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ROLE OF HYPERIMMUNE SERUM IN PROTECTION AGAINST VIRAL HAEMORRHAGIC DISEASE OF RABBITS

(With 2 Figures and 3 Tables)

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تأثير المصل المحتوى على أجسام مناعيه عاليه فى الوقايه ضد مرض النزف الدموى الفيروسى للأرانب

طلبه عبد المطلب

فى هذه الدراسة تم الحصول على نسبة وقايه ١٠٠ ٪ عن طريق حقن أرانب نيوزلندى فى العضل بالمصل المحتوى على أجسام مناعيه عاليه فى نفس الوقت مع الفيروس أو قبل اعطائه ب ٢٤ أو ٤٨ أو ٧٢ ساعة ضد مرض النزف الدموى الفيروسى للارانب. بينما أمكن الحصول فقط على نسبة وقايه ٦٠ ٪ فى مجموعة الارانب التى تم حقنها بالمصل بعد ٢٤ ساعه من الإصابة بالفيرس. وفى ضوابط التجربة بلغت نسبة الوفيات ٨٠ ٪ خلال الفترة من ٢-٥ أيام بعد حقن الفيروس فى العضل وقد لوحظ هزال على الأرانب التى تبقت حتى نهاية التجربة. وبدراسة الصفات البيولوجيه للفيروس عن طريق إختيار تلازن الدم أعطت نتائج إيجابيه بإستخدام كرات الدم الحمراء للإنسان والدجاج، السمان، الحمام، الاغنام، الماعز، الجاموس، البقر، الحمار، الجمال بينما أعطت نتيجة سلبيه مع كرات الدم الحمراء للبط مع وجود تباين فى هذه الأنواع فى نتيجة الإختبار. وقد وجد تدرج فى معدلات نتيجة إختبار تلازن الدم فى أحشاء الأرانب النافقه بعد العدوى بالفيروس كالآتى الكبد - الطحال - الكليتين - الرئتين - القصبه الهوائيه - المخ وأخيرا القلب. وقد كانت نسبة الأجسام المناعيه المضاده بواسطة الإختبار المانع للتلازن الاعلى فى مجموعة الضابط والأقل فى مجموعة الأرانب المحقونة بالفيروس بعد أعطاء المصل المضاد للمرض. وفى محاوله للكشف عن الأجسام المناعيه المرسبه فى المصل بواسطة إختبار الترسيب فى الآجار جل لوحظ عدم وجود خطوط ترسيبيه.

HYPERIMMUNE SERUM, VIRAL HAEMORRHAGIC DISEASE OF RABBITS

SUMMARY

Hyperimmune sera administered intramuscularly (i/m) in 4 months old Newzealand rabbits either simultaneously with the virus or before its injection by 24, 48 or 72 hours give 100% protection against infection with the viral haemorrhagic disease of rabbits while 60% protection was given in rabbits treated with hyperimmune sera 24 hours post infection. I/M administration of the virus killed 80% of the inoculated rabbits within 2-5 days, rabbit that recover was severely emaciated. The virus was able to agglutinate the erythrocytes of human type O, chickens, quails, pigeons, sheep, goats, buffaloes, cattle, donkeys and camels but not ducks, although the haemagglutination (HA) titers varied. The HA titers of various tissues of rabbits which had died of experimental infection were in the folowing order: liver> spleen> kidney> lung> trachea> brain> heart. At the end of experiment the highest haemagglutination inhibition antibody titer (HI) was detected in the only survived rabbit i/m injected with the virus but the lowest was reported in rabbits treated in rabbits treated with hyperimmune sera before infection. There was no detectable precipitating line between the virus and the specific antisera in immunodiffusion test.

INTRODUCTION

Recently viral haemorrhagic disease of rabbits (VHD) is widely distributed in several countries of the world. It is highly fatal disease causing mortality up to 100% *(Fiorettti et al., 1991)*, hence this disease threaten the rabbitries wherever. The disease was firstly reported in China *(Liu et al., 1984)* and it has been reported subsequently in Italy *(Marcato et al., 1988)*, Bulgaria *(Belemezov et al., 1989)*, Austria *(Kolbl et al., 1990)*, Belgium *(Peeters et al., 1990)*. In Egypt the disease was reported by *Ghanem and Ismail (1991) and Salem and Ballal (1992)*. Controlling the outbreaks of the disease either by slaughtering the rabbits and disinfection of the infected area or by vaccination.

Inactivated tissue vaccine induce immunity 3-5 days after vaccination *(Wei et al., 1987; Huang, 1991 and Haralambiev et*

al., 1991). Due to the short incubation period and the course of the disease as well as the required period for animals to be protected against infection after vaccination, the outbreaks causes severe losses so the present investigation was undertaken to investigate the protective value of hyperimmune sera against VHD in addition to investigating some properties of the virus.

MATERIAL AND METHODS

Virus: The virus was previously isolated and identified from outbreaks of rabbits in Assiut Province by *Salem and Ballal (1992).*

Organ suspension preparation: For experimental infection, an approximately 20% suspension of organs (liver, spleen, kidney, lung and brain) of a dead rabbit was prepared. The suspension centrifuged at 6000 rpm for 30 minutes. The supernatant were collected and then added 10,000 I.U penicillin and 10 mg streptomycin /ml suspension leaving for 2 hours before animals inoculation.

Hyperimmune sera: were collected from vaccinated challenged animals *(Salem and El-Zanaty, 1992).* The haemagglutination inhibition antibody titer of this sera was 1:2048.

Experimental animals: Thirty 4 months old Newzealand rabbits were obtained from private farm with no history of VHD outbreaks in this farm and in surrounding areas and before infection the rabbits proved to be serologically negative for VHD, These animals were divided into four main groups:

Group, I: Contain 5 animals inoculated with the virus and hyperimmune sera simultaneously.

Group, II: Contain 5 animals inoculated with hyperimmune sera 24 hours post-infection.

Group, III: Contain 15 animals, each animal inoculated with hyperimmune sera, then divided into three equal subgroups.

Subgroup A: Contain 5 animals, each animal infected with viral suspension 24 hr. after hyperimmune sera inoculation.

Subgroup B: Contain 5 animals, each animal infected with viral suspension 48 hr. after hyperimmune sera inoculation.

Subgroup C: Contain 5 animals, each animal infected with viral suspension 72 hr. after hyperimmune sera inoculation.

Group, IV: Contain 5 animals, each animal infected with viral suspension and kept as control.

N.B

**Both* viral suspension and hyperimmune sera were injected I/M with I ml/animal.

* The duration of experiment is 3 weeks in which the infected animals were observed for clinical signs and mortalities. Dead animals subjected to post mortem examination and organs of liver, spleen, kidney, lung, trachea, brain and heart were collected and suspensions were made for virus detection.

ErythrocyteS: Citrated blood samples were collected from human type O, chicken, pigeon, quail, duck, sheep, goat, buffalo, cattle, donkey and camel. Erythrocyte suspensions were prepared with 1% concentration in phosphate buffer saline solution.

Haemagglutination test (HA test): HA activity of organ suspension was detected using the method described by *Kolbl et al. (1990).*

Immunodiffusion test: Immunodiffusion test was carried out after *Benjamin and Hitchner (1979).*

Haemagglutination inhibition test (HI test): HI test was conducted using the procedures described by *Pu et al. (1985).*

RESULTS

No Clinical signs or mortalities were observed in both animals that inoculated with virus and hyperimmune sera simultaneously and that inoculated with hyperimmune sera before infection by 24, 48 or 72 hours.

Experimentally infected animals (control group) as well as three animals out of 5 in treated group with hyperimmune sera 24 hours post infection showed clinical signs as off food, depression, dyspnea, incoordination, paralysis, convulsions was observed in some animals directly before death. Vaginal bloody discharge was noticed in one animal. One animal died suddenly without showing clinical manifestations, mortality occurred 2-5 days post infection in the control group (4 out of 5 animals) while 3-5 daysafter infection (2 out of 5 animals) was observed in the group II as shown in Table (1). No further deaths occurred until the survivors were necropsied at 21 days post infection.

The necropsied dead animals showed severe tracheitis, congested lungs with minute haemorrhages, enlarged pale liver (Fig. 1). Congested kidneys and petechiae was noticed only in one animal. Heart, brain (Fig. 2)and intestinal blood vessels

were congested, sanguinous fluid was observed in thorax and abdomen in few animals. The virus was detected from the dead animals by HA test.

HA activity of tissue suspensions (liver, spleen, kidney, lung, trachea, brain, heart) to human type O, chicken, quail, buffalo and sheep erythrocytes were more sensitive and give higher agglutination titers than other erythrocytes as shown in Table (2). It is worth to mention that the HA titer in the dead animals infected before 24 hours of hyperimmune sera treatment is usually less than that recorded in dead animals which inoculated with virus only.

The highest HI antibody titers in the survivor animals at the end of experiment were reported in rabbit only injected with virus, followed by rabbits infected before hyperimmune sera treatment, then animals simultaneously inoculated with virus and hyperimmune sera and lastly in animals treated with hyperimmune sera before infection by 24, 48 or 72 hours with minor variation among them as illustrated in Table (3).

DISCUSSION

Viral haemorrhagic disease of rabbits is one of the greatest problems facing rabbitries during the last few years. Several epornitics were recorded in our country in Assiut, Sharkia, Gharbia and Dakuhlia Governorates causing high mortalities in rabbit population in these areas. In rabbits simultaneously injected with virus and hyperimmune sera no clinical signs or mortalities during the observation period were recorded, these results coincide with the findings of *Ghanem and Ismail (1991).*

Also there is no clinical manifestations or mortalities were observed in animals injected with hyperimmune sera 24, 48 or 72 hours before infection. It is obvious in the two previously mentioned groups that the infectivity of the virus was inhibited by hyperimmune sera administration with 100% protection. On the other hand, the rabbits treated with hyperimmune sera 24 hours after infection showed only 60% protection, our observation are in agreement to some extent with those described by *Peschlejski* et al. *(1991).* who mentioned that losses ceased (91. 6% protection) in 6 recently infected groups of rabbits two days after serum treatment.

The clinical manifestation of the viral injected rabbits and to less degree in severity in hyperimmune sera treated rabbits 24 hours after virus injection were off food, dyspnea, depression, incoordination, paralyses, convulsions and death, vaginal bloody discharge and severe emaciation of the recovered

rabbits were also observed. Similar data in naturally and experimentally infected rabbits were reported by *Boucher (1989) and Lee* et al. *(1990).* 80% and 40% mortalities in the viral infected rabbits and those treated with hyperimmune sera 24 hours after infection were recorded. Similar mortality % in natural and experimental infection were reported by *Nowotny* et al. *(1990) and Mocsari* et al. *(1991).*

At necropsy of the dead rabbits, minute haemorrhages in the respiratory organs and occasionally in the kidney were found, discolored liver, severe congestion of heart, brain and intestinal blood vessels, thoracic and abdominal sanguinous fluid with enlargement of the urinary bladder, these lesions to some extent in accordance with those reported by *Liu* et al. *(1984); Boucher (1989); Gregg and House (1989) and Lee* et al. *(1990).*

Organ suspensions from experimentally dead rabbits showed the highest titer of haemagglutinating activity with erythrocytes of human type O, followed by buffalo, chicken, quail and sheep.

Erythrocytes of goat, pigeon, donkey, camel and cattle give very low haemagglutination titer. On the other hand erythrocytes of duck give negative results. Our data inagreement to great extent with those reported by *Liu* et al. *(1984);Ghanem and Ismail (1991) and Salem and Ballal (1992)* for human erythrocytes and with some extent with those reported by *Zhao* et al. *(1988)* for other erythrocytes. On the other hand our results disagree with those reported by *Ghanem and Ismail (1991)* for chicken and animal erythrocytes. Itis worth to mention that higher haemagglutiation titer is usually observed in rabbits infected with virus than treated with hyperimmune sera post-infection. Haemagglutination titers of different organs of dead rabbits were higher with liver, spleen, kidney, lung and trachea extracts than with brain and heart, these results coincide with the findings of *Zhao* et al. *(1988) and Salem and Ballal (1992).*

At the end of the experiment the necropsy of the survivor rabbits showing no clear macroscopic findings, also the HA activity of organ suspensions was not detected except in rabbit of the control group.

There was a minor variation in haemagglutination inhibition antibody titers of necropsied survivor rabbits of different groups. Unsuccessful trial for detecting a precipitin line between the virus and specific antisera was conducted.

Finally we can conclude that the hyperimmune serum play a great role in protection against VHD of rabbits when given

before or at the same time of infection and play a moderate role in protection when used post infection.

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Table (1): Illustrate the route of inoculation, type of treatment, mortality and protection percentage among different groups of experimentally injected rabbits.

Groups	No.of rabbits	Breed	age	route of inoculation	type of treatment	Mortality No.	Mortality %	Protection %
I	5	Newzealand	4 months	I/M	virus with antisera simultaneously	0/5	0	100
II	5	Newzealand	4 months	I/M	virus 24 hr. before sera	2/5	40	60
III A	5	Newzealand	4 months	I/M	sera 24 hr. before virus	0/5	0	100
III B	5	Newzealand	4 months	I/M	sera 48 hr. before virus	0/5	0	100
III C	5	Newzealand	4 months	I/M	sera 72 hr. before virus	0/5	0	100
IV	5	Newzealand	4 months	I/M	virus only	4/5	80	0

Table (2):HA activity of 10% organ suspension of dead animals using human type O, avian and animals erythrocytes.

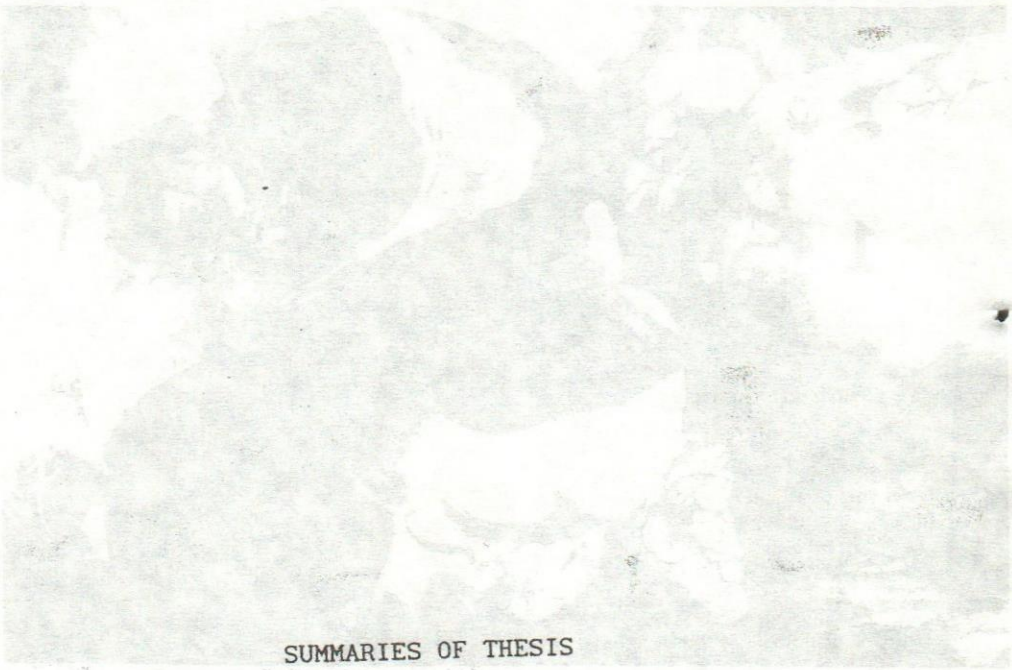
Erythrocytes	HA titers of different organ suspension						
	Liver	spleen	kidney	lung	trachea	brain	heart
I.Human type O	2^{8-10}	2^{7-11}	2^{6-11}	2^{5-9}	2^{4-6}	2^{3-5}	2^{3-4}
II.Bird							
Chicken	2^{5-7}	2^{5-7}	2^{4-5}	2^{3-4}	2^{3}	2^{2}	2^{2}
Quail	2^{5-7}	2^{4-7}	2^{3-4}	2^{3-4}	2^{3}	2^{2}	2^{2}
Pigeon	2^{3-5}	2^{3-4}	2^{2-3}	2^{2-3}	2^{2}	-ve	-ve
Duck	-ve	-ve	-ve	-ve	-ve	-ve	-ve
III.Animals							
Buffalo	2^{6-8}	2^{5-6}	2^{4-5}	2^{4-5}	2^{3-4}	2^{3}	2^{2}
Sheep	2^{5-6}	2^{4-5}	2^{3-4}	2^{3-4}	2^{3}	2^{2}	-ve
Goat	2^{4-5}	2^{3-4}	2^{3}	2^{2}	-ve	-ve	-ve
Donkey	2^{3-4}	2^{2-3}	2^{3}	2^{2}	-ve	-ve	-ve
Camel	2^{3-4}	2^{3}	2^{2-3}	2^{2}	-ve	-ve	-ve
Cattle	2^{2-3}	2^{2}	-ve	-ve	-ve	-ve	-ve

Table (3):Illustrate HI antibody titers in survivor rabbits at the end of experiment.

Groups	No. of rabbits	H I titer
I	5	2^{7-9}
II	3	2^{8-9}
III A	5	2^{7-8}
III B	5	2^{6-8}
III C	5	2^{6-8}
IV	1	2^{10}

Fig. (1)

Fig. (2)



SUMMARIES OF THESIS