Int J Pharm Bio Sci 2015 April; 6(2): (B) 191 - 198

Research Article **Immunobiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

SIGNIFICANCE OF TACROLIMUS MONITORING TO OPTIMIZE IMMUNOSUPPRESSIVE THERAPY

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ABSTRACT

Liver transplantation is the surgical treatment of choice for several irreversible liver diseases where a partially or completely diseased liver is replaced with a healthy liver. The continued use of immunosuppressant causes certain serious consequences such as hepatotoxicity, nephrotoxicity and post-transplant hyperglycemia. Inter individual variation has necessitated frequent monitoring of tacrolimus concentration to prevent toxicity, which is the major clinical challenge encountered in the post-transplant period. This study was carried out with the aim to evaluate the correlation between blood concentration of tacrolimus and the risk/degree of toxicity with time after liver transplant. Our data suggests that monitoring effective immunosuppression is essential to minimize toxicity induced by tacrolimus, especially in the 3-6 month period. Therefore, it is necessary to maintain trough blood concentration in liver transplant recipient's 10-12 ng/ml in first trimester, 8-10 ng/ml in next trimester and 5-8 ng/ml thereafter. Hence, monitoring of tacrolimus concentration of the recipient should be done throughout the period of tacrolimus intake i.e. for life time, to enhance clinical evaluation.

KEYWORDS: Tacrolimus, Immunosuppression, Transplant, Liver, Hepatotoxicity

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INTRODUCTION

Liver transplantation or hepatic transplantation is the replacement of diseased liver with a healthy liver from a donor by the surgical means. Liver transplantation is a viable treatment choice for end-stage liver hepatic disease and acute liver failure. Similar to other allograft,a liver transplant will be rejected and removed by the recipient's immune system unless an immunosuppressive drugs are used. Most of the liver transplant recipient receives corticosteroids plus a calcineurin inhibitor such as tacrolimus or cyclosporine to stop graft rejection[1]. Recent clinical results shows that the tacrolimus is a better choice than the cyclosporine during the first year of transplant[1,2].Tacrolimus (also known as fujimycin or FK506) is a 23-membered macrolide lactone (Figure 1). It is produced by the fermentation broth of *Streptomyces tsukabaesis*[3,4]. It is an immunosuppressive agent belongs to a calineurin inhibitor group which is used in solid organ transplant as a therapeutic drug[5]. Tacrolimus suppresses the natural immunity against a foreign body and thus, prevents rejection in liver, kidney, lung, pancreas and bone marrow recipients[6-8]. Immunophilins, a group of highly conserved proteins, mostly participate in protein folding. Inside cytoplasm, tacrolimus binds with respective cytosolic immunophilins such as FKBP-12 and FKBP-52 (an FK506-binding proteins), a component of the glucocorticoid receptor complex. This complex binds and inhibits the activity of the calcineurin, a calcium/calmodulin-dependent protein phosphatase that is expressed in all mammalian tissues[9]. The said complexes interact with calcium-dependent signal pathway in immunological T-cells. Calcineurin then increases the interference of leads to interference with translocation to the nucleus of various nuclear factors involved in the transcription of cytokine genes. As a result of this inhibition, the transcription of the genes involved in T-cell activation is suppressed, affecting the production of interleukin-2 (IL-2) and various other cytokines and interleukins, such as interleukin-3 (IL-3), interferon-γ (INF-γ), and tumor necrosis factor-α (TNF-α)[10].

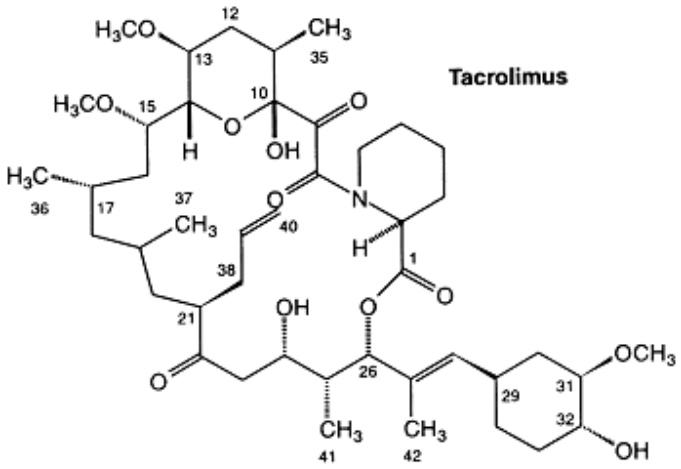

Figure 1
Tacrolimus structure (Adapted from Tanaka HKA, 1987)

Tacrolimus is metabolized by gastrointestinal cytochrome P450 (CYP)3A isoenzyme, 3A4 and 3A5, through O-demethylation and hydroxylation metabolic reaction in the liver and intestinal wall. It is then removed by P-glycoprotein[11]. P-glycoprotein decreases the intracellular concentration of tacrolimus by reversely pumping out the absorbed drug into

intestinal lumen. P-glycoprotein may also regulate access of tacrolimus to CYP3A enzyme and prevent these enzymes from being overcome by high drug concentration in intestine[12-14]. Tacrolimus is repeatedly moved out of intestinal mucosal cell and then passively reabsorbed. 95% tacrolimus matabolites are eliminated by the biliary route11. Increase tacrolimus metabolites concentration in blood can be caused by biliary obstruction[15-16]. During drug therapy with several kinds of drugs like antibiotics, immunosuppressants, etc. there is a direct toxic effect on the liver and immune system and under these conditions the hepatocytes are injured, the intracellular enzymes are released into the circulation and, hence, elevated levels of aminotransfreases and gamma-glutamyltransferase are seen. This is due to the fact that these drugs are metabolized in the liver. Tacrolimus is metabolized in the liver by cytochrome P450 (CYP3A4) enzyme and cleared by the P-glycoprotein. Hence, elevated circulating concentrations of the hepatic enzymes indicate hepatotoxity in post-transplant period[17]. The resultant effects on the transcription and the mRNA degradation of several cytokine genes of interleukin-2; this plays a critical role in T-cell proliferation associated with alloimmune reactions[18].Kidney contains a high concentration of tacrolimus binding proteins. At the cellular level, it has been suggested that tacrolimus nephrotoxicity targets tubular epithelial cells, vascular endothelial cells, arteriolar myocytes, and interstitial fibroblasts. Tacrolimus also have has a direct toxic effect on renal tubules which can be evidenced by the epithelial vacuolization[19].The vascular toxicity of tacrolimus caused by Vasospasm leading to reduced glomerular filtration[20]. Interstitial fibrosis are recognized as another mechanism of tacrolimus nephrotoxicity which leads to narrowing of small arteries and arterioles. As the narrowing proceeds, a linear zone of renal parenchymal vessels is deprived of its nutrition and begins to undergo degenerative changes. The renal parenchymal cells are susceptible to injury because of their relatively low oxygen availability[21-23]. The cytokine TGF-β is likely to involve in the pathogenesis of interstitial fibrosis[24].The toxic effect of tacrolimus on the hormone producing endocrine pancreas is attributable to the selective localisation of FK-binding protein (FKBP)-12 and calcineurin in the pancreatic islets. The intracellular calcium binding protein calmodulin plays an important role in insulin secretion. Tacrolimus binds with calmodulin which may inhibit insulin secretion and cause diabetes mellitus[25-26].

MATERIALS AND METHODS

An analysis of data from 36 patients who has gone for liver transplant has been performed. Subjects who received oral tacrolimus as a main immunosuppressant strategy were included in the study. Analysis of whole blood tacrolimus, Liver function tests (Bilirubin, AST, ALT, ALP, GGT) for monitoring hepatotoxicity, Kidney function tests (blood urea nitrogen, creatinine) for monitoring nephrotoxicity and plasma glucose levels for hyperglycemia were evaluated after 3 months, 6 months and 9 months post-transplant.

Toxicity was ascertained on the basis of the following Tacrolimus levels:
Early post-transplant (up to 3 months) > 15 ng/ml
3 to 6 months >10 ng/ml
6 to 9 months >8 ng/ml
After 9 months >7 ng/dl
Hepatotoxicity were diagnosed on the basis of various enzymes level –
AST/ALT > 60 IU/L
ALP > 140 IU/L
GGT > 95 IU/L

At least two enzymes in the toxic range in two successive readings within a week was considered as hepatotoxicity. Nephrotoxicity was diagnosed when serum creatinine level was measured more than 1.3 mg/dl on two successive readings within a week. Hyperglycemia was considered when blood glucose level was found more than 120 mg/dl in two successive readings within a week.

RESULTS

In this study, we observed whole blood tacrolimus concentration of liver transplant recipients for one year period. Also, we estimated biochemically the liver functions, kidney functions and blood glucose levels throughout this one-year period. We then established correlation between tacrolimus concentration and hepatotoxicity, nephrotoxicity and hyperglycemia. Tacrolimus dose-Liver transplant patients are given tacrolimus post-transplant as per the defined protocol i.e. 0.1-0.3/ mg/kg/day[27].

Tacrolimus levels in post-transplant period

Though appropriate use of immunosuppressants is essential in transplant patients, long term use of immunosuppressive drug causes toxicity. Hence, the relative risks of toxicity and efficacy failure are both related to Tacrolimus concentrations. Table 1 shows tacrolimus concentrations and percentage of subject's distribution at 3rd months, 6th months and 9th months.

Table 1
Whole blood tacrolimus concentration range for different time spans of 3rd months, 6th months and 9th months

Tacrolimus concentration	3rd Month	6th Month	9th Month
3 - 5 ng/ml	3.5%	11%	20%
5.1 – 8 ng/ml	16%	28%	45%
8.1- 10 ng/ml	25%	43%	27%
10– 12 ng/ml	36%	13%	5%
above 12 ng/ml	19.5%	5%	3%

Due to tacrolimus, various kinds of toxicity were notices such as hepatoxic, nepherotoxic and hyperglycemia. In the 3rd month non-toxic subjects were 36.5 % and that of 6th month was 56%. At the end of 9th month non-toxic subjects were almost similar to 6th month i.e., 53 % (Table 2).

Table 2
Prevalence of tacrolimus toxicity for different time spans of 3rd months, 6th months and 9th months

Toxicity	3rd Month	6th Month	9th Month
Hepatotoxic	22%	22%	22%
Nephrotoxic	22%	11%	16.50%
Hyperglycemic	19.50%	11%	8.50%
Nontoxic	36.50%	56%	53%

Three months post-transplant mean tacrolimus concentration was found to be 10.91±4.57 ng/ml; and after 6 months it was 8.00±2.94 ng/ml; where that of after 9 months was 7.13±2.77.

Table 3
Statistical descriptors for the tacrolimus level for 3rd months, 6th months and 9th months

Months	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
3	36	10.914	4.57	0.7617	9.368	12.46	4.7	26.3
6	36	8	2.9419	0.4903	7.005	8.995	2.9	14.7
9	36	7.131	2.7688	0.4615	6.194	8.067	2.7	15.5
Total	108	8.681	3.8487	0.3703	7.947	9.416	2.7	26.3

In each group of patients (patients reviewed during the first 3 months, 3-6 months and 6-9 months), a window of blood tacrolimus levels associated with least toxicity was identified. At the end of 3rd months, 6th months and 9th months this window was identified as 9-11 ng/mL, 8-10 ng/mL and 5-8 ng/mL, respectively. Correlation between tacrolimus levels and toxicity associated parameters were derived in the post-transplant period. During first three months 22% subjects has shown hepatotoxicity, 22% subjects has shown nephrotoxicity and 19.5% subjects has shown hyperglycemia when 53% subject's tacrolimus value were 10-12 ng/ml. After 6 months 22% subjects had hepatotoxicity, 11% subjects had nephrotoxicity and 11% subjects had hyperglycemia when 50% subjects tacrolimus value were 8-10 ng/ml. After 9 months 22% subjects had hepatotoxicity, 16.5% subjects had nephrotoxicity and 8.5% subjects had hyperglycemia when 48.5% subjects tacrolimus value were 5-8 ng/ml. All these levels were lower than the recommended upper limit of trough i.e.20ng/ml. Though the therapeutic range of tacrolimus is defined as 5-20 ng/ml, clinicians are facing dilemma because even within this range several instances of toxicity as well as ineffective immunosuppression were encountered. This could be partly due to inter-individual and intra-individual variations in pharmacokinetics. Moreover, adequate immunosupperssion varies with the time after transplant. Hence, long-term compliance is required to ensure graft survival in transplant patients[27-29]. This is achieved by regular therapeutic monitoring of tacrolimus throughout life in the post-transplant period. The importance of the need for therapeutic monitoring of tacrolimus is further supported by the present study. Drug toxicity in the postoperative period is influenced by the drug-metabolizing capacity of the graft. This capacity depends primarily on the levels and activities of the cytochrome P450 enzymes (P450). P450 enzymes play a central role in the biotransformation of various xenobiotics to more polar compounds, which are readily excreted[30]. The metabolites of a drug can be inactive or less active than the parent compound, although some biotransformation products show enhanced pharmacological or toxicological activity. Any change in the activity of P450 isoforms influences the rate of activation or inactivation of drugs[31]. Lack of functional activity in P450 enzymes need tailored drug treatment in order to avoid excessive drug concentration and toxic effects. Dose adjustment or selection of an alternative drug, which is not a substrate for the polymorphic enzyme, can prevent the development of side-effects in poor metabolizers.Tacrolimus is known to be toxic to the liver so that when the hepatocytes are injured, the intracellular enzymes are released into the circulation. Hence, this effect of tacrolimus on liver function is best observed by activity of alkaline phosphate (ALP), alanine aminotransferase (ALT), aspartate aminotransferase and γ-glutamyltransferase. Tacrolimus binding proteins are present at a high concentration in the kidney. Calcineurin immunoreactivity and enzyme activity in the kidney can be specifically inhibited by tacrolimus. At the cellular level, it has been suggested that tubular epithelial cells, vascular

endothelial cells, arteriolar myocytes, and interstitial fibroblasts are all targets for tacrolimus nephrotoxicity. The occurrence of epithelial vacuolization is evidence of direct toxic effect of tacrolimus on the renal tubule[25]. Vasospasm leading to reduced glomerular filtration appears to be a key element in the vascular toxicity tacrolimus[26]. Interstitial fibrosis are recognized as tacrolimus nephrotoxicity due to narrowing of the small arteries and arterioles. As the narrowing proceeds, a linear zone of renal parenchymal vessels is deprived of its nutrition and, begins to undergo degenerative changes. The renal parenchyma is particularly susceptible to injury because of their relatively low oxygen availability[27-29]. This direct toxicity is characterized by tubular degeneration and atrophy, leading to fibrosis. The cytokine transforming growth factor beta (TGF-β), a well-known activator of collagen transcription, is likely involved in the pathogenesis of interstitial fibrosis. Tacrolimus nephrotoxicity can be said to be present if there is an elevated blood urea nitrogen and/or elevated serum creatinine levels in the post-transplant period. In this study, all subjects with a serum creatinine > 1.3 mg/dl were deemed to have nephrotoxicity. The toxic effect of Tacrolimus on the endocrine pancreas is attributable to the selective localisation of FK-binding protein (FKBP)-12 and calcineurin in the pancreatic islets. The intracellular calcium binding protein calmodulin plays an important role in insulin secretion. Tacrolimus binds with calmodulin which may inhibit insulin secretion and cause diabetes mellitus.

In our analysis, 10–12 ng/ml is the safe therapeutic range of trough levels of tacrolimus for liver transplant recipients in first 3 months, 8-10 ng/ml in the next three months i.e upto 6 months, and 5-8 ng/ml thereafter. Hence, maintaining trough concentrations of tacrolimus within the specified ranges may reduce the degree of complications without compromising efficacy.

CONCLUSION

Inadequate dose of immunosuppressants like tacrolimus is associated with an increased risk for rejection, whereas excessive administration of these immunosuppressants is associated with toxicity. Generally, high tacrolimus concentrations are likely to be required in the initial post-transplant period, but target concentrations need to be reduced over time to minimize toxicity. Our data suggest that monitoring effective immunosuppression is essential to minimize toxicity induced by tacrolimus, especially in the 3-6 month period. In liver transplant recipients, it is necessary to maintain trough blood concentration of 10- 12 ng/ml in first 3 months, 8-10 ng/ml in next three months and 5-8 ng/ml thereafter. Hence, it is necessary to monitor tacrolimus concentration of the transplant recipient throughout the period of tacrolimus intake i.e. for life time.

CONFLICT OF INTEREST

Conflict of interest declared none.

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