13 pages, 3 figures

Letter to the Editor

Artifactual prolongation of the activated partial thromboplastin time by amikacin or gentamicin with ellagic acid, but not silica activated reagent

Short title: Prolongation of the APTT by AMK or GM

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Dear Editors:

Amikacin (AMK) is an aminoglycoside derivative antibiotic that is important for treating some types of drug-resistant bacterial infection. The clinical effectiveness of AMK is correlated with the maximum concentration (C_{max})/minimum inhibitory concentration and once-daily administration is recommended. Adverse effects of AMK include renal failure and ototoxicity, which are correlated with serum AMK concentrations. Therefore, therapeutic drug monitoring is recommended with AMK administration. We observed AMK-induced pseudo-prolongation of the activated partial thromboplastin time (APTT) suggestive of an elevated AMK concentration in an older man with bacteremia and found a proportional relationship between the APTT value and the serum AMK concentration.

The patient was a 74-year-old man who was admitted to our hospital with fever. He had history of diabetes and chronic kidney disease, and had had a urethral catheter for three years because of a neurogenic bladder due to a cerebral infarction. Blood tests revealed a white blood cell count of 16.85×10^9 cells/L (neutrophils, 92.3%); a hemoglobin level of 12.8 g/dL; a hematocrit of 37.0%; and a platelet count of 209×10^9 /L. His coagulation test results were within the normal range with prothrombin time (PT) 12.9 sec (range 11.7-14.8sec),PT-INR 1.09 and APTT: 30.1 sec (range 24-39sec). Blood chemistry tests revealed a creatinine (Cr), blood urea nitrogen, albumin, and C-reactive protein levels of 2,57 mg/dL, 42.4 mg/dL, 3.8 g/dL, and 3.3 mg/dL,

respectively. His serum Cr level was comparable to that of previous blood tests with an estimated glomerular filtration rate of 15 ml/min/1.73 m^2. *Serratia marcescens* was detected in both urine and blood cultures; therefore, his fever was attributed to bacteremia secondary to a urinary tract infection. We administered cefepime, but drug susceptibility testing revealed that the organism was multidrug resistant, and was sensitive only to AMK and sulfamethoxazole and trimethoprim mixture. We switched the antibiotic to AMK at a dose of 15 mg/kg/day once daily. On the second day of AMK treatment, his APTT was markedly prolonged to 67.2 sec at the trough AMK concentration (C_{trough}), and 179.8 sec at the peak concentration (C_{peak}), but his PT remained normal, his fever defervesced and his general condition improved without signs of a bleeding tendency (Figure 1). On day 6, we performed a cross-mixing test to analyze the cause of prolongation of the APTT. The test result revealed a coagulation factor inhibitory pattern (Figure 2A). The activities of the measurable clotting factors were normal except for factor XII which was 31%. Therapeutic drug testing of AMK revealed a C_{trough} and C_{peak} concentrations of 25.9 μg/mL and 77.4 μg/mL, respectively. The dose of AMK was reduced to 10 mg/kg every second day until day 15. The patient's APTT was measured throughout his clinical course by Coagpia APTT-N (SEKISUI MEDICAL CO.,LTD, Japan) reagent with an automated coagulation analyser, CP3000 (SEKISUI MEDICAL CO.,LTD, Japan).

To clarify the mechanism of prolongation of APTT by AMK, we conducted an experiment with plasma from healthy donors. Amikacin sulfate and another aminoglycoside derivative, gentamicin sulfate (GM) were purchased from Fujifilm (Wako Pure Chemical Corporation, Osaka, Japan); plasma was collected from 20 healthy donors and pooled; and the APTT was measured three times independently with

addition of AMK or GM at set concentrations using a different APTT reagent, Thrombocheck APTT (Sysmex, Kobe, Japan). Both Thrombocheck APTT and Coagpia APTT-N reagents contained ellagic acid as the coagulation activator. Measurements were conducted by an automated coagulation analyser, ACL-TOP 500 (IL Japan Co. Ltd., Tokyo) according to manufacturer's instructions. There was a dose-dependent prolongation of the APTT by AMK and GM (Figure 3A), although the extent of prolongation was less marked than that in our patient. We repeated the experiment using two other APTT reagents each containing silica as the coagulation activator with Coag-genesis APTT (LSI Medience, Tokyo, Japan) and HemosIL SynthASil APTT (IL Japan, Tokyo, Japan), but there was little prolongation of the APTT (Figure 3B).

The APTT assay is frequently used to screen for the coagulation pathway. In general, the APTT assay is conducted by adding the commercial APTT reagent to plasma anticoagulated with 3.2% citric acid and incubating the mixture for several minutes, which results in activation of the contact pathway and factor IXa. Calcium chloride is added, resulting in the activation of the intrinsic and common pathways and the time from activation to clot detection is measured in tens of seconds.[1] In the present case, plasma showed an inhibitory pattern on the cross-mixing test.

Selman Abraham Waksmanone discovered the aminoglycoside streptomycin, which is produced by *Streptomyces spp.*[2] After this discovery, many aminoglycoside derivatives were developed and produced. Streptomycin and kanamycin are mainly used for the treatment of tuberculosis; GM and AMK are administered for *Pseudomonas* infection and infections with multidrug-resistant bacteria; and arbekacin is used to treat methicillin-resistant *Staphylococcus aureus* infection. Dose reduction is recommended for patients with renal failure, but our patient had a clinically severe infection with a

multidrug-resistant pathogen, and we continued the administration of AMK with careful monitoring. Aminoglycosides have been reported to have a chelating effect on copper ions in vitro, aside from their antibacterial effects.[3-6] Ellagic acid was reported to form insoluble complexes with copper ion and increase procoagulant activity.[7] Therefore, aminoglycosides chelated copper ions in vitro may inhibit the effects of activators, such as ellagic acid, in concentration-dependent manner, resulting in the prolongation of the APTT. However, we cannot completely rule out the possibility that a part of the elevation of PT, and APTT was attributed to sepsis-induced coagulopathy and/or a lupus anticoagulant because our in vitro experiment showed not so marked elongation of APTT as they were in the patient.

Additional in vitro experiments with pooled plasma from healthy donors spiked with AMK and GM revealed that prolongation of APTT occurred primarily with the ellagic acid-containing reagent, but that the effect of the AMK and GM plasma concentration on the APTT was trivial with the two silica activated reagents. These results suggest that AMK and GM inhibit the effect of ellagic acid, but not silica, on activating the coagulation pathway, causing artifactual prolongation of the APTT. Further investigation is required because there are few published reports of the prolongation of APTT by AMK and GM and clinicians are generally not aware that AMK and GM may be associated with a prolonged APTT in the absence of any coagulation disorders[8,9]. Moreover, we used only one ellagic acid APTT reagent in the in vitro experiment and did not perform the in vitro study on the same analyser as the patient's sample, and did not use the same ellagic acid reagent. This is a limitation because APTTs are known to react differently on the basis of their activator and phospholipid source, therefore, it is possible that the reaction may differ with another

ellagic acid APTT reagent. The mechanism of the different effects of AMK and GM on APTT using ellagic acid-containing and silica activated reagents remains unknown. Thus, Further studies are warranted.

In most hospitals, the measurement of aminoglycoside concentration is outsourced and the results are not reported on the same day. Therefore, the delay in dose adjustment based on the serum drug concentration is a concern. The APTTs in our case were considerably prolonged during administration of AMK, indicating that the AMK concentration exceeded the therapeutic window. However, we only realized this in retrospect. APTT measured using ellagic acid-containing APTT reagents would enable the results to be reported on the same day while awaiting the results of outsourced testing of the plasma aminoglycoside concentration. However, it might be useful to test the APTT using a silica activated reagent that is not susceptible to interference by AMK and GM in patients on AMK or GM if a coagulation disorder is suspected, such as when investigating disseminated intravascular coagulation.

In conclusion, we report a case of AMK-induced artifactual prolongation of the APTT. The mechanism of prolongation is thought to be in vitro interference with the coagulation activator in the ellagic acid containing APTT reagent. This phenomenon is an effect of AMK and GM aside from its antibiotic effect, but it might be indicative of copper ion chelation by AMK or GM.

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Figure legends

Figure 1. Patient's clinical course and treatment

The patient was initially treated for a *Serratia marcescens* urinary tract infection and bacteremia with cefepime, but the antibiotic was changed to amikacin (AMK) at a dose of 15 mg/kg/day based on the antimicrobial susceptibility test result. After administration of AMK, his general condition improved, but his activated partial thromboplastin time (APTT) became markedly prolonged the day after initiating AMK. The extent of prolongation of APTT was associated with the blood AMK concentration.

AMK, amikacin; APTT, activated partial thromboplastin time; C, concentration; Cr, creatinine; CRP, C-reactive protein; WBC, white blood cells

Figure 2. Effect of the amikacin concentration on the activated partial thromboplastin time, measured using a APTT reagent containing ellagic acid as the coagulation activator. To examine the cause of prolongation of the APTT, we performed a cross-mixing test, and indicated inhibitory pattern.

APTT, activated partial thromboplastin time

Figure 3. Scatter plots of in vitro experiments of the correlation between plasma amikacin (AMK) or gentamicin (GM) concentration and the activated partial thromboplastin time (APTT) measured using pooled plasma from healthy donors

A correlation between the APTT and the concentration of AMK (A) and GM (B) was observed with the ellagic acid-containing reagent (Thrombocheck APTT), but not the silica-containing reagent ① (Coag-genesis APTT) and reagent ② (HemosIL SynthASil APTT).

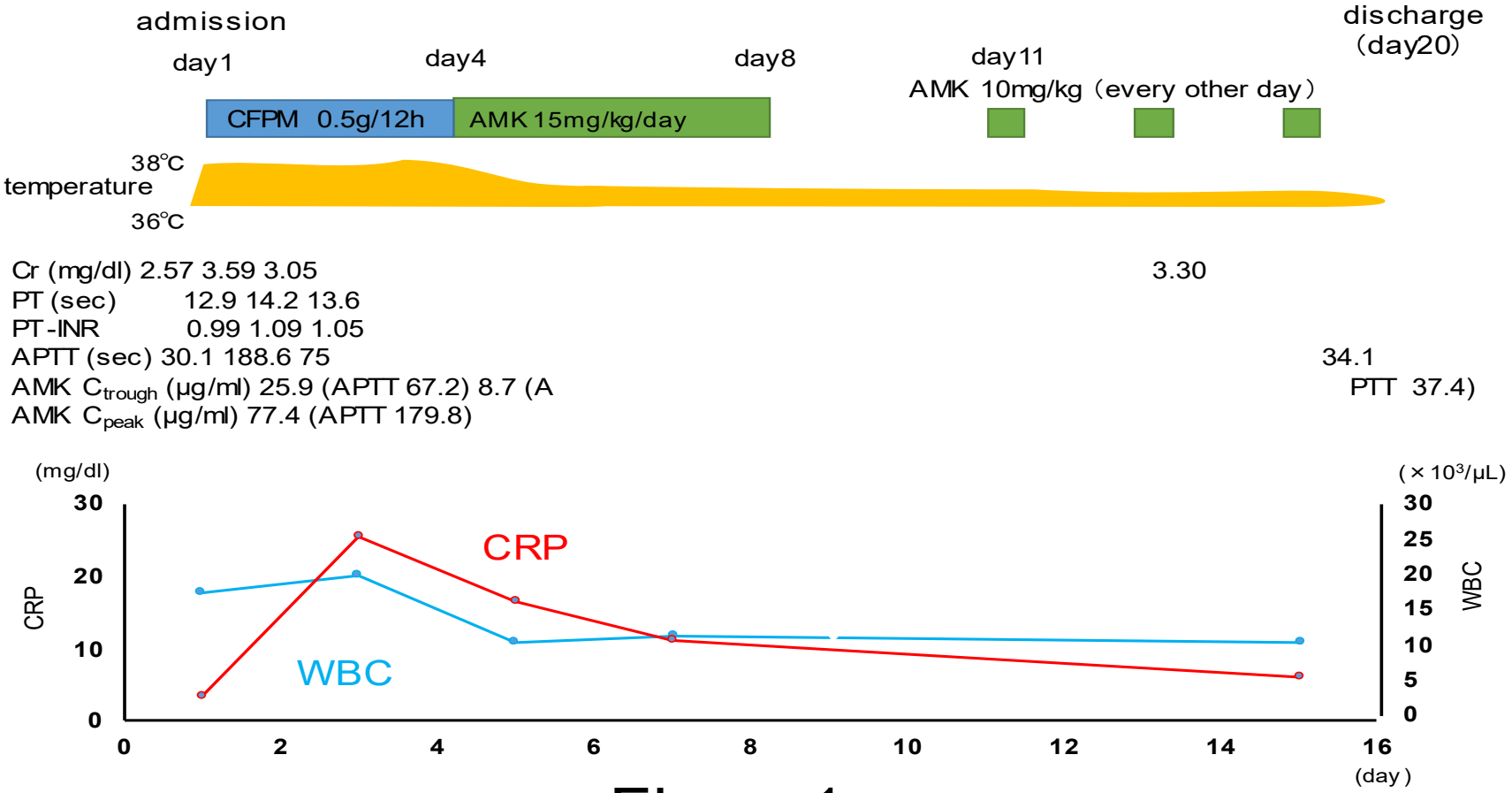

admission
day1
day4
day8
day11
discharge
(day20)
AMK 10mg/kg (every other day)
CFPM 0.5g/12h
AMK 15mg/kg/day
38℃
temperature
36℃
Cr (mg/dl) 2.57 3.59 3.05
3.30
PT (sec) 12.9 14.2 13.6
PT-INR 0.99 1.09 1.05
APTT (sec) 30.1 188.6 75
34.1
AMK Ctrough (μg/ml) 25.9 (APTT 67.2) 8.7 (A
PTT 37.4)
AMK Cpeak (μg/ml) 77.4 (APTT 179.8)
(mg/dl)
(×10³/μL)
CRP
WBC
0
10
20
30
0
5
10
15
20
25
30
0
2
4
6
8
10
12
14
16
(day)

Figure1

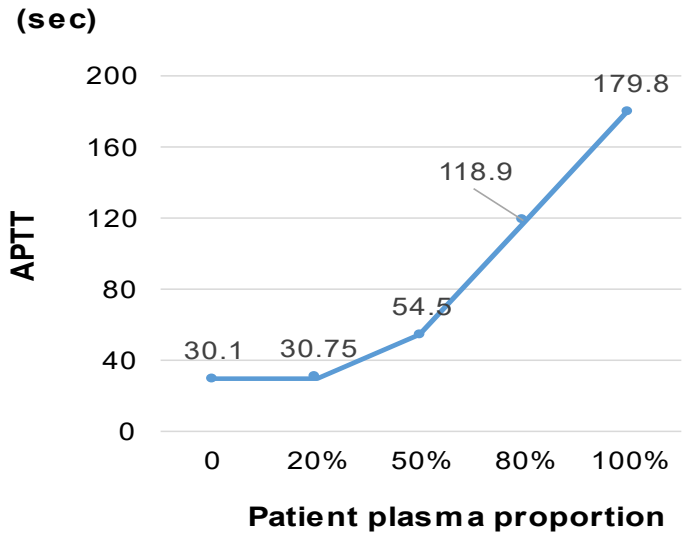
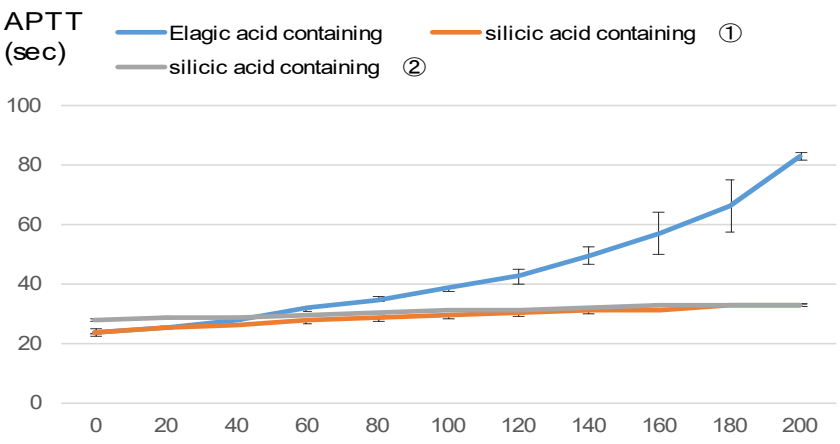

(sec)
200
160
120
80
40
0
APTT
179.8
118.9
54.5
30.1
30.75
0
20%
50%
80%
100%
Patient plasma proportion

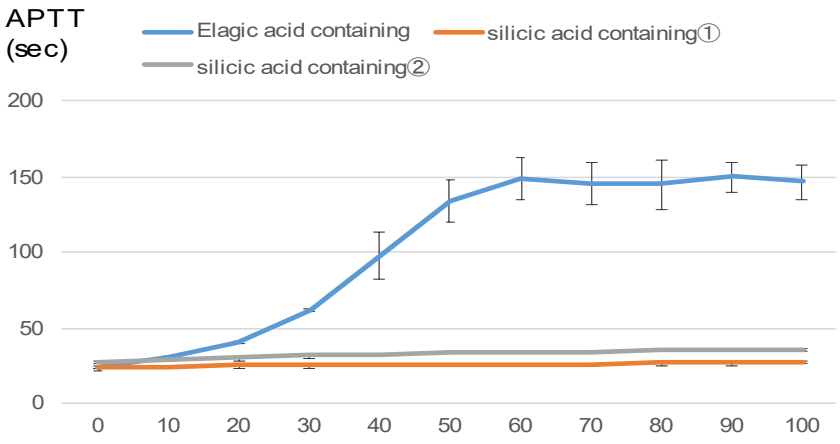
Figure2

A



Concentration of AMK (μg/ml)

B



Concentration of GM (μg/ml)

Figure3