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Efficacy of Antirabies IgG and IgY on Protection of Mice Against Experimental Viral Infection as a Model for Emergency Intervention

By Ahmed Mohamed Albehwar, Abeer Atia Tammam & Amr Ismail Hassan

Veterinary Serum and Vaccine Research Institute, Egypt

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Results: Serum neutralization test revealed that IgY and IgG had rabies antibody titers of 64 and 128 respectively. The tow preparations were tested for determination of their potency in experimentally infected mice with rabies virus on daily intervals post infection.

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Results: Serum neutralization test revealed that IgY and IgG had rabies antibody titers of 64 and 128 respectively. The tow preparations were tested for determination of their potency in experimentally infected mice with rabies virus on daily intervals post infection. It was found that intraperitoneal injection of infected mice with 0.5ml containing 116 mg of IgY and 171 mg of IgG were effective to prevent and overcome the progress of rabies signs when administrated on the 0; 1st; 2nd and 3rd day post exposure to the virus infection and not after that where treated mice on the 4th to 7th day post infection were unable to withstand the virus infection. The use of IgY and IgG with inactivated rabies vaccine showed the same results using IgY and IgG alone while the use of rabies vaccine alone did not provide efficient protection for the treated mice.

Conclusion: The use of anti-rabies IgY and IgG could be recommended as post exposure intervention providing a suitable period of protection until stimulation of the active immunity induced by rabies vaccine. In addition, the preparation of chicken IgY in a non-specific host provides safe, high potent product of lower cost than that prepared in rabbits or other mammals.

Keywords: rabies, vaccine, antirabies IgY, antirabies IgG, emergency.

I. Introduction

Rabies is a zoonotic disease that affects the central nervous system (CNS), provokes acute and fatal encephalitis in its mammal hosts. The disease etiologic agent is the rabies virus which is a neurotropic, RNA virus belonging to the order Mononegavirales, family Rhabdoviridae, genus *Lyssavirus* (1). Transmission of rabies infection usually occurs when infected saliva reaches a bite wound or skin scratches, or breaches mucous membranes. A rare route of rabies infection transmission include aerosol infection as in bat caves, ingestion of an infected carrier and trans-placental infection. Transmission has occurred in man following transplants of corneas taken from infected patients. Not all animals or human bitten contract the infection. The severity, location, and multiplicity of bites inflicted on the victim, biotype of the virus and the susceptibility of the recipient influence the outcome of potential exposure to infection. Bites on the head and neck are associated with the shortest incubation period (2).

Rabies is a serious public health problem in developing countries, especially in Asia. Approximately 35000 to 50000 human deaths occur due to rabies each year (3). Administration of rabies vaccine along with anti-rabies immunoglobulin is known to prevent development of rabies; however, prompt and precise diagnosis is essential for rabies diagnosis, direct immunofluorescence detection of rabies virus antigens has been used worldwide as a rapid and reliable method.

Most rabies-specific antibodies used for diagnosis are made from sera of immunized mammals such as mice, rabbits and goats. However, producing a large amount of specific antibodies from these animals is time-consuming and labor intensive. There is a concern that handling live and large amounts of rabies virus to produce antigen may pose a potential risk of infection to laboratory personnel (4).

Recent advances in molecular biology together with newly invented methods of producing antigen-specific antibodies in egg yolk (IgY) have created new opportunities to develop a safe, convenient and inexpensive way of manufacturing various immunodiagnostics (5.6).

The IgY project which developed uncomplicated techniques for immunization of hens, isolation of egg yolk antibodies, and their applicability in various test systems creates continuous considerations in regard of changing from mammalian derived antibodies to egg yolk antibodies for both, (The National Laboratory for Immunology and diagnostics and the Internationally Orientated Service Laboratory).

Regarding commercially available polyclonal antibodies being produced worldwide it was found that

Author α σ ρ: Veterinary Serum and Vaccine Research Institute, P.O. Box: 131, Abbasia, Cairo, Egypt. e-mails: behwara@gmail.com, svri@idsc.gov.eg

297 from almost 16,000 which is less than 2% of polyclonal antibodies deriving from chicken, and only three from these have been prepared from egg yolk, the rest apparently from chicken sera, it may be concluded that in general, chicken are potent antibody producers. Obviously, the IgY-technology needs further propagation (7).

The present work aims to prepare anti-rabies IgY in chicken egg yolk in addition to preparation of anti-rabies IgG in rabbits and evaluate their efficacy in protection against rabies infection in emergency cases simulated in mice.

II. Materials and Methods

Ethics approval: The experiments were carried out according to the protocol of Institutional Animal Ethics Committee and the authors had a permission of the animal owners at the private farms.

a) Baby hamster kidney cell culture (BHK21)

BHK21 was supplied by DPAVR, VSVRI and used in application of SNT to estimate rabies neutralizing antibody titers in the obtained anti-rabies IgY and IgG preparations.

b) Viruses

i. *Cell culture adapted rabies virus*

Evelyn Rokitnicki Abelseth (ERA) strain of rabies virus adapted to BHK-21 cell line with a titer of 7 $\log_{10}$ $TCID_{50}$ /ml was supplied by Department of Pet Animal Vaccine Research (DPAVR), Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt. It was used in serum neutralization test to estimate the induced antibody titer in immunized chicken.

ii. *Challenge virus strain (CVS)*

Mice brain adapted rabies virus with a titer of 6.5 $\log_{10}$ MLD_{50}/ml was obtained from the DPAVR- VSVRI and used for experimental infection of mice.

c) Experimental Hosts

i. *Chickens*

Fifteen, 35 week-old Rhode Island Red (RIR) hens, were used for egg yolk IgY preparation after their immunization with local rabies vaccine in a dose of 0.5 ml/hen inoculated subcutaneously on a week intervals for 5 successive weeks according to (8). Eggs were collected weekly for 6 weeks from these hens where the anti-rabies IgY were separated and titrated.

ii. *Rabbits*

Ten Bosket rabbits of about 3kg body weight were used for preparation of anti-rabies IgG after their immunization with the local rabies vaccine in a dose of 0.5 /rabbit inoculated subcutaneously on a week intervals for 5 successive weeks according to (8).

Weekly blood samples were collected from chicken and rabbits for 6 weeks for monitoring the levels of induced rabies antibodies in their sera.

iii. *Mice*

Two hundreds and five weaned Swiss Albino mice (3-4 weeks old) were experimentally infected with 0.03 ml of CVS /mouse intramuscularly while five mice are kept without infection.

d) Rabies vaccine

Inactivated cell culture rabies vaccine (ERA strain) was obtained from DPAVR- VSVRI and used for inoculation of chicken and rabbits to prepare anti-rabies IgY from chicken egg yolk and IgG from rabbit sera.

e) Isolation and separation of chicken egg yolk IgY

IgY was separated from egg yolk with PEG-6000 as described by (9). and its protein content was measured as described by (10). Where it was 2.32 g /dl.

f) Separation of rabies IgG from rabbit serum

Rabies IgG was precipitated from rabbit serum using ammonium sulphate according to (11). And its protein content was estimated according to (10), and it was 3.42 g /dl.

g) Quality control testing of the prepared anti-rabies IgY and IgG

Quality control testing of the obtained preparations were carried out following the directions of (3). Including sterility, safety and potency tests.

h) Serum neutralization test (SNT)

SNT was carried out to estimate rabies neutralizing antibodies in test chickens and rabbits as described by (12), and the antibody titer was determined as the reciprocal of the final serum or immunoglobulin preparations dilution which neutralized and inhibited the appearance of the cytopathic effect (CPE) of 100 $TCID_{50}$ of rabies virus.

i) Experimental design

The previously mentioned infected mice were divided into seven groups where each of the first 5 groups included 40 mice, where the 1st and 2nd groups received anti-rabies IgY and IgG using a dose of 0.5 ml/mouse inoculated intraperitoneally containing 116 mg of IgY and 171 mg of IgG respectively on daily intervals started from the day of experimental infection up to 7 days post infection. The 3rd and 4th groups were treated with IgY with rabies vaccine and IgG with rabies vaccine respectively using the same mentioned doses (As in groups 1and 2) where rabies vaccine was inoculated once in a dose of 0.5ml /mouse inoculated intraperitoneally on the same day of infection. The 5th group was treated with the rabies vaccine alone with the same dose.

The 6th group of 5mice was kept infected without any treatment (Control +ve). In addition the

7th group of 5 mice was kept without infection as (Control -ve).

III. Results and Discussion

The present results showed that the prepared anti-rabies IgY and IgG were free from foreign contaminants (aerobic and anaerobic bacteria, fungi and mycoplasma) and safe, inducing no abnormal signs in inoculated mice either generally or at the site of inoculation as shown in Table (1). These findings come in agreement with the recommendations of (13).

Table (1) : Quality control testing of anti-rabies IgY and IgG

Tested preparation	Protein content	Sterility	Safety	Potency
Anti-rabies IgY	2.32g/dl	Sterile	Safe	Potent
Anti-rabies IgG	3.42g/dl			

In addition, estimation of the protein contents in the prepared anti-rabies chicken egg yolk IgY and rabbit IgG revealed that they had levels of 2.32g/dl and 3.42g/dl respectively Table(1). Such high levels of protein contents could be attributed to the formation of antibodies which mainly consisted of globulins as stated by (14), (15), (16) and (17).

Table (2) demonstrated that rabies serum neutralizing antibody titers began to appear in the sera of chickens and rabbits by the 1st week post immunization recorded their highest levels (64 and 128 respectively) by the 5th week. These findings showed that rabbits have higher antibody titers than that of chickens, the thing which could be attributed to the host susceptibility as rabies is a mammal host specific virus (4). It was found that the levels of rabies antibodies in chicken and rabbit sera were increased gradually started from the 1st week post immunization recording their peak by the 5th week. In this respect similar findings were obtained by (18), (19)., (20) and (21). who considered such sera as hyper- immune preparations depending on their high antibody titers where the protective rabies antibody titer is 0.5 IU (about titer of 32).

Table (2) : Rabies neutralizing antibody titers in sera of chicken and rabbits

Tested serum	Mean serum rabies neutralizing antibody titer*/WPI**					
	1WPI	2WPI	3WPI	4WPI	5WPI	6WPI
Chicken	8	16	32	32	64	64
Rabbit	8	16	32	64	128	128

**Antibody titer = the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100TCID$_{50}$ of rabies virus*

***WPI= week post immunization*

On the other hand, rabies neutralizing antibody titers were found to be 64 and 128 in chicken egg yolk IgY and rabbit serum IgG respectively Table (3) in a parallel manner confirming that such preparation could be considered as hyperimmune products against rabies virus.

Table (3) : Rabies neutralizing antibody titers in chicken egg yolk IgY and rabbit IgG preparations

Tested preparation	Mean rabies neutralizing antibody titer*/WPI**					
	1WPI	2WPI	3WPI	4WPI	5WPI	6WPI
Chicken egg yolk IgY	0	16	32	64	64	64
Rabbit IgG	4	8	16	32	64	128

**Antibody titer = the reciprocal of the final immunoglobulin preparations dilution which neutralized and inhibited the CPE of 100TCID$_{50}$ of rabies virus*

***WPI= week post immunization*

Treatment of experimentally rabies infected mice with the prepared anti-rabies IgY and IgG showed that the best time for administration of anti-rabies treatment is from 0 time to 2 days post exposure to virus infection providing 100% protection. This protection rate decreased to 40, 20 and 0% for treatment with IgY and 60, 30 and 0% for treatment with IgG on the 3, 4 and 5 days later as shown in Table (4). Non-treated infected mice showed typical rabies signs represented by paralysis of the hind limbs and tail by the 4th day post infection ended with death while non-infected non-treated mice remained healthy allover the experimental period. These findings come in complete agreement with what reported by (21)., (22). And (17). who concluded that post exposure treatment through passive immunization of the victim should be carried out as soon as possible post exposure to viral infection recommended the same present recorded times. Similar results were obtained in case of infected mice treated with either of IgY or IgG with rabies vaccine while treatment with rabies vaccine alone was unable to protect mice against rabies virus infection the thing

which could be attributed to the fact that stimulation of active immunity require longer time to be detectable; such time was found to be overcame by the passive immunity provided by IgY and IgG.

Table (4) : Potency of chicken egg yolk anti-rabies IgY and rabbit anti-rabies IgG in mice

Group	Received Treatment	Number of survived mice/number of treated mice = protection%							
		0 DOT*	1 DOT	2 DOT	3 DOT	4 DOT	5 DOT	6 DOT	7 DOT
1	IgY	100	100	100	40	20	0	0	0
2	IgG	100	100	100	60	30	0	0	0
3	IgY& rabies vaccine	100	100	100	40	20	0	0	0
4	IgG &rabies vaccine	100	100	100	60	30	0	0	0
5	Rabies vaccine alone	These mice were unable to withstand the virus infection where the active immunity required longer time to be effective (0%)							
6	Infected &not treated mice showed typical rabies signs stared by the 4th day post infection (control +ve) (0%)								
7	Non infected &non treated mice remained healthy allover the experimental period (control –ve)								

**DOT= day of treatment post infection*

IV. Conclusion

Depending on the obtained results through the present work, it could be concluded that both of anti-rabies chicken egg yolk IgY and rabbit serum IgG are able to withstand rabies infection when they are administrated on the optimum time post exposure as simulated in mice and further studies are in need to evaluate such preparations in farm animals. In addition, the preparation of chicken IgY in a non-specific host provides safe, high potent product of lower cost than that prepared in rabbits or other mammals where a huge amount of IgY could be obtained through the egg production life of hens with easily housing and simple management requirements.

Author's contribution

Ahmed Mohamed Albehwar immunized hens and rabbits with rabies vaccine and collected egg of hens and serum of the rabbits and prepared the IgY and IgG solutions, applied SNT on the obtained preparations to determine the antibody titers, evaluated the results and revised the data and write the research.

Abeer Atia Tammam estimated the IgY and IgG content in the obtained preparations and made the quality control testing on both of them besides sharing in application of SNT and writing the research.

Amr Ismael Hassan made experimental Infection of mice then immunized them with the obtained preparations to determine their protection rates and analyzed and tabulated the obtained data. All authors read and approved the final manuscript.

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Competing interests: The authors declare that they have no competing interests.

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كفاءة إميونوجلوبيولينات السعارIgG و IgY لوقاية الفئران من عدوى الفيروس كنموذج للتدخل الاضطرارى

أحمد محمد البهوار, عبير عطية تمام, عمرو إسماعيل حسن

معهد بحوث الأمصال واللقاحات البيطرية- العباسية- القاهرة

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تم خلال هذا العمل تحضير IgY وIgG مضادين لفيروس السعار فى مح بيض الدجاج ومصل الأرانب حيث وجد أن محتوى الجلوبيولين هو 2.32 جرام / ديسيلتر و 3.42 جرام/ ديسيلتر على التوالى وكان معيار أجسام السعار المناعية الخلطية (باستخدام اختبار المصل المتعادل) 64 و128 فى IgY و IgG على التوالى موازيالمثيله فى أمصال الدجاج والأرانب المستخدمة فى تحضير هذه المستحضرات.

ولقد وجد أن كلا المستحضرين خالى من الملوثات قبل الحقن وآمن عند حقنه فى الفئران السويسرية وعند الحقن فى الفئران المعدية تجريبيا بفيروس السعار الضارى (المؤقلم على أمخاخ الفئران) بتجويف البطن على فترات يومية بعد العدوى، تبين أن أنسب وقت للتدخل بهذه المستحضرات (بتوفير مناعة سلبية) هو من وقت التعرض للإصابة وحتى اليوم الثانى معطيا نسبة حماية 100% بينما تقل هذه النسبة إلى 40، 20 ثم 0 % عند العلاج باستخدام IgY وإلى 60، 30 ثم 0% عند استخدام IgG فى الأيام 3،4، 5 بعد التعرض للعدوى.هذا وقد تم الحصول على نفس النتائج حال استخدام كل من IgY وIgG مع اللقاح فى حين لم يكفى استخدام اللقاح وحده لحماية الفئران ضد عدوى الفيروس حيث أن الأمر يتطلب وقتا أطول لاستحداث مناعة إيجابية تكفى للتغلب على الفيروس 0وعلى ذلك يمكن االتوصية باستخدام أحد المستحضرين مع اللقاح حال التعرض للعقر من حيوانات مصابة.

كما يمكن القول بأن كل من المستحضرين له القدرة على التغلب على عدوى السعار خاصة عندما يتم التدخل به فى الوقت المناسب،إلا أن تحضير IgY فى مح بيض الدجاج يستلزم جهد وتكلفة أقل وبكميات أكبر من إنتاج IgG فى الأرانب إضافة إلى كونه محضر فى عائل غير أساسى الأمر الذى يزيد من أمان تحضيره واستخدامه.