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CLINICAL, BIOCHEMICAL AND HEMOSTATIC ADVERSE REACTIONS IN YOUNG, MIDDLE AGED AND GERIATRIC HORSES IN CONSEQUENCE OF THEIR USE IN ANTI-TETANIC SERA PRODUCTION

M. M. EL-HANAFY and A. EL-SAYED
Department of Internal Medicine and Infectious Diseases
Faculty of Veterinary Medicine Cairo University

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SUMMARY

The immunization of horses with tetanus toxins for the production of antitetanic sera was associated with significant vital changes, a significant rise of body temperature and a slightly significant increase of pulse and respiratory rates in young, middle aged and geriatric horses. All horse groups showed different degrees of local reactions at the sites of injection of tetanus toxins; beginning from diffuse, large, hot, tender, and painful swelling swellings extending from the thoracic inlet to the head at the injected side of the neck which subsided gradually to multiple local abscesses formation at the sites of injections followed by complete sloughing of the skin with ulcer formation covered by creamy yellow pus.

The immunological investigations showed significant increase in the total leukocytic count with concomitant increase of both polymorphnuclear and band cells in all horses groups and the quantitive estimation of tetanus toxins antibodies revealed better response of young horses than middle aged and geriatric horses .

The hematological investigations revealed a significant decrease in hemoglobin as well as RBCs count with concomitant decrease in MCHC in geriatric horses more than young and middle aged ones. Significant increase in prothrombin time and significant decrease in activated partial thromboplastine time as well as platelets count were recorded in geriatric horses.

Serum biochemistry revealed a significant decrease in total proteins with concomitant decrease in albumin in middle aged and geriatric horses groups. Serum AST was significantly increased in all horse groups and total bilirubin was significantly increased in middle aged and geriatric horses.

INTRODUCTION

Active immunization against infectious diseases is important. However, agents that provide passive immunity thus remain essential biologicals. The most important of these are equine antisera against rabies, tetanus, diphtheria, and snake antivenins. Since 1891 horses have been used by some organizations for the production of prophylactic and therapeutic antisera for the human welfare, where horses are subjected to a process of active immunization by toxins, toxoid, and different venoms for the production of antitoxins or antivenoms. Several traditional sources of equine antisera are becoming depleted as a result of economic disincentives; poor reputation based on the high adverse reaction rates of the old, unpurified products; and the activities of animal rights activists who object to the use of horses as blood donors (Wilde et al., 1996). The present investigation is designated to study the adverse reactions in young, middle aged and geriatric horses regarding clinical, biochemical and hemostatic alterations in consequence of their use for anti-tetanic sera production.

MATERIAL AND METHODS

1. Horses:-

Forty apparently healthy horses belonging to the laboratory animal farm of the Egyptian Organization for Biological Products and Vaccines, (VACSERA) were used for the production of commercial hyperimmune sera.

They were classified into 4 groups according to their ages to study the pathophysiological, clinical, and immunological changes in response to tetanus antisera production.

Group I: Ten horses less than 5 years old, as control group

Group II: Ten prevaccinated horses less than 5 years old

Group III: Ten prevaccinated horses less than 10 years old

Group IV: Ten prevaccinated horses less than 15 years old

2. Toxins and immunization schedule:-

Tetanus toxins: the toxins used in the present study were prepared and titrated by the Egyptian Organization for Biological Product and Vaccines Laboratories. (VACSERA) according to the methods described by Keleser and Schoening (1948).

The young, middle aged and geriatric horses groups were vaccinated with tetanus toxins, for the production of commercial hyperimmune sera according to the following program.

Day	Dose
1	75 ml
4	120 ml
7	150 ml

The tetanus toxins were injected subcutaneously in alum hydroxide adjuvant, each ml contains 20 flocculation units, each flocculation unit is equal to 1000 minimum lethal dose (MLD), the MLD is the dose of toxins sufficient to kill a guinea pig of about 350 grams body weight.

The experimental horses were confined in the stable during the whole experimental period, each horse was fed on concentrated mixture (2 kg pelleted concentrate, 1.5 kg horse bean and 1 kg yellow corn) divided on two times a day and green food was offered in between.

3. Samples and measured parameters:-

A total of 80 whole blood and serum samples were collected three days following the end of the immunization program. A detailed clinical assessment was applied for each horse.

Blood samples were collected by jugular vein-puncture into heparinized vacutainers, immediately mixed gently and kept in ice box. These samples were used for the determination of total leukocytic (WBCs), differential leukocytic and blood platelets counts according to Schalm et al., (1975).

Part of each blood sample was taken at the same time on plain vacutainer, left to coagulate at room temperature and was put in the refrigerator till the clot was retracted. The serum was removed by a Pasteur pipette and clarified by centrifugation at 3000 R.P.M for 15 min. Each individual serum sample was divided into two aliquots then kept in a deep freeze at -20°C until the different assays were carried out. The sera obtained were used for the determination of total protein, albumin, total bilirubin, aspartate aminotrasferase (AST), alkaline phosphatase (PAL), creatinphosphokinase (CPK), urea, creatinine, sodium, potassium, and antitetanic IgG.

Quantitative estimation of antitetanic sera was assayed by direct enzyme linked immunoassay according to Voller and Desavingy (1981).

Other whole blood samples were collected into citrated tube. These samples were used for the determination of fibrinogen, prothrombin time, and activated partial thromboplastin time according to the method described by Schalm et al., (1975).

The obtained results were statistically analyzed using (SXW) statistics for windows.

RESULTS

I. General health condition

a) Systemic reactions;

The injection of horses with tetanus toxins antigen for the production of anti-tetanic sera was

associated with significant vital signs changes as presented in table (1), there was a significant rise of body temperature accompanied by a significant increase of pulse and respiratory rates in all injected horse groups.

These systemic alterations were accompanied by moderate anorexia, muscle weakness, variable degrees of CNS inhibition, as dullness, depression, lethargy and apathy as well as congestion of visible mucous membranes and sweating from time to time which slightly normalized the animalis condition.

Table (1): General health conditions in horses in consequence of their use in anti-tetanic sera production.

Measured Parmeters	Control horses	young horses	Middle aged horses	Geriatric horses
Respiration /m	16.5 ± 0.73	20.6 ± 0.82***	19.9 ± 0.88**	20.1 ± 0.74**
Pulse /m	30.5 ± 1.27	38.1 ± 2.40***	38.5 ± 2.41**	36.0 ± 1.33***
Temperature C°	37.5 ± 0.09	38.3 ± 0.14	38.0 ± 0.07***	37.9 ± 0.12**

The results are presented as mean ± S.E.
*P < 0.05; **P < 0.02; ***P < 0.01 when compared to the control group

b) Local reactions:

The horses showed different degrees of local reactions at the sites of tetanus toxins injection. Some cases showed diffuse, large, hot, tender, and painful swellings extending from the thoracic inlet to the head at the injected side of the neck. These swellings subsided gradually to multiple local abscesses formation and complete sloughing of the skin with ulcer formation covered by a creamy yellow pus.

II. Hematological alterations

Table (2): Hematological alterations in horses in consequence of their use in anti-tetanic sera production.

Measured Parmeters	Control horses	young horses	Middle aged horses	Geriatric horses
HB gm%	14.5 ± 0.42	13.3 ± 0.31**	13.0 ± 0.48**	10.6 ± 0.39***
RBCs X 10^6/µl	8.71 ±0.31	7.51 ± 0.26***	7.58 ± 0.25***	6.53 ± 0.22***
PCV %	42.4 ± 1.62	42.0 ± 1.65	41.3 ± 1.51***	35.9 ± 0.70***
MCV fl	47.4 ± 0.65	56.1 ± 2.12***	54.7 ± 1.51	49.0 ± 5.46
MCHC gm%	35.1 ± 0.70	31.5± 1.16	31.5 ± 0.70***	30.4 ± 1.18***

The results are presented as mean ± S.E.
*P < 0.05; **P < 0.02; ***P < 0.01 when compared to the control group.

III. Immunological alterations

Table (3) Immunological alterations in horses in consequence of their use in anti-tetanic sera production

Measured Parameters	Control horses	Young horses	Middle aged horses	Geriatric horses
WBCs /µl	5307 ± 120	8300 ± 329***	8850 ± 257***	8177 ± 149***
Neutrophil /µl	2751 ± 81.5	5200 ± 268***	5386 ± 153***	4842 ± 117***
Esinophil /µl	111 ± 10.8	111 ± 9.89	120 ± 6.06	100 ± 7.52
Basophil /µl	0.00	0.00	0.00	0.00
Lymphocyte/µl	2332 ± 56.8	2346 ± 108	2818 ± 149	2708 ± 8306
Monocyte /µl	108 ± 6.75	129 ± 10.3	106 ± 4.71	115 ± 8.45
Band cell /µl	65.7 ± 8.18	466 ± 74.1***	477 ± 54.1***	344 ± 30.6***
Antitetanic sera IgG IU/ml	203 ± 73	28186 ± 1011***	25320 ± 934***	24320 ± 1032***

The results are presented as mean ± S.E.

*P < 0.05; **P < 0.02; ***P < 0.01 when compared to the control group

IV. Biochemical alterations

Table (4) Biochemical alterations in horses in consequence of their use in anti-tetanic sera production

Measured Parameters	Control horses	Young horses	Middle aged horses	Geriatric horses
T.P. gm%	6.60 ± 0.06	6.61 ± 0.07	6.43 ± 0.04*	6.01 ± 0.12***
Albumin gm%	3.29 ± 0.12	3.04 ± 0.04	2.80 ± 0.07**	2.77 ± 0.08**
Globulin gm%	3.38 ± 0.19	3.50 ± 0.11	3.61 ± 0.09	3.25 ± 0.11
T. bilirubin mg%	0.67 ± 0.08	0.59 ± 0.08	1.04 ± 0.1*	1.48 ±0.08***
AST U/L	95.9 ± 5.21	153 ± 4.48***	145 ± 7.06***	125 ± 9.62***
Alkaline U/L	140 ± 10.3	146 ± 9.5	151 ± 8.12	138 ± 10.0
CK U/L	22.5 ± 2.54	21.8 ± 2.24	21.8 ±2.24	19.1 ± 2.34
Urea mg%	17.7 ± 2.01	18.7 ± 1.88	23.4 ± 1.89**	25.7 ± 1.13***
Creatinin mg%	0.59 ± 0.09	0.61 ± 0.05	0.77 ± 0.04	1.08 ± 0.14*
Na mEq/L	152 ± 3.07	148 ± 1.95	143 ± 3.09	142 ± 3.10***
K mEq/L	4.00 ± 0.13	4.11 ± 0.12	4.14 ± 0.15	4.13 ± 0.10

The results are presented as mean ± S.E.

*P < 0.05; **P < 0.02; ***P < 0.01 when compared to the control group

V. Haemostatic alterations

Table (5) Haemostatic alterations in horses in consequence of their use in anti-tetanic sera production

Measured Parameters	Control horses	Young horses	Middle aged horses	Geriatric horses
PT sec	11.7 ± 0.29	11.9 ±0.35	12.7 ± 0.33	12.9 ± 0.35*
PTT sec	41.6 ± 0.74	38.2 ± 0.72*	39.1 ± 1.62	34.8 ± 1.06***
Platelets x 10^3/µl	227 ± 6.55	225 ± 6.90	194 ± 10.4	193 ± 6.86***
Fibrinogen mg%	705 ± 25.1	706 ± 20.9	699 ± 18.1	716 ± 26.1

The results are presented as mean ± S.E.

*P < 0.05; **P < 0.02; ***P < 0.01 when compared to the control group

DISCUSSION

I. General health condition

a) Systemic reactions:

The resulting systemic reactions due to tetanus vaccination (Table 1) could be by post-vaccination reactions and the horses suffered from hypersensitivity reaction type III either in the form of localized Arthus reaction and/or systemic serum sickness. This could be explained on the basis that, following the tetanus toxins injection, foreign proteins appear in the circulation; these foreign proteins include tetanus toxins, immune complexes, and destructed tissues which encounter macrophages. The later secret the interleukin 1, which acts on the thermoregulatory centre in the hypothalamus to increase prostaglandin synthesis. The increased prostaglandin synthesis in the hypothalamus elevates the thermostatic set point which increases heat production and decrease heat loss from the animal's body causing fever (Atkins and Wood 1995, and Radostits et al., 2002). Also interleukin 1 acts on the skeletal muscles inducing protein catabolism and thus mobilizes a pool of available amino acids for increased immunoglobulin synthesis by B cells. This eventually results in muscle weakness (Tizard 1987). As reported by Stites et al., (1997) interleukin 1 acts on the CNS increasing the synthesis of sleep inducing factor, which is responsible for the CNS inhibition manifestations.

b) Local reactions:

Agnew and Myers (1994) have described the inflammatory reactions following the repeated antigen injection as Arthus reaction, which is one of the clinical forms of hypersensitivity reaction type III. The reaction is initiated following antigen injection to an animal's body firstly by neutrophils adherence to the vascular endothelium, followed by emigration through the wall of small blood vessels, and as the reaction progresses, severe destruction of vascular walls resulting in hemorrhage and edema leading to swelling. During this process large quantities of neutrophils proteolytic enzymes are released into the tissues. These enzymes mediate the tissue damage seen in the Arthus reaction.

II. Hematological alterations

Regarding the obtained results of hematology, all animals showed a significant decrease in hemoglobin concentration, as well as RBCs count with concomitant decrease in MCHC (main corpuscular hemoglobin concentration).These changes can be attributed to the destruction of red cell in response of tetanolysin toxins of tetanus in all horse groups (Collee et al., 1989 and Ernst and Yuan chung 1990). The more pronounced anaemia detected in the geriatric and middle aged horses is encouraged by chronic liver dysfunction caused by hepatic amyloidosis (Abdelkader et al., 1991).

III. Immunological alterations

There was a highly significant increase in the total white blood cells count with concomitant increase of both polymorphnuclear leukocytes and band cells in injected horses. These leukocytic responses could be attributed to:

* The stimulation of granulopeisis and neutrophils chemotaxis by the activated complements components, due to immune-complexes formation (Roitt 1994 and Stites et al., 1997).
* Macrophage's interleukin 1, which acts directly on the bone marrow stimulating the release of neutrophils into the circulation (Eppstein et al., 1989 and Kampschmidt and Mesecher, 2000).
* Stress induced neutrophilia. (Cannon et al., 1998). Stress of immunization may also play a role in the induction of neutrophilia (Schalm et al., 1975), where the endogenous corticoids increased in secretion in response to the immunization exhausting stress.

As shown in table (3) the level of antitetanic sera shoot, up in all horses groups but young horses showed better response than middle aged and geriatric horses.

IV. Biochemical alterations

As presented in table (4) there was a slight significant decrease in the total proteins with concomitant decrease in albumin in middle aged and geriatric horses groups.

The decrease in serum albumin is probably due to coagulative necrosis of hepatocytes and deposition of amyloid material replacing the hepatocytes which decreased the albumin production in the animal's body (Abdelkader et al., 1991).

Another factor leading to the development of hypoalbuminaemia in these horses is the continuous loss of albumin in the urine as a result of nephropathy and loss of selective permeability of the renal glomerulie due to proliferative glomerulonephritis as a sequel to the deposition of immunocomplexes. Timoney (1985) reviewed that, the renal tissuees are is the most deteriorated tissues following the repeated exposure to the same antigen with pre-existing high level of circulating antibodies, the immune-complex is formed within the circulation followed by the deposition of immune-complexes in the glomeruli followed by leukocytic cell accumulation leading to proliferative glomerulonephritis .

The non significant increase in total globulins is possibly due to the increase of different globulin fractions as serum amyloid A, Ceruloplasmin, C reactive proteins, haptoglobin, and ferritin, which are called acute phase proteins and classified as α globulins (Kaneko et al., 1997). In addition, the increase in γ globulins is due to their production in response to immunization (Kaneko et al., 1997).

Serum AST was significantly increased in all horse groups. The increase in the serum AST is

probably due to coagulative necrosis of hepatocytes and deposition of amyloid material replacing the hepatocytes as well as muscular changes in response to tetanus toxins (Radostits et al., 2002).

Toxemia resulting from injection of tetanus toxins, products of tissue destruction and immune-contplexes also contributed to the degenerative changes in the vital organs and the elevation of serum enzymes.

The slight increase in total bilirubin in middle aged and geriatric horses may be attributed to hepatopathy caused by coagulative necrosis of liver cells or amyloidosis and destruction of red cells under the effect of tetanolysin toxins of clostridium tetani (William et al., 1968).

Significant hyponatremia was recorded in geriatric horse groups, the condition could be attributed to nephropathy

Although there was an increase in the urea and creatinin level but they were still in the normal levels in all horse groups

V. Haemostatic alterations

As presented in table (5), there was a significant increase in prothrombin time, a significant decrease in activated partial thromboplastine time and platelets count in the geriatric horses group. The increase in the prothrombin time may be due to coagulative necrosis of hepatocytes and deposition of amyloid material replacing the hepatocytes (Abdelkader et al., 1991).

Wintrobe et al., (1974) made a correlation between activated partial thromboplastin time (PTT) and the severity of tissue damage. The thromboplastin is a tissue factor (factor III) which finds its way to the circulation following tissue damage especially endothelial lining of blood vessels. This explains the significant decrease in the activated partial thromboplastin time especially in geriatric horse's group.

Thrombocytopenia developed in geriatric horses may be attributed to the formation of immune complexes in the circulation, which induces platelets aggregation and accelerates platelets destruction leading to immune-mediated thrombocytopenia (Roitt 1994 and Stites et al. 1997).

It could be concluded from the present results that, the immune response of middle aged and young horses in antitetanic sera production is much better than old horses.

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