



Vipera ammodytes bites treated with antivenom ViperaTab[®]: a case series with pharmacokinetic evaluation



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INTRODUCTION

In the southeastern parts of Europe *Vipera a. ammodytes* (*V. a. ammodytes*) and *Vipera berus* (*V. berus*) are the only medically important poisonous snakes. Differentiation of their bites based on clinical presentation is very difficult and unreliable. In the past this was not a concern, since snakebites were successfully treated with Viperfav[™] (Aventis Pasteur, France) or European viper venom antiserum (Zagreb antivenom) (Institute of Immunology, Croatia) as formulations containing equine F(ab')₂ fragments that are either specific for both venoms, either clinically proved to be safe and effective for the treatment of *V. a. ammodytes* and *V. berus* envenomings. Due to current shortage in Viperfav[™] and Zagreb antivenom availability, *V. a. ammodytes* and *V. berus* bites have recently been treated with ViperaTAB[®] (MicroPharm Limited, UK) composed of ovine Fab fragments as active principle against the venom of *V. berus* only. Its therapeutic convenience for use against *V. a. ammodytes* venom-induced toxicity in human has not been described yet. Neutralisation efficacy has been proved preclinically, although at much weaker level in comparison to protective potential exhibited by *V. ammodytes*-specific antivenoms.

RESULTS

Case No. 1

- postbite t [h]
- 0 A 60-year-old man was bitten in the thenar of the hand by *V. a. ammodytes*.
- 1 Upon hospitalisation the patient was faint, dizzy, confused, tachycardic (100/min) and hypotensive (85/50 mmHg). Edema of the affected hand was extending up to a fifth of his forearm.
- 3 The patient was somnolent, tachycardic (100/min), normotensive (110/80 mmHg) and tachypneic (30/min). He felt strong pain at the bite site. Local edema with erythema extended up to a half of his forearm. Laboratory tests showed leucocytosis (22×10⁹/L), thrombocytopenia (26×10⁹/L), coagulopathy with prolongation of prothrombin time (0.41) and increased D-dimer (10469 µg/L). The patient had rhabdomyolysis (myoglobin: 1101 µg/L; creatine kinase: 7.9 µkat/L) and acute renal failure (creatinine: 113 mmol/L).
- 4.5 ViperaTAB[®] was given.
- 7 Follow up studies revealed increase of platelet count (84×10⁹/L) and improvement of coagulopathy (prothrombin time: 0.48) and rhabdomyolysis (myoglobin: 677.6 µg/L).
- 12 Transient improvements were followed by profound 2nd thrombocytopenia (18×10⁹/L), coagulopathy (prothrombin time: 0.24) and extension of local pain, edema, erythema, lymphangitis, petechiae and ecchymosis up to the upper arm.
- 14 Second dose of ViperaTAB[®] was given.
- 16 Platelet number normalised (177×10⁹/L) again, while coagulopathy improved with prothrombin time reaching 0.53. However, local oedema and erythema extended further to the shoulder and the patient developed neurological signs (bilateral ptosis, ophthalmoplegia and dysphagia).
- 21.5 Viperfav[™] was given. Local swelling extension was effectively reduced. There were no neurological signs anymore.

ELISA analysis of serum samples taken 3 h after the bite revealed the venom level of 160 ng/mL and the Atxs level 9.5 ng/mL. These and subsequent serum concentrations are presented in Fig. 1. Antivenoms ViperaTAB[®] and Viperfav[™] serum concentrations measured by ELISA are presented in Fig. 1, while ViperaTAB[®] pharmacokinetic data is summarised in Table 1.

Case No. 2

- postbite t [h]
- 0 An 83-year old man was bitten in the dorsal side of the right foot by *V. a. ammodytes*. Immediately after the bite he felt pain and within a few minutes the right foot started to swell.
- 4 Upon hospitalisation the patient was in extreme pain, confused and hypertensive (200/100 mmHg). Oedema, lymphangitis and haematoma were extending up to a third of the lower leg. There were no neurological symptoms. The initial laboratory tests showed leucocytosis (21×10⁹/L), thrombocytopenia (21×10⁹/L), slight rhabdomyolysis (myoglobin: 97.6 µg/L) and coagulopathy with increased D-dimer (5428 µg/L). Prothrombin time was at lower normal value (0.70).
- 5.5 ViperaTAB[®] was given. The extension of local signs stopped.
- 7.5 Platelet count increased (136×10⁹/L). Afterwards, profound thrombocytopenia reappeared (18×10⁹/L) with nadir 30-70 h after bite.
- 72 Second dose of ViperaTAB[®] was given. Platelet number increased to 94×10⁹/L.
- 74 Platelet count decreased to 70×10⁹/L again and remained between 70 and 140×10⁹/L until the seventh day, when it finally normalised.

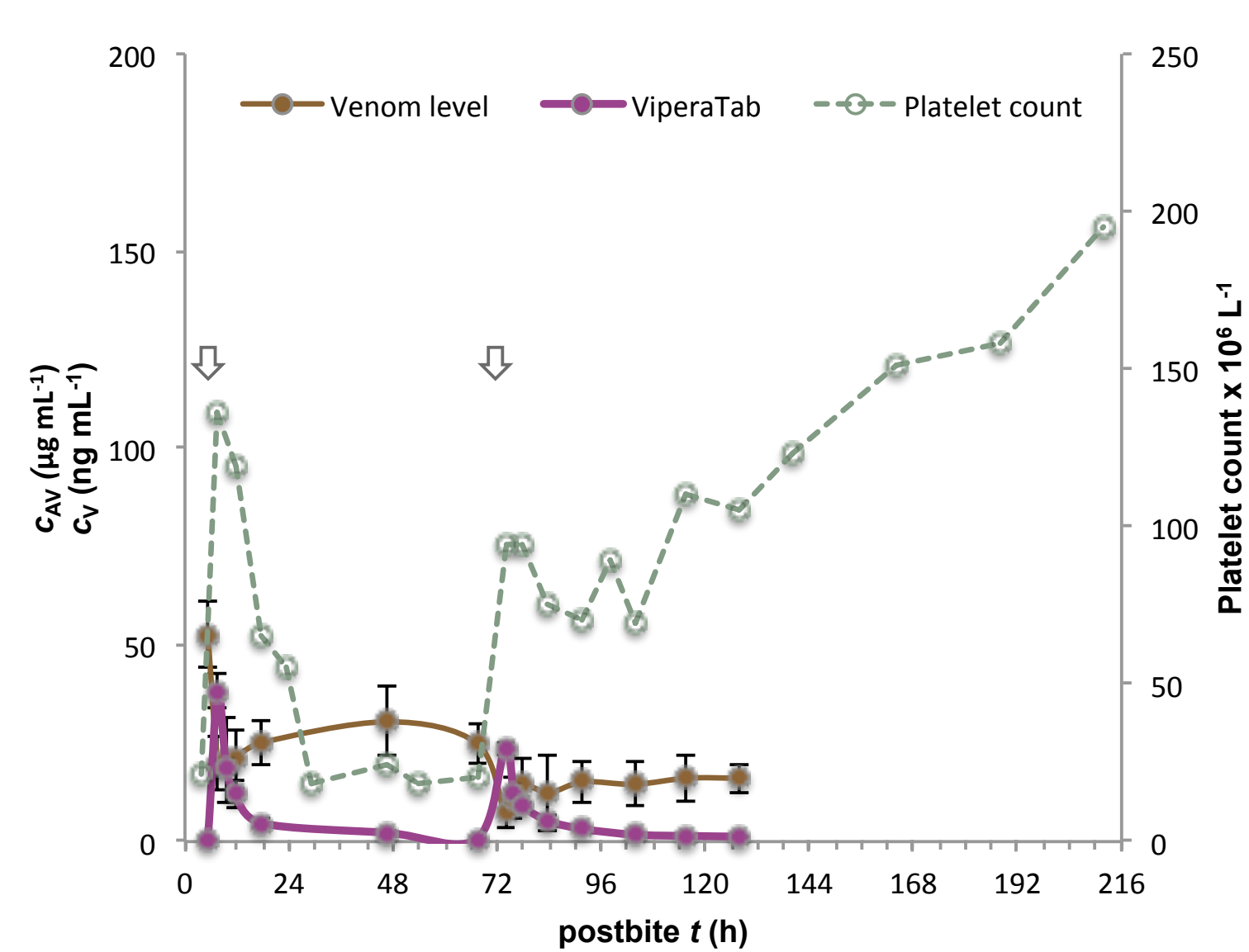


Figure 2. Analysis of the serum of the patient #2 bitten by *V. a. ammodytes* and treated with ViperaTAB[®]. Concentration of venom in the serum (c_v), platelet count and serum concentrations of ViperaTAB[®] (c_{AV}). Error bars represent 95% CI. Antivenom application is denoted by arrow.

ELISA analysis of serum samples taken 4 h after the bite revealed venom level of 50 ng/mL. Serum concentrations are presented in Fig. 2. None of the analysed sera samples for Atxs in this patient gave absorbance values higher than those obtained for the simultaneously assayed negative control. Antivenom ViperaTAB[®] serum concentrations measured by ELISA are presented in Fig. 2, while ViperaTAB[®] pharmacokinetic data is summarised in Table 1.

CONCLUSIONS

In *V. a. ammodytes* bitten patients ViperaTAB[®], a monospecific *V. berus* antivenom with weak preclinical cross-reactivity with *V. ammodytes* venom, reduces moderate swelling and temporarily improves systemic effects, except neurological symptoms.

ViperaTAB[®] application induces *V. ammodytes* venom level decrement, but it does not affect serum concentration of neurotoxic Atxs.

ViperaTAB[®] doses in *V. a. ammodytes* bites should be higher and given repeatedly despite its maximum 55-hour long elimination half-life.

No adverse effects of ViperaTAB[®] were noticed in *V. a. ammodytes* bitten patients.

AIM

For the first time we present cases of several *V. a. ammodytes* snakebites that occurred in Slovenia and were treated with ViperaTAB[®] – *V. berus*-specific antivenom, whose pharmacokinetics has been measured and correlated with clinical picture.

MATERIALS AND METHODS

V. ammodytes venom, neurotoxic ammodytoxins (Atxs) and Fab fragment levels in sera samples of three patients envenomed by *V. a. ammodytes* snakebite and treated with ViperaTAB[®] were determined by the respective in-house ELISA assays.

In addition, pharmacokinetic analysis of the antivenom Fab fragments was carried out. Pharmacokinetic analysis of the measured concentrations was performed using PKSolver add-in software (version 2.0, China Pharmaceutical University, Nanjing, China) for Microsoft Excel [1]. Concentration-time data was fitted either to one-, two- or three-compartment model. Akaike information criterion (AIC) and Schwarz criteria (SC) were used for comparison of goodness of their fit [2].

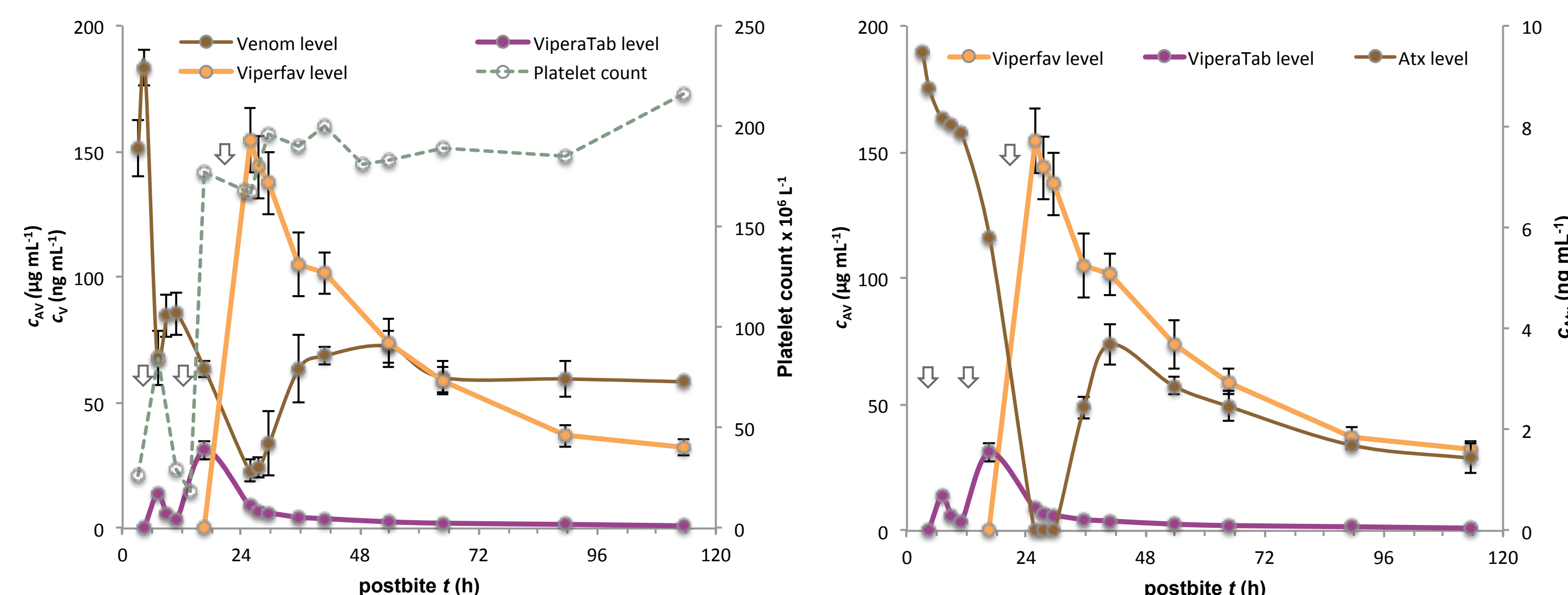


Figure 1. Analysis of the serum of the patient #1 bitten by *V. a. ammodytes* and treated with ViperaTAB[®] and Viperfav[™]. Concentration of venom in the serum (c_v), platelet count and serum concentrations of ViperaTAB[®] (c_{AV}) and Viperfav[™] (c_{AV}) (A). Serum concentrations of Atxs (c_{Atxs}), ViperaTAB[®] (c_{AV}) and Viperfav[™] (c_{AV}) (B). Error bars represent 95% CI. Antivenom application is denoted by arrow.

Case No. 3

- postbite t [h]
- 0 73-year old man was bitten in the left thumb by *V. a. ammodytes*. Immediately afterwards he felt pain. His right arm started to swell and become erythematous.
- 6 Local pain, oedema, erythema and lymphangitis extended up to the elbow. On admission to the hospital the patient was tachycardic (124/min) and normotensive (150/90 mmHg).
- 7 The initial laboratory tests showed slight thrombocytopenia (133×10⁹/L) and increased D-dimer (3639 µg/L).
- 14 Local signs extended above the elbow. Platelet number further decreased (113×10⁹/L). ViperaTAB[®] was given. After application the extension of local signs stopped. The follow up studies revealed an increase of platelet count (147×10⁹/L).

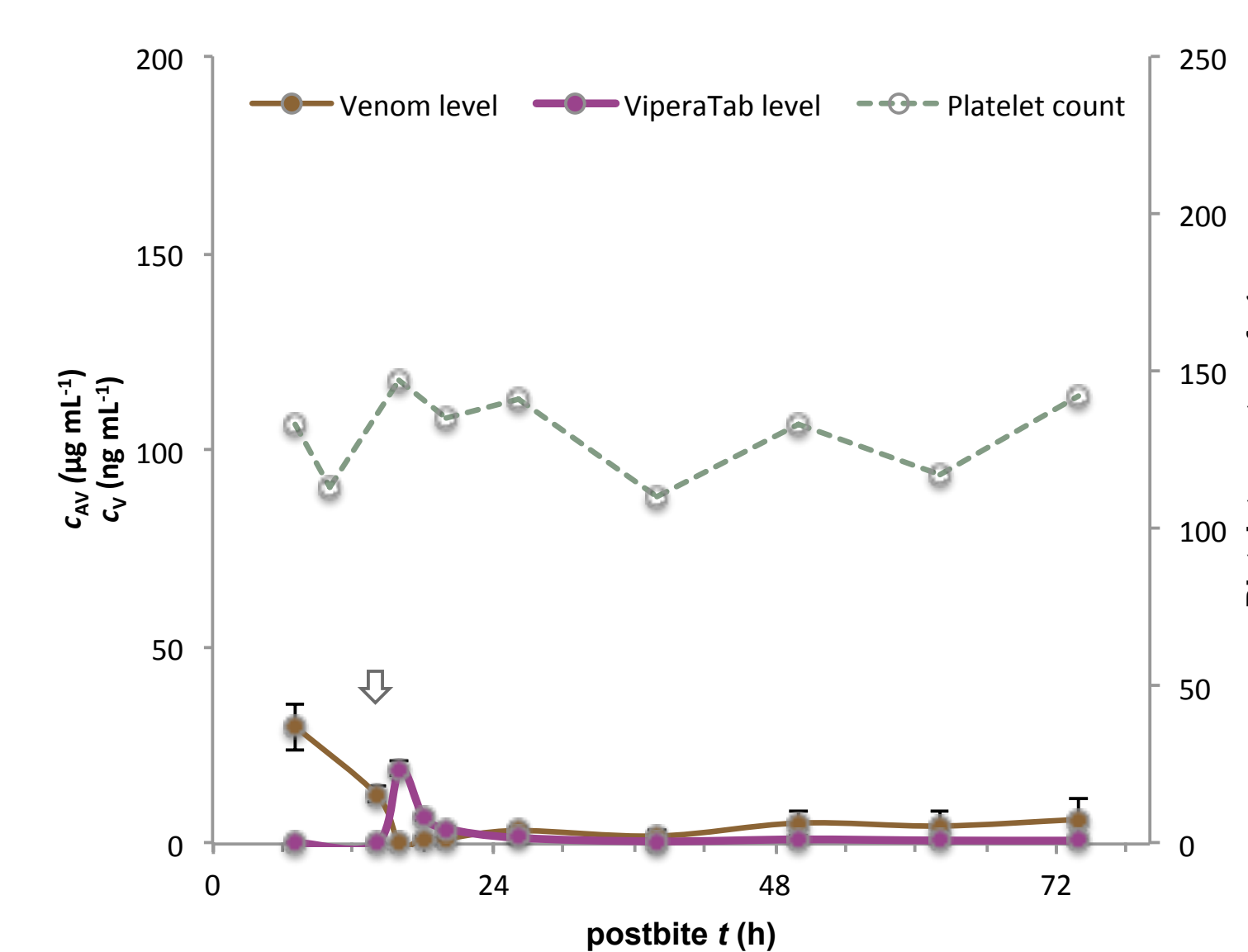


Figure 3. Analysis of the serum of the patient #3 bitten by *V. a. ammodytes* and treated with ViperaTAB[®]. Concentration of venom in the serum (c_v), platelet count and serum concentrations of ViperaTAB[®] (c_{AV}). Error bars represent 95% CI. Antivenom application is denoted by arrow.

ELISA analysis of serum samples taken 7 h after the bite revealed the venom level of 40 ng/mL (Fig. 3). Measurable Atxs quantity was not detected in the patient's serum. Antivenom ViperaTAB[®] serum concentrations are presented in Fig. 3 and ViperaTAB[®] pharmacokinetic data in Table 1.

Pharmacokinetic data

Table 1. ViperaTAB[®] pharmacokinetic data.

	Case #1	Case #2 1 st dose	Case #2 2 nd dose	Case #3
$t_{1/2\alpha}$	3.2 h	1.6 h	1.2 h	1.2 h
$t_{1/2\beta}$	55.9 h	22.2 h	14.1 h	45.3 h
V_{ss}	252.9 mL / kg	154.5 mL / kg	118.3 mL / kg	524.2 mL / kg
MRT	59.0 h	19.6 h	13.0 h	39.1 h
AUC _∞	666.8 (µg h) / mL	373.1 (µg h) / mL	323.5 (µg h) / mL	198.7 (µg h) / mL
AUMC	39347.1 (µg h ²) / mL	7311 (µg h ²) / mL	4207.5 (µg h ²) / mL	7760.0 (µg h ²) / mL
CL	4.3 (mL / h) / kg	7.9 (mL / h) / kg	9.1 (mL / h) / kg	13.4 (mL / h) / kg

$t_{1/2\alpha}$, distribution half-life; $t_{1/2\beta}$, elimination half-life; V_{ss} , steady-state volume of distribution; MRT, mean residence time; AUC_∞, area under the curve at $t = \infty$; AUMC, area under the first(-order) moment curve; CL, systemic clearance.

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