients recovered after Tacrolimus was withdrawn and the basal immunosuppressant was switched from Tacrolimus to CsA (cases 3, 4 and 5). However, one of these patients subsequently suffered from acute cellular rejection, probably occurred due to the reduction in the CNIs dose (case 3). Both CsA and Tacrolimus are highly lipophilic and considerable portions of these compounds are bound to the lipoprotein fractions in the blood, unusually large amounts of CNIs may remain unbound or free in patients with low cholesterol levels. If the blood-brain barrier is impaired, the unbound CNIs might be able to cross the blood-brain barrier, resulting in an increased uptake of CNIs in the brain. Based on this concept, we hypothesized that exogenously supplied lipids can prevent lipophilic CNIs from crossing the blood-brain barrier, 20% soybean oil (Intralipos®; Otsuka Pharmaceutical Factory, Japan) was continuously administered at a dose of 0.1–0.2 ml/kg/h for 2-3days to

5 patients with neurotoxicity, regardless of its severity (cases 6, 7, 8, 9, and 10). The neurological symptoms of all these 5 patients showed remarkable improvement after starting treatment with intravenous injection of soybean oil without discontinuing or even reducing the dose of CNIs. During the observation period, no adverse effects caused by soybean oil treatment or any episode of ACR were encountered in these patients.

The mechanism underlying CNI-induced neurotoxicity has not been clearly elucidated. Several investigators have suggested the presence of some disturbance in the blood-brain barrier (*6, 8, 11*). Analysis of the cerebrospinal fluid after liver transplantation demonstrated the presence of CNIs and their metabolites and that high-dose corticosteroids inhibit metabolism of CNIs in the liver. Both these observations suggest a direct toxic effect of CNIs on neural tissues. The CNIs may gain access to the neural tissues either due to membrane toxicity or indirectly through the disrupted blood-brain barrier associated with severe hepatic failure. However, we could not precisely differentiate CNI-induced neurotoxicity from the dementia/mental derangement associated with hepatic coma – a common complication in patients with acute liver failure.

In the patients suffering from neurotoxicity, serum cholesterol levels was significantly

lower in the patients with neurotoxicity than in those without neurotoxicity at the time that neurotoxicity developed. It is well known that both CsA and Tacrolimus are highly lipophilic, and the lipophilic nature of both substances implies that they do not rapidly enter the brain tissue (*10, 11*). In blood, approximately 40% of the CsA is taken up by erythrocytes. Most of the remaining 60% is bound to the lipoprotein fractions, which include triglycerides, phospholipids, free cholesterol, cholesterol esters, and apolipoproteins. Apparently, less than 10% of the CsA is not bound to lipoproteins or erythrocytes (*8, 12, 13*). In the presence of hypolipidemia, unusually large amounts of CNIs may remain unbound or free. If the blood-brain barrier is impaired, the unbound CNIs might be able to cross it, resulting in an increased uptake of CNIs in the brain. In addition, there exists a strong evidence suggesting that the average FK-binding protein (FKBP) level in brain tissues is 10–40 times higher than that in the immune tissues (*14*). A combination of high FKBP levels in the brain and the increased Tacrolimus content in the brain tissues might result in local Tacrolimus-associated toxicity. Based on this concept, we hypothesized that exogenously supplied lipids can prevent lipophilic CNIs from crossing the blood-brain barrier. Consistent with the hypothesis, all the 5 patients who were administered 20% soybean oil continuously (at a rate of 0.1–0.2 ml/kg/h) showed a dramatic improvement in

the symptoms of neurotoxicity. This finding encourages us to further investigate a possible therapeutic approach of using lipid supplementation for the CNI-associated neurotoxicity in liver transplant recipients.

In conclusion, we propose a possible therapeutic approach of exogenous supply of lipids for neurotoxicity in liver transplant recipients. This use of this therapy may help in avoiding insufficient immunosuppressive therapy because neither dose reduction nor substitution of CNIs would be then required.

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Table 1: Patient characteristics

	No neurotoxicity group (n = 35)	Neurotoxicity group (n = 10)	*P* value
Age at LTx (years)	51.4 ± 9.6	49.1 ± 8.4	0.49
Sex (male/female) (n/n)	22/13	5/5	
Primary diagnosis (n)	Cirrhosis 12 HCC 16 Others 7	Cirrhosis 6 HCC 3 Others 1	
Operation time (min)	724.8 ± 134.0	685.4 ± 71.3	0.38
Time to extubation (days)	2.9 ± 2.8	2.2 ± 1.6	0.49
GRWR	1.00 ± 0.28	0.90 ± 0.23	0.32
CsA/Tacrolimus (n)	8/27	3/7	

Abbreviations used in the table: LTx, liver transplantation; HCC, hepatocellular carcinoma; GRWR, graft-to-recipient body weight ratio; CsA, cyclosporine. Data are expressed as average values ± SD.

Table 2: Clinical parameters

	No neurotoxicity group (n = 35)	Neurotoxicity group (n = 10)	*P* value
	Averaged data of POD7	Averaged data of at the time of neurotoxicity developed	
Total bilirubin (mg/dl)	5.2 ± 4.0	6.2 ± 2.9	0.50
AST (IU/l)	81.0 ± 51.2	82.0 ± 63.6	0.96
ALT (IU/l)	246.0 ± 168.6	178.1 ± 141.2	0.25
Ammonia (μmol/l)	34.0 ± 7.7	33.4 ± 16.0	0.89
CsA trough (ng/ml)	204.3 ± 74.9	221.1 ± 111.8	0.61
Tacrolimus trough (ng/ml)	9.3 ± 3.9	11.1 ± 4.6	0.33
Total cholesterol (mg/dl)	97.5 ± 21.6	73.5 ± 18.0	<0.01

Abbreviations used in the table: POD, postoperative days; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CsA, cyclosporine. Data are expressed as average values ± SD.

Table 3: Characteristics of patients suffering from neurotoxicity

Case	Age (years)	Sex	Indication for transplantation	Immunosuppressant used	Onset of neurological	Neurological symptoms	Immunosuppressive treatment for	Duration of symptoms
1	46	F	Alcoholic cirrhosis	Tacrolimus	POD3	Mental status change	Dose of Tacrolimus reduced	9 days
2	57	M	HBV cirrhosis/HCC	CsA	POD6	Mental status change	Dose of CsA reduced	4 days
3	52	M	Primary biliary cirrhosis	Tacrolimus	POD7	Confusion, seizures	Tacrolimus withdrawn, switched to CsA	5 days
4	32	F	Autoimmune hepatitis	Tacrolimus	POD3	Altered level of consciousness	Tacrolimus withdrawn, switched to CsA	3 days
5	38	M	HBV cirrhosis	Tacrolimus	POD7	Headache Tremor	Tacrolimus withdrawn, switched to CsA	2 days
6	59	F	HCV cirrhosis	CsA	POD10	Altered level of consciousness	CsA unchanged + soybean oil	2 days
7	54	F	HCC	Tacrolimus	POD7	Mental status change	Tacrolimus unchanged + soybean oil	2 days
8	52	M	HCV cirrhosis	CsA	POD6	Altered level of consciousness	CsA unchanged + soybean oil	2 days
9	51	F	Primary biliary cirrhosis	Tacrolimus	POD18	Neuralgia	Tacrolimus unchanged + soybean oil	1 day
10	50	M	HBV cirrhosis/HCC	Tacrolimus	POD4	Mental status change	Tacrolimus unchanged + soybean oil	1 day

Abbreviations used in the table: M, male; F, female; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; CsA, cyclosporine; POD, postoperative day.