Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.0071-17
Intern Med 57: 1265-1268, 2018
http://internmed.jp

【 CASE REPORT 】

False Elevation of the Blood Tacrolimus Concentration, as Assessed by an Affinity Column-mediated Immunoassay (ACMIA), Led to Acute T Cell-mediated Rejection after Kidney Transplantation

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Abstract:

Tacrolimus is the most commonly used immunosuppressant. Because of its narrow therapeutic range, it is necessary to frequently monitor its concentration. We report the case of a 25-year-old man who underwent kidney transplantation whose tacrolimus concentrations, as measured by an affinity column-mediated immunoassay, were falsely elevated. As we reduced the dose of tacrolimus, the recipient developed T cell-mediated rejection. Using the same blood samples, an enzyme-multiplied immunoassay technique showed that the patient's levels of tacrolimus were extremely low. A further examination indicated that the false increase in the tacrolimus concentration was likely due to an unknown interfering substance. We administered methylprednisolone and antithymocyte-globulin. The patient's serum creatinine level decreased and remained stable after these treatments.

Key words: affinity column mediated immunoassay, ACMIA, kidney transplantation, tacrolimus, T cell-mediated rejection

(Intern Med 57: 1265-1268, 2018)
(DOI: 10.2169/internalmedicine.0071-17)

Introduction

Tacrolimus is the most frequently prescribed immunosuppressive drug for kidney transplantation. The accurate measurement of whole-blood tacrolimus concentration is essential for achieving therapeutic immunosuppression and minimizing toxicity in kidney transplant recipients. An affinity column-mediated immunoassay (ACMIA) is one of the most common methods for tacrolimus measurement and does not require the pretreatment extraction of whole blood samples, thereby reducing both the assay time and the individual immunoassay techniques that must be applied (1). However, interference has been reported to affect the accuracy of measurements by the ACMIA method, with falsely elevated tacrolimus concentrations reported in both laboratory and clinical settings (1-6).

In this report, we describe a case in which a kidney transplant patient's blood tacrolimus concentration, as measured by the ACMIA method, was falsely elevated. As a consequence, the patient developed acute rejection. Several examinations suggested that an unknown substance had caused interference in the tacrolimus assay; however, we were unable to identify the substance.

Case Report

The patient was a 25-year-old man who developed end-stage renal disease due to mesangial proliferative glomerulonephritis. He had no past history of autoimmune disease, including rheumatoid arthritis, and no history of blood transfusion. He underwent ABO-incompatible living kidney

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Received: August 15, 2017; Accepted: September 19, 2017; Advance Publication by J-STAGE: December 27, 2017
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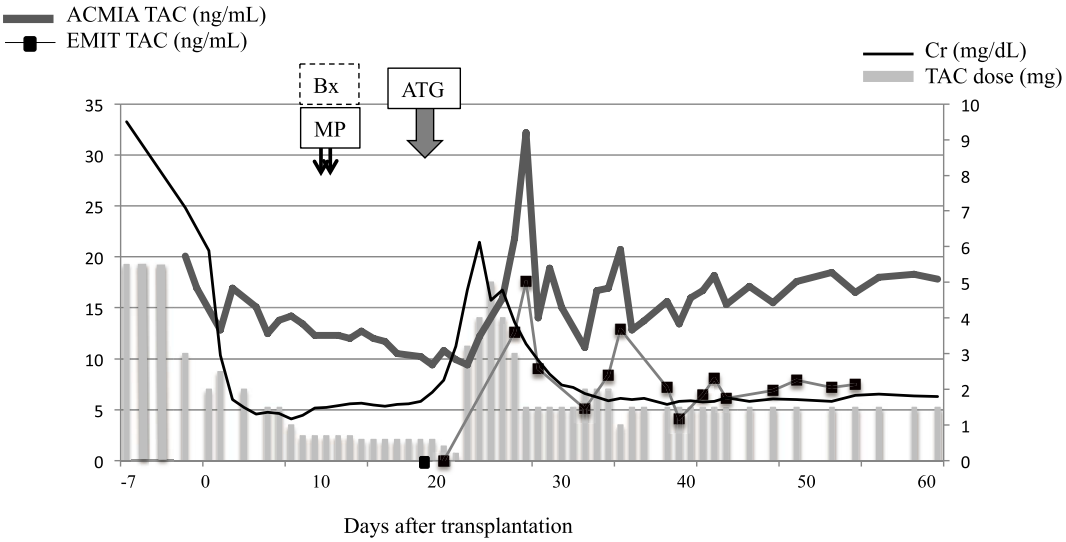

Figure. **The clinical course, tacrolimus concentration, and tacrolimus dosage. ACMIA: affinity column mediated immunoassay, ATG: anti-thymocyte globulin, Bx: biopsy, EMIT: enzyme-multiplied immunoassay technique, MP: methylprednisolone, TAC: tacrolimus**

Table. **Comparison of Tacrolimus Concentrations Assessed Using ACMIA and EMIT.**

	Sample 1 (POD 20)	Sample 2 (POD 22)
Whole blood by ACMIA (ng/mL)	8.3	7.2
Plasma by ACMIA (ng/mL)	7.4	7.7
EMIT (ng/mL)	undetectable	undetectable

ACMIA: affinity column mediated immunoassay, EMIT: enzyme-multiplied immunoassay technique, POD: postoperative day

transplantation one year after the initiation of hemodialysis therapy. The donor was his mother. The patient received induction immunosuppressive therapy, which included basiliximab and rituximab, followed by maintenance immunosuppression with tacrolimus (Graceptor®; Astellas Pharma, Tokyo, Japan), mycophenolate mofetil (CellCept®; Roche, Basel, Switzerland), and methylprednisolone (Medrol®; Pfizer, Tokyo, Japan). Tacrolimus was administered at an initial dose of 0.1 mg/kg/day. The blood concentration levels were subsequently monitored by an ACMIA with the Dimension Xpand Plus, Flex Cartilage TACR (Siemens Healthcare Diagnostics, Munich, Germany) to adjust the tacrolimus dose. The target trough levels were 8-10 ng/mL within one week, 6-8 ng/mL between two and three months, and 4-6 ng/mL at four months after transplantation. The operation was successfully performed without complications, and the patient's serum creatinine (Cr) level was stable at approximately 1.1 mg/dL. After starting tacrolimus treatment, the patient's serum trough levels of tacrolimus, as measured by ACMIA, were high (10-16 ng/mL), despite the low dosage (Figure). We attempted to adjust the dosage of tacrolimus according to the patient's high trough levels, but 12 days after transplantation, the patient's serum Cr increased from 1.2 mg/dL to 1.6 mg/dL. An allograft biopsy showed acute T cell-mediated rejection, type IIA (Banff 2013; i2, t2, v1, g0, ptc0, ci0, ct0, cv0, cg0, ptcbm0, ah0, aah0, c4d0) (7), and the patient was treated with high-dose methylprednisolone and rabbit antithymocyte globulin. We reanalyzed his blood samples by the enzyme-multiplied immunoassay technique (EMIT) using a Viva-E® system, EMIT 2000 Tacrolimus Assay (Siemens Healthcare Diagnostics). The tacrolimus concentration was under the detection limit when measured by the EMIT, while the ACMIA still showed a concentration as high as 10.8 ng/mL. The rejection was apparently caused by abnormally low tacrolimus concentrations, and it was suspected that an unknown factor had interfered with the ACMIA.

First, as tacrolimus is mainly distributed in erythrocytes and is normally not detected in the plasma (8-12), we measured the levels of tacrolimus in two samples of whole blood and plasma using the ACMIA and EMIT (Table). The ACMIA suggested that the plasma tacrolimus concentration was elevated. These results indicate that some interfering substances were present in the patient's plasma.

To determine whether the false positive results were due to the presence of heterophilic antibodies, such as human anti-mouse antibody (HAMA), we incubated the blood samples with heterophilic blocking tubes (HBT, Scantibodies Laboratory, Santee, USA) and reanalyzed them on a Dimension Xpand Plus (13). The titer of the tacrolimus would decrease after the use of HBT if interfering heterophilic antibodies were present; however, the patient's tacrolimus concentration was 7.6-7.7 ng/mL (after this pretreatment), which suggested that it was unlikely that a heterophilic antibody had interfered with the assay.

We also measured the blood level of cyclosporine A on a Dimension® RxL system (Siemens Healthcare Diagnostics) to exclude interference due to the presence of endogenous β-galactosidase or antibodies directed toward β-

galactosidase, since these can also interfere with the ACMIA (5). If anti-β-galactosidase antibodies are present in a sample, a false-positive cyclosporine A signal can occur, despite the fact that the patient never received the drug. However, no β-galactosidase activity was detected in the patient's samples, which showed that the falsely elevated tacrolimus concentration was not caused by anti-β-galactosidase antibodies (or endogenous β-galactosidase activity). Rheumatoid factors (i.e., IgG, IgM, and IgA) are also reported to cause falsely elevated tacrolimus levels (14), but they were all within the normal ranges. Although we were unable to detect the specific factor using several assays, we concluded that an unknown substance had interfered with the ACMIA.

We immediately increased the dose of tacrolimus and began monitoring the patient's tacrolimus concentration with the EMIT; thereafter, the patient's tacrolimus concentration remained stable. His serum Cr level gradually increased to 6.1 mg/dL by twenty days after transplantation. A single emergency hemodialysis treatment was required; however, his Cr subsequently decreased and remained stable at approximately 1.5 mg/dL. He was discharged from the hospital at three months after transplantation. At five years after transplantation, his kidney functions and tacrolimus concentrations were stable and a protocol biopsy showed no evidence of rejection.

Discussion

We reported a case in which a kidney transplant recipient's whole-blood tacrolimus concentrations were falsely elevated due to interference in the ACMIA. We hypothesized that this was caused by unknown interfering substances. Several reports have indicated that the tacrolimus concentrations in kidney and liver transplant recipients were falsely elevated when measured using the ACMIA (1-6). The monitoring of the blood tacrolimus concentration is important because of its narrow therapeutic and toxic ranges and its variable pharmacokinetics (8). The most accurate technique for measuring the tacrolimus concentration is liquid chromatography coupled with mass spectrometry (LC-MS). Since LC-MS is time-consuming, immunoassay-based methods (1) [*i.e.*, ACMIA, EMIT, and chemiluminescence immunoassay (CLIA)], are most frequently used in clinical settings due to their convenience. The ACMIA and EMIT are both enzyme immunoassays that utilize antigen-antibody reactions, measuring the absorbance of the resulting color reaction (15). ACMIA requires no sample pretreatment procedures; thus, the time that is required to perform the assay is significantly reduced (16). At present, the ACMIA is widely used. The EMIT, however, requires the pretreatment of samples (*i.e.*, deproteinization) and takes more time than the ACMIA; the results of EMIT are also dependent on the laboratory technician's manipulation. However, extraordinarily elevated concentrations of tacrolimus have not been reported with the EMIT, and it is thought to have sufficient precision to be used in the clinical setting (1). The CLIA is the most precise of the three immunoassays and shows good performance in measuring low concentrations of tacrolimus; it is currently replacing the ACMIA and EMIT in the clinical field (17).

Several studies have reported that falsely elevated whole-blood tacrolimus concentrations could be attributed to the presence of an endogenous antibody that interacts with the reagent. Anti- β-galactosidase antibody (4), anti-tacrolimus monoclonal antibody (3), HAMA, and rheumatoid factor (14) have been reported to cause false elevation. These antibodies are produced in response to infection, blood transfusion, biological drugs, and autoimmune disorders. Most of the cases involving tacrolimus interference are presented in sporadic case reports and the overall frequency is unclear. In our case, the patient was not positive for rheumatoid factor, HAMA, or β-galactosidase. However, the antibodies that can interfere with the reagent have not all been identified (2, 3). As tacrolimus is mainly distributed in erythrocytes and is normally not detected in the plasma, measuring the samples of whole blood, plasma and washed erythrocytes is useful for determining whether or not the titer is falsely elevated (8-12). Furthermore, area under the blood concentration-time curve monitoring before transplantation could be a clue to diagnosing this phenomenon.

The elevation of tacrolimus trough concentrations in kidney transplant recipients has major implications. The therapeutic window for tacrolimus is narrow, and high concentrations can result in adverse effects, such as renal toxicity. When a high concentration of tacrolimus is detected by an ACMIA for no apparent reason in a patient without toxic symptoms, laboratory staff and physicians should consider the possibility of interference by an antibody interacting with the reagent. Misinterpretation of the result, as we have demonstrated, can lead to acute rejection.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

We would like to express our gratitude to Siemens Healthineers Japan (Tokyo, Japan) for their experimental support and Drs. Naohiro Toda, Kei Sekizawa, Kaori Kojima, Yuka Nakamura, Takashi Chiba, Yusuke Imaizumi, Rie Ohkubo, Yuiko Kamei, Natsuho Sano, Tomoko Kawanishi, Toshie Ogawa, Yasutomo Abe, and Mariko Endo (Department of Nephrology, Tokyo Metropolitan Health and Medical Treatment Corporation Okubo Hospital, Tokyo, Japan) for their clinical support in this report.

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