# *Trimeresurus* venom inhibition of anti-HPA-1a and anti-HPA-1b antibody binding to human platelets

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A solid-phase red cell adherence assay was used to demonstrate the specific inhibitory effect of seven species of *Trimeresurus* snake venom on the binding of HPA-1a- and HPA-1b-specific platelet antibodies. *Trimeresurus* venom did not inhibit the binding of HLA-, HPA-3a-, HPA-3b-, HPA-4a-, HPA-5a-, and HPA-5b-specific platelet antibodies. Venom from other genera of snakes, including representatives from *Agkistrodon, Ancistrodon, Bitis, Bothrops, Bungarus, Causus, Crotalus, Dendroaspis, Ecis, Micrurus, Naja, Notechis, Ophiophagus, Pseudechis, Sepedon (Hemachatus),* and *Vipera*, all failed to specifically inhibit anti-HPA-1a and HPA-1b binding. These results may indicate that the component in *Trimeresurus* snake venom previously reported to bind to the platelet GPIIb-IIIa complex, inhibiting fibrinogen binding, binds close to the HPA-1a and HPA-1b epitopes. ***Immunohematology*** 1995;11:129–132.

Snake venoms are composed of complex mixtures of proteins that have many different biological effects on mammalian cardiac and circulatory systems. Nearly all snake venoms produce phospholipase $A_2$, which splits the $\alpha$-positioned unsaturated fatty acid chains from phospholipids, exerting a lytic effect on cell membranes, including those of platelets.[1] Some snake venoms also contain 5-nucleotidases that split purine- and pyrimidine-nucleoside-5-mono- and diphosphates, which can inhibit platelet aggregation due to ADP, sodium arachidonate, or collagen.[2]

There are also less well-characterized nonenzymatic venom components that act on platelets. Trigramin, a cysteine-rich, single-chain polypeptide with a molecular weight of 9,000 daltons isolated from *Trimeresurus gramineus* snake venom specifically binds to the platelet IIb-IIIa complex, thus preventing fibrinogen binding.[3]

We were interested in determining if *Trimeresurus* venom components were capable of preventing the binding of human antibodies specific for the glycoprotein IIb-IIIa complex found on platelets. To avoid sensitivity and quantitation problems associated with the platelet aggregation procedures, immobilization of platelet monolayers in a solid-phase red cell adherence (SPRCA) assay[4] was used to measure the inhibitory effect of *Trimeresurus* venoms on glycoprotein IIb-IIIa-specific antibody binding.

## Materials and Methods

One hundred frozen human plasma and serum samples, with previously determined platelet antibody specificities, were thawed and retested by SPRCA to confirm their reaction strengths and specificity (Capture-P Ready Screen, Immucor, Inc., Norcross, GA). All test sample reactions were scored independently by two investigators, and only those samples with mean score values greater than or equal to 6 were used in venom inhibition experiments. SPRCA reactions were scored according to Stone et al.[5] The specificities of the samples are shown in Table 1.

**Table 1.** Specificity of antisera used in venom inhibition experiments

| Specificity | Number |
|---|---|
| Anti-HLA | 42 |
| Anti-HPA-1a | 42 |
| Anti-HPA-1b | 4 |
| Anti-HPA-3a | 4 |
| Anti-HPA-3b | 3 |
| Anti-HPA-4a | 1 |
| Anti-HPA-5a | 2 |
| Anti-HPA-5b | 2 |

The snake venoms (Sigma Chemical Co., St. Louis, MO) were stored frozen, and fresh working solutions were prepared immediately prior to use. Working venom solutions were 1.0 mg/mL in phosphate-buffered saline (PBS), pH 7.4, and were kept on ice until the addition to platelet monolayers. Snake venom from 42 species of snakes, representative of the major groups of venomous snakes found in the world, were tested for inhibition of platelet-specific antibody binding. The venoms are listed in Table 2.

**Table 2.** Snake venoms tested for inhibition of anti-platelet antibody binding

| Scientific Name | Common Name |
|---|---|
| *Agkistrodon contortis laticintus* | Broad-banded copperhead |
| *Agkistrodon contortis mokasen* | Northern copperhead |
| *Agkistrodon contortix contortix* | Southern copperhead |
| *Agkistrodon halys blomhoffii* | Japanese mamushi |
| *Agkistrodon piscivorus leukostoma* | Western cottonmouth moccasin |
| *Agkistrodon piscivorus piscivorus* | Eastern cottonmouth moccasin |
| *Ancistrodon bilineatus* | Tropical moccasin |
| *Bitis arietaus* | Puff adder |
| *Bitis candalis* | Horned puff adder |
| *Bitis gabonica* | Gaboon viper |
| *Bitis nasicornus* | Riverjack |
| *Bothrops atrax* | Fer de lance |
| *Bothrops jararaca* | South American pit viper |
| *Bothrops neuwiedi* | |
| *Bothrops nummifer* | Jumping viper |
| *Bungarus caeruleus* | Indian krait |
| *Bungarus fasciatus* | Banded krait |
| *Causus rhombeatus* | Rhombic night adder |
| *Crotalus horridus articaudatus* | Canebrake rattlesnake |
| *Crotalus horridus horridus* | Timber rattlesnake |
| *Crotalus viridis viridis* | Prairie rattlesnake |
| *Dendroaspis angusticeps* | Eastern green mamba |
| *Dendroaspis jamesoni* | Jameson's mamba |
| *Dendroaspis polylepis* | Black mamba |
| *Ecis carinatus* | Saw-scaled viper |
| *Lachesis muta* | Bushmaster |
| *Micrurus fulvius* | Eastern coral snake |
| *Naja nigricollis crawshawii* | Spitting cobra |
| *Notechis ater niger* | Peninsula tiger |
| *Notechis ater serventyi* | Chappell Island tiger |
| *Ophiophagus hannah* | King cobra |
| *Pseudechis porphyriacus* | Common black |
| *Sepedon (Hemachatus) hemachatus* | Ringhals cobra |
| *Trimeresurus albolabris* | White-lipped tree viper |
| *Trimeresurus elegans* | |
| *Trimeresurus flavoviridis* | Okinawan habu |
| *Trimeresurus okinavenisis* | Okinawan pit viper |
| *Trimeresurus popeorum* | Pope's tree viper |
| *Trimeresurus purpureomaculatus* | Mangrove viper |
| *Trimeresurus stejnegeri* | Chinese green tree viper |
| *Vipera ammodytes* | Southern European sand viper |
| *Vipera russelli* | Russell's viper |

Using accepted phlebotomy techniques, group O blood was collected from human volunteers into glass vacutainer tubes containing citrate-phosphate-dextrose-adenine (CPDA) anticoagulant. The tubes of blood were centrifuged in a Sorval GLC-2B at 650 × g for 7 minutes. Platelet-rich plasma (PRP) was collected and added to clean 16 × 100 mm glass test tubes. The PRP was centrifuged in a Serofuge-II (Clay Adams, Becton Dickinson & Co., Parsippany, NJ) for 30 seconds on high to pellet contaminating red blood cells (RBCs). The platelet-containing supernatant was poured into a new glass test tube, and 10 mL of platelet wash and storage solution (PWSS, Immucor, Inc.) were added. Following gentle mixing, the test tube was centrifuged for 4 minutes on high to pellet the platelets. The supernatant was poured off, and the platelets were washed three more times as described above. Following the last wash, the platelet pellet was resuspended in PWSS to a concentration of 200,000/$\mu$L.[5]

One hundred $\mu$L of the platelet suspension were added to chemically modified U-bottom microtitration strip wells (Modified Capture-P, Immucor, Inc.). The microtitration strip wells were centrifuged at 150 × g for 5 minutes to create platelet monolayers. Excess platelets were removed by washing with PBS, pH 7.4. Platelet monolayers were used immediately in venom inhibition experiments.

One hundred $\mu$L of venom solution were added to platelet monlayers in duplicate. Following a 30-minute incubation at 37°C, the microtitration strips were washed with PBS, pH 7.4. Fifty $\mu$L of human serum or plasma containing platelet-specific antibodies and 100 $\mu$L of a low-ionic-strength solution (Capture-LISS, Immucor, Inc.) were added to venom-treated wells. Following a 20-minute incubation at 37°C, the microtitration strip wells were washed with PBS, pH 7.4. The presence of IgG immunoglobulin molecules was determined by adding 50 $\mu$L/well of anti-IgG-coated human RBCs (Capture-P Indicator Cells, Immucor, Inc.) and centrifuging the microtitration strips for 1 minute at 600 × g. In the case of a positive reaction, the migration of the anti-IgG-coated RBCs to the bottom of the wells was impeded, as anti-IgG bridges were formed between the indicator RBCs and the platelet-bound antibodies. In negative tests, where there was an absence of platelet antigen-antibody reactions due to venom effects, the indicator cells could not be impeded during their migration, and pelleted to the bottom of the wells as tightly packed, well-defined red cell buttons. Venom pretreatment that resulted in reducing SPRCA score values to 0 (negative reaction) were considered significant.

## Results

One hundred serum or plasma samples of known platelet specificity were tested on platelet monolayers previously incubated with various snake venoms. Inhibition of antibody binding was demonstrated on platelet monolayers incubated with venom from *A. halys blomhoffii, B. arietaus, B. atrax, T. albolabris, T. elegans, T. flavoviridis, T. okinavenisis, T. popeorum, T. purpureomaculatus,* and *T. stejnegeri*, as shown in Table 3, but not by the other venoms listed in Table 2. The venom from *A. halys blomhoffii, B. arietaus,* and *B. atrax* abolished all antibody binding, regardless of antibody specificity. These results may be due to the

**Table 3.** Inhibitory effect of snake venom on binding of platelet antibodies

| Venom source | Anti-HLA | Anti-HPA-1a | Anti-HPA-1b | Anti-HPA-3a | Anti-HPA-3b | Anti-HPA-4a | Anti-HPA-5a | Anti-HPA-5b |
|---|---|---|---|---|---|---|---|---|
| *A. halys blomhoffii* | 42/42 | 42/42 | 4/4 | 4/4 | 3/3 | 1/1 | 2/2 | 2/2 |
| *B. arietaus* | 42/42 | 42/42 | 4/4 | 4/4 | 3/3 | 1/1 | 2/2 | 2/2 |
| *B. atrax* | 42/42 | 42/42 | 4/4 | 4/4 | 3/3 | 1/1 | 2/2 | 2/2 |
| *T. albolabris* | 0/42 | 30/42 | 4/4 | 0/4 | 0/3 | 0/1 | 0/2 | 0/2 |
| *T. elegans* | 0/42 | 30/42 | 4/4 | 0/4 | 0/3 | 0/1 | 0/2 | 0/2 |
| *T. flavoviridis* | 0/42 | 28/42 | 4/4 | 0/4 | 0/3 | 0/1 | 0/2 | 0/2 |
| *T. okinavenisis* | 0/42 | 29/42 | 4/4 | 0/4 | 0/3 | 0/1 | 0/2 | 0/2 |
| *T. popeorum* | 0/42 | 30/42 | 4/4 | 0/4 | 0/3 | 0/1 | 0/2 | 0/2 |
| *T. purpureomaculatus* | 0/42 | 30/42 | 4/4 | 0/4 | 0/3 | 0/1 | 0/2 | 0/2 |
| *T. stejnegeri* | 0/42 | 30/42 | 4/4 | 0/4 | 0/3 | 0/1 | 0/2 | 0/2 |

*Note:* Number of sera or plasma samples inhibited by venom pretreatment/total number of samples with indicated specificity

fact that venom phospholipases were totally destroying the antigenic activity of the platelet membrane.

Of greater interest was the specific inhibitory activity of *Trimeresurus* venom on sera demonstrating specificity for HPA-1a and HPA-1b antigens. Approximately 70 percent of the HPA-1a-specific sera (30/42) and 100 percent of the HPA-1b-specific sera (4/4) were inhibited by *Trimeresurus* venom pretreatment. *Trimeresurus* venom pretreatment did not inhibit the binding of antisera specific for HLA, HPA-3a, HPA-3b, HPA-4a, HPA-5a, or HPA-5b antigenic determinants.

## Discussion

Previous studies have demonstrated that *Trimeresurus* venom components bind to the platelet glycoprotein IIb-IIIa complex, preventing fibrinogen attachment. We were interested in determining if components from *Trimeresurus* venom could also inhibit the binding of human antibodies specific for the platelet glycoprotein IIb-IIIa complex, which is a receptor complex for fibrinogen.

A broad spectrum of snake venoms were tested for their inhibitory effect on the binding of platelet-specific antibodies. Of the 42 species of snake venom tested, 10, including all seven species of *Trimeresurus* venom tested, were found to be effective in inhibiting the binding of platelet-specific antibodies. Venom from *A. halys blomhoffii, B. arietaus,* and *B. atrax* inhibited all antisera tested, including sera specific for HLA and platelet glycoprotein antigens other than HPA-1a and HPA-1b, indicating nonspecific destruction of platelet antigens by the venom. This generalized degradation of platelet antigenic activity may be due to venom phospholipase activity.

The specific inhibition of approximately 70 percent of the anti-HPA-1a and 100 percent of the anti-HPA-1b sera by *Trimeresurus* venom suggests the possible binding site(s) of the venom component(s) are near the HPA-1a and HPA-1b antigenic epitopes on the GPIIIa molecule of the GPIIb-IIIa complex. Further evidence is provided by the lack of inhibition of antibodies directed toward other epitopes found on the GPIIb-IIIa complex. Anti-HPA-3a and anti-HPA-3b, specific for epitopes on GPIIb, and anti-HPA-4a, specific for an epitope on GPIIIa, were not inhibited by *Trimeresurus* venom. It is unclear why approximately 30 percent of the HPA-1a-specific sera were not inhibited by *Trimeresurus* venom. This lack of inhibition could be due to a subpopulation of HPA-1a epitopes with different conformations that are not susceptible to *Trimeresurus* venom.

The four examples of HPA-5a and HPA-5b sera, specific for platelet GPIa epitopes and all 42 HLA-specific sera were not inhibited by prior incubation with *Trimeresurus* venom. These results provide further evidence that the inhibitory effect of *Trimeresurus* venom is specific for antibodies directed toward HPA-1a and HPA-1b epitopes, and is not a nonspecific effect due to phospholipases or other enzymatic activity. Further experiments with purified *Trimeresurus* venom component(s) and platelet glycoproteins may provide further evidence to support this hypothesis.

## References


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