## Supplemental material: methods

## Surgical techniques

Surgery was performed in an operating room designed and prepared to accommodate ovine subjects and standard aseptic surgical procedures.

For Groups 2 and 3, male and female adult sheep from 45 to 72 kg received no food for 12 hours prior to induction of anesthesia. Prior to surgery, the sheep were sedated using xylazine (1 mg/kg I.M.), anesthetized with tiletamine/zolazepam (10 mg/kg I.V.) and maintained with isofluorane (1.5 to 2%) in 100% O2 and butorfanol (0.1 mg/kg I.V.) asanalgesic, and intubated to prevent aspiration in the event of regurgitation [23]. Heart rate, blood pressure and respiration were monitored and hydration in all 3 groups was maintained with a continuous IV infusion of isotonic saline [23]. In addition, Group 3 animals received replacement volume of saline equal to that of thoracic duct lymph removed. The jugular vein was cannulated for blood samples [21] and the cannula was flushed with heparin solution to avoid clotting. Rumenotomy was performed to avoid pasture bloat. In Group 3, Evans blue dye was injected SC in the left hindlimb to facilitate visualization and cannulation of the thoracic duct. Cannulation was achieved by an incision into the left neck with the head recumbent to identify the thoracic duct at its termination into the venous system. The duct was ligated just before entry into the vein to distend the vessel and a 22 gauge angiocath (Becton Dickinson and Company, Mexico) was inserted and secured with suture for collection. After 6 hours, all animals were euthanized with intravenous pentobarbital [23, 24]. Subjects in Group 1, which did not involve the use of venom, were assessed without anesthesia or surgery [23] and were donated to a farm after the procedure.

## Determination of Micrurus fulvius venom concentration by sandwich immunoassay

Venom concentrations in lymph, serum, urine, and tissues were determined by sandwich immunoassay as described previously. Briefly, immunopurified antibodies were obtained by passing rabbit immune serum against whole *M. fulvius* venom through a Sepharose-4B column with covalently attached (CNBr chemistry) whole *M. fulvius* venom. The resulting antibodies were used both as capture (non-biotinilated) and as detection (biotinilated) antibodies. The signal was developed with Streptavidin-Peroxidase conjugate and ABTS. The standard curve had a lower limit of quantification of 1.3 ± 0.4 ng/ml. This assay was able to detect all components of venom that can be detectable by SDS-PAGE and RP-HPLC, without discrimination between PLA2 and other proteins contained in the venom[8].

## Determination of AV concentration by sandwich immunoassay

AV concentrations in lymph, serum, urine, and tissues were determined using a sandwich immunoassay. Briefly, Maxisorp (NUNC Inc., USA) 96-well plates were coated with immunopurified polyclonal antibodies from serum of sheep hyperimmunized against horse F(ab´)2. A 0.5% gelatin solution was applied as the blocking solution. For detection, commercial rabbit antibodies against horse F(ab´)2 peroxidase conjugate were used and the assay was revealed with ABTS. For quantification, a standard curve was established using 1:2 serial dilutions, starting with an AV concentration of 3 µg/ml. The standard curve had a lower limit of quantification of 0.36 (0.08) µg/ml.

## Determination of concentration by sandwich immunoassay of 3FTxs from Micrurus fulvius venom

A sandwich immunoassay for non-toxic 3FTx of *Micrurus fulvius* venom[1] in ovine serum samples was developed [26]. Immunopurified antibodies were obtained by passing rabbit immune serum against whole *M. fulvius* venom through a Sepharose-4B column with covalently attached (CNBr chemistry) RP-HPLC purified 3FTx [1]. The resulting antibodies were used both as capture (non-biotinilated) and as detection (biotinilated) antibodies. Signal was developed with Streptavidin-Peroxidase conjugate and ABTS. The assay had a lower limit of quantification of 1.2 (0.3) ng/ml. This assay detected only proteins of approximately 7 kDa, excluding detection of PLA2 or other proteins of different molecular weight detectable by SDS-PAGE.

## Pharmacokinetic parameters

For group 1, AV pharmacokinetic was fitted to a tri-compartment analysis described by equation 1.

(1)

Half-lives were calculated using equations 2, 3, and 4.

(2)

(3)

Apparent volume of serum (central) compartment (Vc) was estimated using equation 4.

(4)

Total exposure to venom and AV that reaches systemic circulation was measured using the area under the curve (AUC), calculated by trapezoidal numerical integration (equation 5).

(5)

The fraction of the dose administered SC that reaches systemic circulation was expressed as bioavailability (F), calculated with equation 6, using the mean of AUCiv for venom reported previously [8].

(6)

Clearance (CL) describes the process of irreversible elimination of venom and AV from systemic circulation and was calculated as follows:

(7)

Mean residence time (MRT) refers to average time that venom and AV stays in the body; it is defined by:

(8)

The area under the first moment curve (AUMC) was estimated using trapezoidal numerical integration:

(9)

Volume of distribution at steady state (Vss) refers to apparent volume of distribution when the concentration vs. time profile curve reaches equilibrium:

(10)

Maximum concentration (Cmax), and the last concentration measured at the end of experiment (Cz), were taken directly from experimental data, for both venom and AV.

For group 3, the quantity of venom and AV in each lymph sample was calculated by multiplying the concentration obtained in each sample by the corresponding volume, and the cumulative amount was estimated by adding together the quantities previously calculated. Total recovery of venom was calculated as the sum of systemic availability in blood (F) and cumulative fraction of the dose recovered in lymph.

Statistical comparisons between treatment groups were conducted for all parameters using the Student t-test.