Drug Metabolism and Pharmacokinetics

Research Article

Title
Mechanisms of lower maintenance dose of tacrolimus in obese patients

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Running Title
Lower maintenance dose of tacrolimus in obese patients
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Abbreviations
Alanine aminotransferase, ALT; analysis of variance, ANOVA; aspartate aminotransferase, AST; area under the concentration curve, AUC; bioavailability, F; cytochrome P450, CYP; elimination rate constant, k\text{e}; high-performance liquid chromatography, HPLC; inducible nitric oxide synthase, iNos; interleukin-6, IL-6; mean residence time, MRT; oral clearance, CL\text{tot}/F; P-glycoprotein, P-gp; terminal elimination half-life time, t\text{1/2}; total body clearance, CL\text{tot}; tumor necrosis factor-α, TNF-α; volume of distribution at steady-state, Vd\text{ss}
Summary

A retrospective analysis suggested that blood tacrolimus concentrations were consistent among patients with a body mass index (BMI) < 18.5 (lean), normal (≥18.5 and <25) and ≥ 25 (overweight or obese). The average maintenance dose of tacrolimus in patients with BMI ≥ 25 were significantly lower compared with that in patients with a BMI of less than 25. Lean and obese Zucker rats fed a normal diet were given tacrolimus intravenously or orally. The blood concentrations of tacrolimus in obese rats were significantly higher than those in lean rats after administration via both routes. The moment analysis has suggested that CL_{tot} and V_dss of tacrolimus were not significantly different between lean and obese rats. The bioavailability was higher in obese rats, compared with that in lean rats. The protein expression of Cyp3a2 in the liver was significantly decreased in obese rats, compared with lean rats, while P-gp in the small intestine was also significantly decreased in obese rats. These results suggested that the steady-state trough concentration of tacrolimus in obese patients were well maintained by relatively low dose compared with that in normal and lean patients, presumably due to increased bioavailability.

Key words
Cyp3a2, Obesity, P-gp, Pharmacokinetics, Tacrolimus
Introduction

Obesity has become a serious problem throughout the world, being closely associated with increased incidences of serious medical conditions, including hypertension, diabetes, dyslipidemia, and cardiovascular disease. The World Health Organization has estimated that 35% of adults aged 20 and over were overweight and 11% were obese in 2008.1) The physiology of obese and non-obese subjects is markedly different, and obesity is associated with changes in regional blood flow and plasma lipoprotein concentrations, as well as increased cardiac output and fat mass. Obese subjects also show increased activity of cytochrome P-450 2E1 (CYP2E1), but the effect of obesity on the activity of other isozymes, such as CYP3A4, CYP1A2, CYP2C9, CYP2C19, and CYP2D6, remains controversial.2) Thus, the various physiological changes associated with obesity may lead to altered pharmacokinetics of many drugs. However, it is often difficult to find obese subjects willing to participate in clinical trials during drug development, so information concerning the effect of obesity on drug pharmacokinetics is quite limited.3)

Tacrolimus, a calcineurin inhibitor, is widely used as an immunosuppressive drug to prevent rejection after transplantation. However, it shows large pharmacokinetic variability among individuals and has a narrow therapeutic index, so monitoring of blood concentrations of the drug is important for dosage regimen design and optimization of therapy.4,5) Tacrolimus is oxidatively metabolized by CYP3A and is also a substrate of
the drug efflux pump P-glycoprotein (P-gp) encoded by the MDR1 (ABCB1) gene, so there is potential for drug-drug interactions. We have reported that high-dose steroid therapy decreased blood tacrolimus concentration owing to induction of P-gp and CYP3A in the liver and small intestine. Intestinal P-gp expression is also closely related to variation in the pharmacokinetics of tacrolimus during immunosuppressant therapy after living-donor small bowel or liver transplantation. In obese humans and rodents, the mRNA or protein expression levels of cytochrome P450 (CYP) and various transporters are altered in a variety of tissues, including liver and small intestine. Therefore, there is good reason to think that the pharmacokinetics of tacrolimus would be altered in obese subjects. It is important to understand these changes in order to administer the drug safely and effectively to obese patients.

In this study, we first examined retrospectively the pharmacokinetics of tacrolimus in outpatients in our hospital and evaluated the relation between trough concentration and obesity. The results indicated that the blood concentration of tacrolimus is increased in obese patients. In order to understand the mechanisms of this obesity-related increase, we then examined changes in expression of drug-metabolizing enzymes and transporters, focusing on Cyp3a2 in liver and P-glycoprotein in small intestine, in Lepr<sup>Δ/Δ</sup>/Lepr<sup>Δ/Δ</sup> Zucker rats, which are a rat model of obesity due to genetic leptin receptor deficiency.
Methods

Retrospective study in humans

We examined the records of patients treated at Kanazawa University Hospital (Kanazawa, Japan) during the period 2009-2011.

We identified outpatients who had received tacrolimus therapy and whose blood tacrolimus concentration had been measured at our institution. We excluded patients who had received steroid pulse therapy or drugs that could have influenced the pharmacokinetics of tacrolimus, and those who had suffered serious liver damage or were less than 15 years old. The following parameters were recorded: age, sex, body weight, body height, dose of tacrolimus, steady-state trough concentration of tacrolimus, aspartate aminotransferase (AST) activity, and alanine aminotransferase (ALT) activity. The body mass index (BMI) was calculated for each patient as body mass (kg) divided by the square of height (m). To examine the influence of physical size, we compared the steady-state trough concentration of tacrolimus (ng/mL) standardized by dose (mg) (C/D ratio) in lean (BMI: <18.5), normal (BMI: ≥18.5 and <25), and overweight or obese (BMI: ≥25) patients.

This study was approved by the ethics committee of Kanazawa University Hospital (No. 1135).

Chemicals

Tacrolimus injection solution (Prograf injection, 5 mg/mL) was
purchased from Astellas Pharma, Inc. (Tokyo, Japan). Pentobarbital and 
diethyl ether were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All 
other chemicals were of analytical or high-performance liquid 
chromatography (HPLC) grade.

Animals

Nine-week-old male lean (+/+) (220-230 g) and obese (Lepr<sup>fa</sup>/Lepr<sup>fa</sup>) 
Zucker rats (330-340 g) were purchased from Japan SLC, Inc. (Hamamatsu, 
Japan). Rats were housed under a 12-hour light, 12-hour dark cycle. 
Beginning at 9 weeks of age, rats were fed normal diet for up to 18 weeks, 
and their body weight was monitored. AST activity, ALT activity, 
triglyceride, and total cholesterol were measured by Oriental Yeast Co., Ltd. 
(Tokyo, Japan). All animal procedures were carried out in accordance with 
the standards set forth in the Guidelines for the Care and Use of Laboratory 
Animals at the Takara-machi campus of Kanazawa University.

Tissue preparation

After collection of blood samples, animals were killed humanely, and the 
liver, kidney, fat and small intestine were removed. The small intestine was 
divided equally into three parts, the upper, middle and lower parts, in 
descending order. Portions of liver and small intestine were stored at -80°C 
until use.

Pharmacokinetic studies of tacrolimus
Rats (18 weeks of age) were fasted for 12 h prior to tacrolimus administration, but water was given freely. The rats were anesthetized with pentobarbital (30 mg/kg), and tacrolimus injection solution (1 mg/kg) in saline was intravenously administered from the jugular vein. Blood samples (250 µL each) were collected from the opposite jugular vein at designated time intervals. Similarly, tacrolimus injection solution (3.2 mg/kg) in saline was orally administered via a gastric tube.

Measurement of blood concentration of tacrolimus

Blood concentration of tacrolimus was measured quantitatively by using the tacrolimus kit of the Dimension® clinical chemistry system (Siemens Japan K.K., Tokyo, Japan) with Dimension® Xpand plus-HM (Siemens Japan K.K.). This method was validated between 1.2 ng/mL and 30.0 ng/mL. The cross-reactivities with metabolites of tacrolimus were 15% for M-1, and less than 5% for other metabolites. The samples were measured after dilution when blood tacrolimus concentration was above 30.0 ng/mL.

Pharmacokinetic analysis

Pharmacokinetic parameters (AUC₀-∞, MRT and t₁/₂) were estimated by model-independent moment analysis using Napp version 2.01 software. The terminal log-linear part of each concentration-time curve was identified, and the elimination rate constant (kₑ) was determined from the common log-transformed data using linear regression analysis of at least three data points from the terminal portion of the blood concentration-time plots. The
area under the concentration-time curve of tacrolimus from time 0 to infinity after intravenous or oral administration (AUC$_{0-\infty}$) was calculated using the log-linear trapezoidal rule: the concentration from the last measured point to infinity was incorporated by using a correction term. The total body clearance (CL$_{\text{tot}}$) after intravenous administration and oral clearance (CL$_{\text{tot}}$/F) after oral administration was calculated as Dose/AUC, where Dose represents the dose administered. The volume of distribution at the steady state (Vd$_{\text{ss}}$) was calculated by multiplying CL$_{\text{tot}}$ by MRT. Bioavailability (F) of tacrolimus was calculated as the dose-normalized AUC after oral administration divided by the mean dose-normalized AUC after intravenous administration.

Preparation of intestinal crude membrane fraction and hepatic microsomes

Intestinal crude membrane fraction was prepared as described previously,$^{20}$ according to the method of McCullagh et al. with slight modifications.$^{21}$ In brief, a small portion of mucosa of the small intestine was suspended in a buffer consisting of 210 mM sucrose, 2 mM EGTA, 40 mM NaCl, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 30 mM HEPES, pH 7.4, and homogenized in a Polytron-type homogenizer. Then, 2 mL of homogenate was mixed with 1.5 mL of 1.17 M KCl solution containing 58.3 mM tetrasodium pyrophosphate, kept on ice for 15 min, and centrifuged at 230,000 g for 75 min. The resultant pellet was suspended in a buffer consisting of 10 mM Tris-HCl, pH 7.4, and 1 mM EDTA and centrifuged again at 230,000 g. The obtained pellet was resuspended in the
same buffer and ultrasonically dispersed. Then 4% SDS was added. A clear supernatant was obtained after centrifugation at 15,000 \( g \).

Hepatic microsomes were prepared by differential centrifugation. In brief, the liver was homogenized in 0.1 M KCl, 0.1 M Tris-HCl and 1 mM EDTA (pH 7.4), and the homogenate was centrifuged at 9,000 \( g \) for 15 min. The supernatant was subjected to two additional centrifugation steps at 100,500 \( g \) for 60 min and suspended in TGE buffer containing 20% glycerol, 10 mM Tris-HCl and 1 mM EDTA (pH 7.4). The microsomal fraction was stored at -80°C until needed.

**Western blot analysis**

Protein concentration was determined by means of BCA assay (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer’s protocol. Proteins (20 μg/lane) were separated by SDS-PAGE (7.5% polyacrylamide gel), and transferred onto a polyvinylidene difluoride membrane (Millipore Corporation, Billerica, MA) at 60 V for 240 min. Ponceau S staining confirmed equal efficiency of transfer to the membrane (data not shown). For detection of rat P-gp, Cyp3a2 and β-actin proteins, the membrane was incubated in phosphate-buffered saline containing 0.1% Tween 20 (PBS-T) and 5% skim milk for blocking, and then incubated overnight at 4°C with primary antibody in PBS-T. The membranes were washed three times with PBS-T, and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Bands were visualized by using the enhanced chemiluminescence detection method with 20x LumiGLO®.
Reagent and 20x Peroxide (Cell Signaling Technology, MA, USA). Experiments were repeated at least two times and 3 or 4 independent lanes of blot were quantitated by densitometry using a LAS·4000 (Fujifilm, Tokyo, Japan). The primary antibodies used were mouse C219 monoclonal antibody (COVANCE, NJ, USA), rabbit anti-CYP3A2 antibody (Enzo Life Sciences, NY, USA) and anti-β-actin antibody (Cell Signaling Technology). The secondary antibodies were horseradish peroxidase-conjugated anti-mouse or anti-rabbit antibodies (Cell Signaling Technology). In the case of small intestinal samples, Western blot analysis was performed for each segment.

Statistical analysis

Statistical analysis of the data was performed with Microsoft Excel 2010 for Windows and GraphPad Prism 5J (GraphPad Software Inc., San Diego, CA, USA). The unpaired Student’s $t$-test was used for comparison between two independent groups. For multiple comparisons, one-way analysis of variance (ANOVA) with Dunnett’s post hoc test was performed for each group. The number of determinations is noted in each table and figure. $P$ values of less than 0.05 were considered significant in all analyses.
Results

Blood concentration of tacrolimus in humans

Among outpatients who received tacrolimus therapy in our hospital, 26 (5/21, male/female) were in the lean group, 92 (30/62) were in the normal group, and 17 (3/14) were in the overweight or obese group. Age distribution was not different among the three groups. Average numbers of body weight were 44.2 ± 5.4 kg in the lean group, 55.2 ± 9.2 kg in the normal group, and 70.3 ± 10.5 kg in the overweight or obese group, and were significantly different between the normal and overweight or obese group (p < 0.05), respectively. We examined the relation between the BMI and C/D ratio of tacrolimus. Average doses of tacrolimus, that was 3.8 ± 2.5 mg/day in the lean group, 3.3 ± 2.2 mg/day in the normal group, and 2.2 ± 0.5 mg/day in the overweight or obese group, and that in the overweight or obese group was significantly lower compared with the normal group (p < 0.05). Trough concentration of tacrolimus was 4.8 ± 2.5 ng/mL in the lean group (n = 141), 4.8 ± 2.4 ng/mL in the normal group (n = 764), and 4.3 ± 1.7 ng/mL in the overweight or obese group (n = 111), and was not significantly different among the three groups. The C/D ratios of tacrolimus were also not significantly different among three groups (Table 1).

Biochemical parameters in rats

Phenotypic and biochemical parameters of lean and obese Zucker rats are shown in Table 2. At 18 weeks of age, the body, liver and epididymal fat
weights of obese rats were significantly higher than those of lean rats \((p < 0.05)\). No difference was observed in serum ALT and AST between lean and obese rats. However, serum total cholesterol and triglyceride concentrations were significantly higher in obese rats than in lean rats \((p < 0.05)\).

Pharmacokinetics of tacrolimus in rats

The blood concentration-time profiles of tacrolimus after intravenous bolus administration of tacrolimus (1 mg/kg) in rats are shown in Figure 1. The blood concentrations of tacrolimus were significantly increased at several time points in obese rats compared with lean rats. The blood concentration-time profiles of tacrolimus after oral administration of tacrolimus (3.2 mg/kg) in rats are shown in Figure 2. The blood concentrations of tacrolimus were higher in obese rats than in lean rats up to 24 hr. The pharmacokinetic parameters of tacrolimus are shown in Table 3. After intravenous administration, the AUC value was significantly higher in obese rats than in lean rats \((p < 0.05)\). The \(\text{CL}_{\text{tot}}\) and \(\text{Vd}_{\text{ss}}\) values were not significantly different between two groups, although the \(\text{Vd}_{\text{ss}}\) per kg body weight of rats was significantly lower in obese rats than that in lean rats \((p < 0.05)\). The MRT and \(t_{1/2}\) values showed no difference between the lean and obese groups. The AUC value was also significantly higher in obese rats than in lean rats after oral administration, and the \(\text{CL}_{\text{tot}}/\text{F}\) value in obese rats showed decreasing trend than that in lean rats \((p = 0.05)\). The bioavailability in obese rats was increased over 2-fold, compared to that in lean rats.
Protein expression levels of Cyp3a2 and P-gp in liver and small intestine

Western blot analysis was performed to assess the effect of obesity on the protein expression levels of Cyp3a2 and P-gp in liver and small intestine. Specific bands were detected at around 54 kDa for Cyp3a2 and at around 170 kDa for P-gp. The protein expression of Cyp3a2 was significantly decreased in the liver of obese rats compared with lean rats ($p < 0.05$), but the expression of P-gp in the liver was not changed (Figure 3). On the other hand, the protein expression of Cyp3a2 in the small intestine was not changed between obese and lean rats, while the protein expression of P-gp was significantly decreased at the upper and middle sites of the small intestine in obese rats compared with lean rats ($p < 0.05$) (Figure 4).
Discussion

Obesity changes the expression levels of drug-metabolizing enzymes and drug transporters.\textsuperscript{12-16} However, there is relatively little information about how the pharmacokinetic and pharmacodynamic characteristics of drugs change in obese patients. Immunosuppressant therapy is indispensable for organ transplantation, and precise control of the blood concentration is necessary to prevent rejection. It has been suggested that the disposition kinetics of cyclosporine A is changed in obesity; for example, the distribution volume of cyclosporine A was decreased in obese subjects\textsuperscript{22} and the bioavailability was decreased in obese rats fed a high-fat diet.\textsuperscript{23} In clinical practice, tacrolimus plays as important a role as cyclosporine A in preventing rejection of transplanted organs. It was recently reported that overweight renal transplant recipients were prone to develop high trough levels of tacrolimus (> 15 ng/mL).\textsuperscript{24}

Trough concentration of tacrolimus at the steady-state was not significantly different among the three groups. Although that in the overweight or obese group was slightly lower compared with the other group, the blood tacrolimus concentrations were controlled nicely by the therapeutic drug monitoring and appropriate dose adjustment. Actual dose of tacrolimus was significantly lower to achieve therapeutic window in the overweight or obese group compared with the normal group. Although the C/D ratio of tacrolimus in the overweight or obese group was slightly higher compared with the other groups, there was no significant difference among
three groups. It may be ascribed to the fact that the trough concentration of tacrolimus in the overweight or obese group was lower compared with other groups.

Therefore, we next examined whether the pharmacokinetic change of tacrolimus in humans could be reproduced in rodents. When tacrolimus injection solution was administered either orally or intravenously to lean and obese Zucker rats, the blood concentration of tacrolimus was significantly increased in the obese group, compared with the lean group (Figures 1, 2). The $\text{CL}_{\text{tot}}$ and $\text{Vd}_{\text{ss}}$ were not significantly different between the obese group and the lean group (Table 3), although the $\text{Vd}_{\text{ss}}$ per kg body weight of rats was significantly lower in obese rats than that in lean rats ($p < 0.05$). In healthy subjects, 98.8% or more of tacrolimus is bound to plasma proteins such as lipoproteins, globulins, $\alpha_1$-acid glycoprotein and albumin.$^{30,31}$ In the present study, we found that the serum concentrations of total cholesterol and triglycerides were significantly higher in obese rats than in lean rats (Table 2), confirming the finding of high levels of serum lipoproteins such as HDL and LDL in obese Zucker rats.$^{32}$ Therefore, the reduction of $\text{Vd}_{\text{ss}}$ per body weight in obese Zucker rats may be a consequence of the decrease in the unbound fraction of tacrolimus due to increased lipoprotein concentration.

In human blood, about 85% of tacrolimus is bound to erythrocytes.$^{30,31,33}$ Zahir et al have reported that hematocrit and red blood cell count significantly influenced the percentage of tacrolimus associated with erythrocytes and the fraction unbound of tacrolimus was correlated with
α1-acid glycoprotein and high density lipoprotein cholesterol concentrations.\textsuperscript{33} There are several reports suggesting that erythrocyte counts are increased in obese patients compared with non-obese patients,\textsuperscript{34-36} although no clear effect of obesity on hematocrit has been reported. Moreover, laboratory data provided by the breeder of Zucker rats suggested no significant difference in erythrocyte counts or hematocrit between lean and obese Zucker rats. Effects of obesity on the distribution of tacrolimus into blood cells may affect blood pharmacokinetics of tacrolimus. Further study is needed to address this issue.

It is well known that tacrolimus is a substrate of both P-gp and CYP3A.\textsuperscript{6-8} In rats, tacrolimus is mainly metabolized by Cyp3a2,\textsuperscript{7} the counterpart of human CYP3A4. So, we compared the protein expression levels of Cyp3a2 and P-gp in liver and small intestine of lean and obese Zucker rats. The protein expression of Cyp3a2 in the liver of obese Zucker rats at 20 weeks of age was significantly decreased compared with that of lean rats (Figure 3). This is consistent with previous reports that CYP3A-associated testosterone 6β-hydroxylase activity was decreased in obese male Zucker rats\textsuperscript{37} and the expression of CYP3A was reduced at both the mRNA and protein levels in obese mice fed a high-fat diet.\textsuperscript{13} However, the CL\textsubscript{tot} of tacrolimus was not significantly different between lean and obese rats (Table 3). It may be ascribed to that the Cyp3a2 activity per whole liver was not changed between two groups, because the liver was enlarged in the obese rats.

On the other hand, it has been reported that tacrolimus is absorbed
rapidly and predominantly from the upper site of the small intestine.\textsuperscript{38,39} We found that protein expression of P-gp in the upper and middle sites of the small intestine was significantly decreased in obese Zucker rats compared with lean Zucker rats, whereas protein expression of Cyp3a2 showed no difference between the two groups (Figure 4). In accordance with this result, low protein expression of P-gp has been reported in small intestine of obese rats.\textsuperscript{40} We have reported that the bioavailability of tacrolimus showed a 3-fold increase in mdr1a knockout mice compared with that in normal mice.\textsuperscript{41} Therefore, we consider that the high bioavailability of tacrolimus in obese rats is a result of increased intestinal absorption of the drug because of the decreased protein expression of the efflux pump, P-gp.

Down-regulation of P-gp expression in small intestine of obese Zucker rats is likely to be associated with inflammation in these rats. It was reported that expression of the proinflammatory molecules such as tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), interleukin-6 (IL-6) and inducible nitric oxide synthase (iNos) in adipose tissue is increased in obese patients compared with non-obese patients.\textsuperscript{42-45} Release of these proteins from visceral fat may induce an inflammatory state in the small intestine. In fact, several reports have suggested that intestinal inflammation is linked to obesity in humans and diet-induced obese rodents.\textsuperscript{46-49} P-gp expression is decreased in inflamed intestinal epithelium of patients with gastrointestinal disorders, such as Crohn's disease and ulcerative colitis.\textsuperscript{50} Also, iNos, a mediator of inflammation, in the ileum is involved in the decreasing of ileal P-gp.

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expression in streptozotocin-induced diabetic mice.\textsuperscript{51} Further, TNF-\(\alpha\) decreased the activity of P-gp in Caco-2 cell lines.\textsuperscript{52} From these evidence, inflammatory proteins in obesity may down-regulate the expression of P-gp in the small intestine. However, there has been few report on change of P-gp expression in obesity, except that Ulvestad et al. have reported no significant correlation between body mass index and protein expression of P-gp in humans.\textsuperscript{16} As the C/D ratio of tacrolimus in the overweight or obese patients was not significantly different from that in the normal patients, P-gp expression in obese patients may not so greatly down-regulated to be affected as with that observed in obese rats.

In conclusion, our results suggested that the steady-state trough concentration of tacrolimus in obese patients were well maintained by relatively low dose compared with that in normal and lean patients, presumably due to increased bioavailability. Therefore, in clinical practice, careful therapeutic drug monitoring and dosage design may be especially important in obese patients.

Acknowledgement

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References


27) Li, D., Lu, W., Zhu, J.Y., Gao, J., Lou, Y.Q. and Zhang, G.L.: Population pharmacokinetics of tacrolimus and CYP3A5, MDR1 and


40) Sugioka, N., Haraya, K., Fukushima, K., Ito, Y. and Takada, K.: Effects of obesity induced by high-fat diet on the pharmacokinetics of


Legends

Figure 1
Blood concentration-time profiles of tacrolimus after intravenous administration of tacrolimus (1 mg/kg) to lean (open circles) and obese (closed circles) rats.
Values are expressed as means ± S.E. (n = 6).
* Significant differences from the lean group at p < 0.05; Student’s unpaired t-test.

Figure 2
Blood concentration-time profiles of tacrolimus after oral administration of tacrolimus (3.2 mg/kg) to lean (open circles) and obese (closed circles) rats.
Values are expressed as means ± S.E. (n = 5).
* Significant differences from the lean group at p < 0.05; Student’s unpaired t-test.

Figure 3
Western blot analysis of protein expression of P-glycoprotein and Cyp3a2 in the liver of lean and obese rats.
Values are expressed as means ± S.E. (n = 6).
* Significant differences from the lean group at p < 0.05; Student’s unpaired t-test.
Figure 4

Western blot analysis of P-glycoprotein and Cyp3a2 in the upper, middle and lower sites of the small intestine of lean and obese rats.

Values are expressed as means ± S.E. (n = 5).

* Significant differences from the lean group at $p < 0.05$; Student’s unpaired $t$-test.
Table 1

*Characteristics and steady-state trough concentration/dose ratio of tacrolimus in humans*

<table>
<thead>
<tr>
<th>BMI (kg/m²)</th>
<th>&lt; 18.5</th>
<th>≥ 18.5, &lt; 25</th>
<th>≥ 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>26</td>
<td>92</td>
<td>17</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>5/21</td>
<td>30/62</td>
<td>3/14</td>
</tr>
<tr>
<td>Age (years)</td>
<td>51 ± 16</td>
<td>50 ± 17</td>
<td>50 ± 17</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>44.2 ± 5.4 *</td>
<td>55.2 ± 9.2</td>
<td>70.3 ± 10.5 *</td>
</tr>
<tr>
<td>Total number of measurements</td>
<td>141</td>
<td>764</td>
<td>111</td>
</tr>
<tr>
<td>Trough concentration of tacrolimus (ng/mL)</td>
<td>4.8 ± 2.5</td>
<td>4.8 ± 2.4</td>
<td>4.3 ± 1.7</td>
</tr>
<tr>
<td>Dose of tacrolimus (mg/day)</td>
<td>3.8 ± 2.5</td>
<td>3.3 ± 2.2</td>
<td>2.2 ± 0.5 *</td>
</tr>
<tr>
<td>Tacrolimus C/D ratio ([ng/mL]/[mg/day])</td>
<td>1.88 ± 1.67</td>
<td>1.87 ± 1.27</td>
<td>2.05 ± 0.86</td>
</tr>
</tbody>
</table>
Values are expressed as means ± S.D.

* Significant differences from BMI ≥18.5, BMI <25 group at $p < 0.05$; one-way ANOVA with Dunnett's post hoc test.
Table 2

*Phenotypic and biochemical parameters of Zucker rats*

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>368.7 ± 24.1</td>
<td>598.1 ± 27.2 *</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>9.4 ± 1.2</td>
<td>17.4 ± 2.2 *</td>
</tr>
<tr>
<td>Epididymal fat weight (g)</td>
<td>3.4 ± 1.1</td>
<td>15.5 ± 1.0 *</td>
</tr>
<tr>
<td>ALT (IU/mL)</td>
<td>72.0 ± 15.2</td>
<td>93.7 ± 23.6</td>
</tr>
<tr>
<td>AST (IU/mL)</td>
<td>167.2 ± 97.3</td>
<td>169.0 ± 108.2</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>77.7 ± 4.5</td>
<td>126.3 ± 11.2 *</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>39.8 ± 15.7</td>
<td>322.5 ± 88.8 *</td>
</tr>
</tbody>
</table>

Data are means ± S.D. and were obtained from 18-week-old, fasted, lean and obese Zucker rats (n = 6-9).

* Significant differences from the lean group at p < 0.05; Student’s unpaired t-test.
Table 3

*Pharmacokinetic parameters of tacrolimus after intravenous and oral administration to lean and obese Zucker rats*

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>i.v.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0→∞&lt;/sub&gt; (ng hr/mL)</td>
<td>888 ± 50</td>
<td>1381 ± 90 *</td>
</tr>
<tr>
<td>CL&lt;sub&gt;tot&lt;/sub&gt; (mL/min)</td>
<td>6.80 ± 0.37</td>
<td>7.57 ± 0.56</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>5.49 ± 0.21</td>
<td>5.77 ± 0.28</td>
</tr>
<tr>
<td>V&lt;sub&gt;dss&lt;/sub&gt; (L)</td>
<td>2.24 ± 0.15</td>
<td>2.61 ± 0.23</td>
</tr>
<tr>
<td>V&lt;sub&gt;dss&lt;/sub&gt; (L/kg)</td>
<td>6.29 ± 0.44</td>
<td>4.26 ± 0.33 *</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (hr)</td>
<td>4.35 ± 0.43</td>
<td>5.39 ± 0.24</td>
</tr>
<tr>
<td><strong>p.o.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0→∞&lt;/sub&gt; (ng hr/mL)</td>
<td>257 ± 107</td>
<td>800 ± 119 *</td>
</tr>
<tr>
<td>CL&lt;sub&gt;tot/F&lt;/sub&gt; (mL/min)</td>
<td>125 ± 31</td>
<td>42 ± 4</td>
</tr>
<tr>
<td>F (%)</td>
<td>8.30 ± 3.49</td>
<td>18.60 ± 2.53 *</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E. (n = 5-6). Lean (368.7 ± 24.1 g) and obese (598.1 ± 27.2 g) Zucker rats were administered tacrolimus
intravenously (1 mg/kg) and orally (3.2 mg/kg). Blood concentration of tacrolimus was analyzed as described in the Material and Methods.

* Significant differences from the lean group at $p < 0.05$; Student’s unpaired $t$-test.
Figure 1

Blood concentration of tacrolimus (ng/mL) vs. Time (hr)
Figure 2

Blood concentration of tacrolimus (ng/mL) vs. Time (hr)
Figure 4

A. Upper

- **P-gp**
- **Cyp3a2**
- **β-actin**

B. Middle

- **P-gp**
- **Cyp3a2**
- **β-actin**

C. Lower

- **P-gp**
- **Cyp3a2**
- **β-actin**

Bar graphs showing the expression levels of P-gp, Cyp3a2, and β-actin in lean and obese groups for A, B, and C regions. The graphs indicate a significant difference in expression levels between lean and obese groups, marked by an asterisk (*) and a p-value of 0.08 for the C region.