

THE USE OF THE ANTI-VENOM SPECIFIC ANTIBODIES ISOLATED FROM DUCK EGGS FOR INACTIVATION OF THE VIPER VENOM

UTILIZAREA ANTICORPILOR POLICLONALI SPECIFICI ANTI-VENIN IZOLAȚI DIN OUĂLE DE RAȚĂ ÎN INACTIVAREA VENINULUI DE VIPERĂ

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The activity of specific anti-venom can be demonstrated using protection test in laboratory mice. Our study aimed to emphasize the possibility of viper venom inactivation by the antibodies produced and isolated from duck eggs and also to the activation concentration of these antibodies. The venom used for inoculation was harvested from two viper species (*Vipera ammodytes* and *Vipera berus*). The immunoglobulin extract had a better activity on the venom from *Vipera berus* compared to the venom from *Vipera ammodytes*. This could be the result of a better immunological response, as consequence of the immunization with this type of venom, compared to the response recorded when the *Vipera ammodytes* venom was used. Besides the advantages of low cost, high productivity and reduced risk of anaphylactic shock, the duck eggs also have high activity up to dilutions of 1/16, 1/32, respectively, with specific activity and 100 surviving in individuals which received 3 x DL50.

Key words: viper venom, antibodies, duck eggs, protection test

Introduction

Since 1894 the anti-venom serums were produced. The French pathologist, Dr. Albert Calmette was the first who demonstrated the venom protection in animals when immunization was performed with low venom doses were used. Calmette produced the first *Naja kaouthia* antiserum against venom in mule. In 1895 the first antiserum produced in equine saved several individuals with severe bites.

The activity of specific anti-venom can be demonstrated using protection test in laboratory mice. Our study aimed to emphasize the possibility of viper venom inactivation by the antibodies produced and isolated from duck eggs and also to the activation concentration of these antibodies.

Material and Methods

Producing of the anti-venom antibodies. Two Pekin ducks were immunized with viper venom for producing antibodies. The venom used for inoculation was delivered from the bio base of the Department of Ecology from the University of agricultural Sciences and Veterinary Medicine Cluj – Napoca, and was harvested from two viper species (*Vipera ammodytes* and *Vipera berus*). Stock solutions 1 mg/mL in saline solution were prepared from venom. 500 µL from each viper specie mixed with aluminum hydroxide as adjuvant, for amplification of the immune response, were used for inoculation. 4 rappel immunizations were performed at 2 weeks interval. In the end of the immunization period, the eggs from the immunized ducks were harvested, and antibodies were isolated using the previously described method of dilution with distilled water and precipitation with ammonia sulfate. The isolated antibodies were re-suspended in a minimum volume of saline solution and photometrically quantified. The obtained mixture had 24.55 mg IgY /mL content.

The protection test

Laboratory mice, 20 g weight each were used for the protection test. They were subcutaneous injected with venom – anti-venom mixture.

Serial dilutions, in micro-method, within the interval 1:2, 1:4, 1:8, 1:16 etc., were obtained from the immunoglobulin extract obtained from the duck eggs. The dilutions were performed with saline solution for realizing the antigen – antibody complexes.

After dilution, an equal venom quantity was added within two experimental variants:

Ist Variant: 5 x DL 50

IInd Variant: 3 x DL 50

The mixture was incubated for 60 minutes at 37⁰C, and then inoculated in mouse. Two mice, individually marked, were inoculated with each dilution. Control inoculations were also performed, 2 positive control mice inoculated only with venom and 2 negative control mice inoculated only with immunoglobulin extract. The mice were monitored at 2 hours interval during first 12 experimental hours. After 24 hours from inoculation the monitoring started.

Results and Discussions

After mice inoculation with venom doses incubated with dilution obtained from immunoglobulin extract, they were monitored for 24 hours.

When 5 x DL 50 venom dose was used, 100% mortality of the individuals from positive control and experimental groups was recorded. Only the individuals who received immunoglobulin extract, from negative control group, survived.

When 3 x DL 50 dilutions were used, mortality was not recorded in all cases. The results are synthesized in the following table:

Table 5.5

The mortality recorded in mice as result of the protection test performed for emphasizing the activity of the immunoglobulin extract

| Venom 3 x DL 50 | Positive control | Negative control | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 | 1:512 |
|-----------------------------|---------------------|---------------------|-----|-----|-----|------|------|------|-------|-------|-------|
| <i>Vipera berus</i> | 2 | - | - | - | - | - | - | 1 | 2 | 2 | 2 |
| <i>Vipera ammodytes</i> | 2 | - | - | - | - | 1 | 2 | 2 | 2 | 2 | 2 |

The mixture venom and immunoglobulin extract had different action function of the venom origin. When *Vipera berus* venom was used, 100% surviving was recorded in tested individuals up to the dilution of 1:32, while the use of the dilution of 1:64 had as result 50% surviving in tested individuals. Higher dilutions of the immunoglobulin extract led to 100% mortality in treated individuals.

The use of the *Vipera ammodytes* venom led to 100% surviving in tested individuals up to the dilution of de 1:16, while the use of the dilution of 1:32 had as result 50% surviving in tested individuals. Higher dilutions of the immunoglobulin extract led to 100% mortality in treated individuals.

The immunoglobulin extract had a better activity on the venom from *Vipera berus* compared to the venom from *Vipera ammodytes*. This could be the result of a better immunological response, as consequence of the immunization with this type of venom, compared to the response recorded when the *Vipera ammodytes* venom was used.

Besides the advantages of low cost, high productivity and reduced risk of anaphylactic shock, the duck eggs also have high activity up to dilutions of 1/16, 1/32, respectively, with specific activity and 100 surviving in individuals which received 3 x DL50.

Conclusions

Other advantages of the aviary antibodies resulted from the protection test performed in laboratory mice with the aim of emphasizing the activity of the anti-venom from *Vipera ammodytes* and *Vipera berus*. Besides high activity, the obtained antibodies have low cost, high productivity and reduce the risk of anaphylactic shock recorded when other anti-venom serums are used.

Bibliography

1. **Akita EM., Nakai S.** (1992) – Immunoglobulin from egg yolk: Isolation and purification. *J. Food Sci.*, 57:626-634
2. **Covaciu Manuela, Florina Olaru, V. Băc, I. Petrescu** (2004) – Studiul comparativ al imunoglobulinelor obținute din ouăle de păsări prin diferite tehnici de purificare, *Analele Societății Naționale de Biologie Celulară*, Vol. IX, Nr. 2, pag. 267-271
3. **Schade R, Pfister C., Halatsch R., Henklein P** (1991) – Polyclonal IgY antibodies from chicken egg yolk – an alternative to the production of mammalian IgG type antibodies rabbits. *Atla* 19: 403-419
4. **Maya Devi C., Vasantha Bai M., Krishnan L.K.** (2002) – Development of viper venom antibodies in chicken egg yolk and assay of their antigen binding capacity, *Toxicon.*, 40:857-861