False Positive Blood Tacrolimus Concentration in Microparticle Enzyme Immunoassay

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The difference in the blood concentration of tacrolimus between the assay methods, microparticle enzyme immunoassay (MEIA) and enzyme linked immunosorbent assay (ELISA) was observed in a liver transplant recipient with anemia. MEIA provided significantly higher concentration than those of ELISA (7.8 ± 1.9 vs. 5.0 ± 1.8 ng/ml, $p < 0.05$) while the patient had low haematocrit $< 25\%$. The difference, however, was not observed during the periods with haematocrit $> 25\%$. This observation suggested that unknown tacrolimus levels generated from difference in assay methods gave incorrect blood tacrolimus during anemia. False positive concentration of tacrolimus ranging 0.1–3.3 ng/ml was observed in MEIA applying to the blood samples obtained from the patients without receiving tacrolimus. The false positive tacrolimus increased in the samples with lower haematocrit, suggesting that MEIA gave incorrect blood tacrolimus during anemia. Since MEIA potentially overestimates the tacrolimus levels, ELISA should be used for blood tacrolimus monitoring in the patients with anemia.

Key words false positive; tacrolimus; microparticle enzyme immunoassay; anemia

Tacrolimus, an immunosuppressive agent, requires blood concentration monitoring to achieve appropriate dosage regimen in management of the patients with organ transplantation and autoimmune diseases.^{1,2} Two enzyme immunoassays, enzyme linked immunosorbent assay (ELISA) and microparticle enzyme immunoassay (MEIA), have been used for routine determination of whole blood tacrolimus.^{3,4} The characteristics of monoclonal anti-tacrolimus antibodies used in these two immunoassays are almost same and there is no difference in the cross reactivities to tacrolimus metabolites.⁵

We currently observed a difference in the determination of tacrolimus when MEIA (MEIA-II Tacrolimus, IMX; Abbott, Chicago, IL, U.S.A.) and ELISA (PRO-Trac II, DiaSorin, Stillwater, MN, U.S.A.) were simultaneously applied to blood concentration monitoring for tacrolimus in a liver transplant recipient with anemia. As shown in Table 1, there was no difference in tacrolimus levels between MEIA and ELISA in the period of haematocrit values $> 25\%$. However, the tacrolimus levels determined by MEIA were significantly higher than those of ELISA ($p < 0.05$) in the period of haematocrit values $< 25\%$ (Table 1). Difference in the tacrolimus levels between MEIA and ELISA was also enlarged in this period ($p < 0.01$, Table 1). When the haematocrit value decreased by 17%, difference in the concentration determined by MEIA and ELISA was almost double (6.9 ng/ml in MEIA vs. 3.2 ng/ml in ELISA). Since liver and kidney functions

and medications did not differ between both periods, the enlarged difference between MEIA and ELISA during the low haematocrit was considered to be incorrect tacrolimus concentration generated from MEIA. The tacrolimus was given to maintain the blood concentration of 5.0–7.0 ng/ml determined by MEIA in this case. The graft did not work well and the patient received second liver transplantation. Since we used MEIA for routine tacrolimus monitoring, it was considered that the immunosuppression of this case might be insufficient due to the sub-therapeutic levels of tacrolimus.

To confirm the difference in determining tacrolimus between MEIA and ELISA, we applied both assays to whole blood samples obtained from patients who were not treated with tacrolimus. A total of 191 samples collected from 69 patients (18 kidney transplant, 15 nephrosis, 6 aplastic anemia, 5 bone marrow transplant, 4 chronic articular rheumatism, 3 pneumonitis, 2 liver transplant, 2 systemic lupus erythematosus, and 14 others) were tested. MEIA determined tacrolimus at the concentration of 0.1–3.3 ng/ml in 93 samples (0.1–0.5 ng/ml for 54 samples and 0.6–3.3 ng/ml for 39 samples). All samples showed under the detection limit (0.5 ng/ml) when the ELISA was applied to same blood samples.

We compared the characteristics of the samples between negative ($n=98$) and false positive-tacrolimus (> 0.5 ng/ml, $n=39$) in MEIA. A significant difference was observed in the haematocrit value. False positive samples had a lower haematocrit ($39.5 \pm 5.7\%$ vs. $26.9 \pm 9.4\%$, $p < 0.001$). No difference was observed in other biochemical data and patients' gender, age, and medication between negative and positive samples.

As shown in Fig. 1, false positive tacrolimus increased in the samples obtained from the patients of aplastic anemia

Table 1. Difference in the Tacrolimus Concentration between the Samples with Low and High Hematocrit Values in a Liver Transplant Recipient

	Ht (%)	Tacrolimus concn. (ng/ml)		False positive concn. (ng/ml)
		MEIA	ELISA	MEIA–ELISA
Ht $< 25\%$ ($n=10$)	$21.4 \pm 2.8^*$	7.8 ± 1.9	$5.0 \pm 1.8^{\#}$	$2.8 \pm 0.7^*$
Ht $> 25\%$ ($n=11$)	30.7 ± 3.3	7.9 ± 3.4	6.1 ± 3.3	1.7 ± 0.7

Significant difference was observed in comparison with Ht $> 25\%$; $*p < 0.01$. Significant difference was observed in comparison with MEIA; $\#p < 0.05$.

Fig. 1. Inverse Correlation between Hematocrit Values and False Positive Tacrolimus Concentration

The samples were collected from the patients of aplastic anemia ($\blacktriangle$), bone marrow transplantation ($\times$) and other diseases ($\bullet$).

and bone marrow transplantation. Inverse correlation was found between hematocrit and false positive concentrations ($r = -0.72$, $p < 0.05$). We further examined the effects of hematocrit values on false positive tacrolimus in diluted blood samples with plasma. False positive concentrations were dramatically increased by the dilution (data not shown). Plasma samples showed false positive concentration of 4.4—5.5 ng/ml in MEIA.

DISCUSSION

We observed the incorrect blood concentration of tacrolimus in the blood level monitoring for the liver transplant recipient with anemia. Incorrect concentration was considered due to false positive tacrolimus in MEIA. The mechanism for the determination of false positive concentration in MEIA is unclear. However, it is clear that the false positive tacrolimus was not caused by the cross reactivities of the monoclonal antibody with tacrolimus metabolites and other medications contained in the blood samples, because no one received tacrolimus and the medication for the patients did not differ between negative and positive samples.

The difference in the procedures between MEIA and ELISA is to use micro particle beads. We hypothesized that the false positive tacrolimus was generated from the reaction between deproteinized blood and tacrolimus antibodies on the micro particle beads used in MEIA. Neither MEIA manufacturer's instructions nor cited medical literatures provide the information about the reaction in detail.

There were several reports that the MEIA consistently determined higher tacrolimus concentration than the ELISA.⁶⁾ It has been considered that one reason explaining this observation was difference in the cross reactivities of anti-tacrolimus antibody against to endogenous compounds or drugs concomitantly administered. Present results suggest

that the false positive tacrolimus has also associated with the overestimation for blood tacrolimus concentrations in MEIA.

Current immunosuppressive therapy for organ transplantation is to use low dose tacrolimus in special cases, pregnancy⁷⁾ and human immunodeficiency virus (HIV)-positive patients,⁸⁾ and in the weaning process of immunosuppressive drugs.⁹⁾ The blood tacrolimus for these patients are maintained 4—8 ng/ml. In such therapeutic drug monitoring, the false positive tacrolimus in MEIA could not be neglected when the blood concentrations are evaluated for making dosage regimen. False positive concentrations potentially provide the incorrect blood concentrations, which lead to underestimate dose for tacrolimus. We, therefore, emphasize that ELISA should be used instead of MEIA for the monitoring of blood tacrolimus in the patients treated with low dose tacrolimus, especially with anemia.¹⁰⁾

REFERENCES

- 1) Wallemacq P. E., Reding R., *Clin. Chem.*, **39**, 2219—2228 (1993).
- 2) Van Thiel D. H., Wright H., Carroll P., Abu-Elmagd K., Rodriguez-Rilo H., McMichael J., Irish W., Starzl T. E., *Am. J. Gastroenterol.*, **90**, 771—776 (1995).
- 3) Lee J. W., Sukovaty R. L., Farmen R. H., Dressler D. E., Alak A., Bekersky I., *Ther. Drug Monit.*, **19**, 201—207 (1997).
- 4) Garg U. C., Austin G., Barnes C., Hamilton M., *Clin. Chem.*, **44**, 1783—1785 (1998).
- 5) Alak A. M., *Ther. Drug Monit.*, **19**, 338—351 (1997).
- 6) Cao Z., Linder M. W., Jevans A. W., Brown G., Valdes R., *Clin. Chem.*, **45**, 1868—1870 (1999).
- 7) Wu A., Nashan B., Messner U., Schmidt H. H., Guenther H. H., Niesert S., Pichlmayr R., *Clin. Transplant.*, **12**, 454—464 (1998).
- 8) Sheikh A. M., Wolf D. C., Lebovics E., Goldberg R., Horowitz H. W., *Transplantation*, **68**, 307—309 (1999).
- 9) Takatsuki M., Umemoto S., Inomata Y., Egawa H., Kiuchi T., Fujita S., Hayashi M., Kanematsu T., Tanaka K., *Transplantation*, **72**, 449—454 (2001).
- 10) Mathias R. S., *Pediatr. Nephrol.*, **11**, 355—357 (1997).