Original article

The evolution of some blood parameters in hypovolemia conditions in rabbits

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Abstract

The shock is a general, non-specific pathological process, caused by the sudden action of very brutal pathogens, a situation for which the body has no reserves for qualitative and quantitative compensation-adaptation. The objective of our experiment was to make an evaluation of the changes in some hematological and biochemical parameters of the blood, during some hypovolemic evolutions, in the rabbits. Twenty New Zealand White rabbits we used. An IDEXX ProCyte Dx Hematology Analyzer was applied to perform hematological determinations. An IDEXX VetTest Chemistry Analyzer was used to perform blood biochemistry determinations. The data obtained were statistically analyzed, calculating the Media and Standard Deviation (SD), using the Microsoft Excel application. At the same time, the statistical significance of the differences between the batches was calculated based on the t test (Student) using the Microsoft Excel application. The study revealed a decrease in the number of red blood cells and leukocytes per unit volume of blood ($p < 0.05$) in the case of group 2 and an increase in glucose, triglycerides ($p < 0.05$).

Experimental hypovolemia induced in the conditions of our experiment determined: an obvious posthemorrhagic anemia, a significant leukopenia mainly 6 hours after the production of hypovolemic shock and a significant hyperglycemia, manifested mainly 12 hours after the induction of hypovolemia.

Key words: blood parameters, hematology, hypovolemia, rabbits

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Introduction

The shock is a general, non-specific pathological process, caused by the sudden action of very brutal pathogens, a situation for which the body has no reserves for qualitative and quantitative compensation-adaptation. Shock is a serious threat to life, acting by severely decreasing tissue perfusion (hypoperfusion), which endangers the existence and function of all organs and tissues. During the shock process, there is a discrepancy established between the capacity of the blood vessels and the circulating blood volume, which leads to a decrease in tissue perfusion. The result translates into hypoxia, inducing serious metabolic effects.

One of the most common forms of shock is hypovolemic shock, which is usually a consequence of severe bleeding, a major cause of death in patients with polytrauma (Holzrichter et al.1987, Weiss and Loh 1999, Porth 2005, Porter et al. 2013). Lower blood volume leads to decreased diastolic filling at the ventricular level. This causes a drop in blood pressure and tissue perfusion. The body uses its compensatory resources in the first moments of hypovolemic shock, thus stimulating the secretion of catecholamines. At the same time, the production of antidiuretic hormone/vasopressin (ADH) increases. These events are able to restore for the time being the level of blood pressure in the vital organs (heart, lungs and brain), which also benefit from a redistribution, in their favor, of blood flow (Kovách et al. 1972, Hamar et al. 1979, Nunez and Cotton 2009).

The compensatory mechanisms have low effectiveness, not being sustainable. Thus, in these cases, in the absence of an adequate therapeutic approach, severe hypoxia is established, which can lead to the patient's death (Bailey1985, Gutierrez et al. 2004).

Reducing bleeding-induced mortality is a current topic from a medical research perspective. This topic is approached both experimentally and clinically (Sondeen et al. 2007, Cho et al. 2009). Animal models are essential in researching topics related to hemorrhagic shock. Three types of experimental models studying hemorrhagic shock were imagined: fixed-volume hemorrhage, fixed-pressure hemorrhage, and uncontrolled hemorrhage. Experimental techniques have also been applied that combine the experimental models mentioned above (Humphreys et al. 1985, Capone et al. 1995, Yu et al. 2008). Most of the research undertaken concluded that an ideal and at the same time clinically relevant experimental model, focused on hemorrhagic shock, should impose severe hemorrhages, which can become uncontrollable (Tabsh et al. 1986, Majde 2003). However, this way of working is difficult to accept from an ethical point of view (Tsukamoto et al. 1995). So in our research we approached the study model that involves the induction of hypovolemia by hemorrhage with fixed volume.

Our research is part of the efforts to understand and reveal the processes following hypovolemic shock, in all et its aspects. Thus, the objective of our experiment was to make an assessment of changes in blood hematological and biochemical parameters, during hypovolemic shock evolution in rabbits.

Materials and Methods

The study was conducted in the spring season. In our experiment we used 20 New Zealand White rabbits with body weights of 3.5±0.2 kg. The rabbits were separated into 2 experimental groups: group 1 (Control; n=10) and group 2 (Experimental; n=10).

During the experimental period the rabbits were housed in cages adapted to their species. The cages were kept indoors. The animals were exposed to a cyclical variation of the ambient temperature placed between 18-22° C. The relative humidity varied between 40 and 50%. Food and water were administered at will. The food consisted of compacted feed prepared according to a special recipe for rabbits, which contained 18% protein, 14% fiber, 2% fat and 2600 kcal/kg feed. The rabbits were kept in a lighting regime of about 10 hours/24 hours.

On the day of the experiment, for each animal from both groups, 0.25 ml of blood was collected from the auricular vein. These samples were taken in heparin tubes and stored for laboratory determination.

Afterwards, from the rabbits in lot no. 2, a volume of blood 35 ml (about 12% of the volume) was extracted from the femoral vein, thus inducing a hypovolemic status of the animals in this experimental group.

At 6 hours and then 12 hours after the above-mentioned treatment, blood samples of 0.25 ml of blood were collected again from the rabbits in the two experimental groups, in order to carry out the laboratory determinations.

The blood samples collected were used to determine the following parameters: the number of red blood cells and the number of leukocytes in the blood. We also monitorized blood glucose and triglyceride levels.

An IDEXX ProCyte Dx Hematology Analyzer was used to perform hematological determinations. An IDEXX VetTest Chemistry Analyzer was also used to perform blood biochemistry determinations.

The data obtained were statistically analyzed, calculating the Media and Standard Deviation (SD), using the Microsoft Excel application. At the same time, the statistical significance of the differences between the batches was calculated based on the t test (Student), (Rao, 1999), using the Microsoft Excel application.
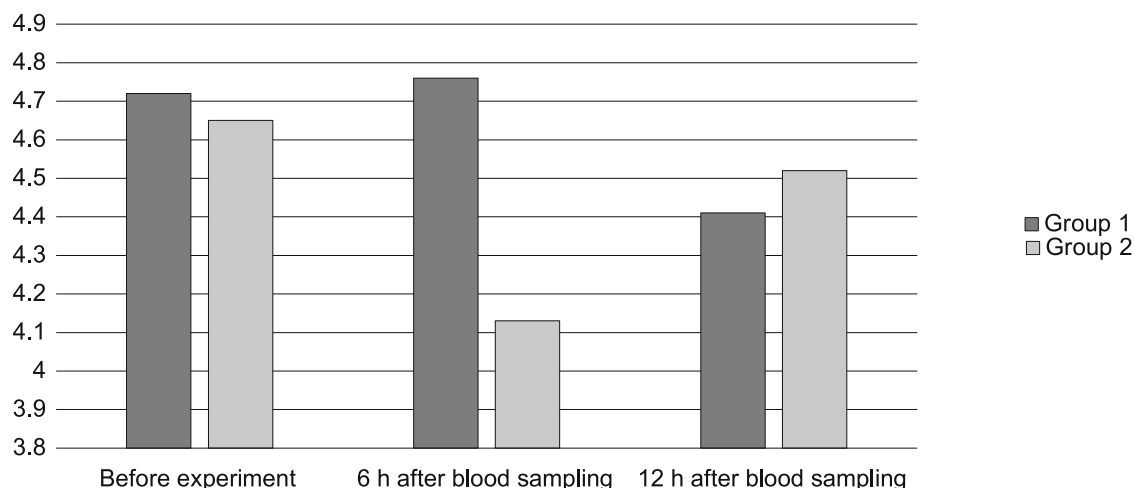
Fig. 1. Average red blood cell count/mm³, in rabbits from the two experimental groups.

Table 1. Average number of red blood cells (M/μL), leukocytes leukocyte count (K/μL), blood glucose (mg/dl) and triglycerides (mg/dl) in the blood in the two experimental groups of rabbits.

Lot no.	Before the experiment (Media ± SD)	At 6 o'clock from the induction of hypovolemia (Media ± SD)	At 12 o'cloc kfrom the induction of hypovolemia (Media ± SD
	Average number of red blood cells (M/μL)		
1.	4.72±0.23	4.76±0.19	4.41±0.21
2.	4.65±0.32	4.13*±0.18	4.52±0.16
	Average leukocyte count(K/μL)		
1.	5.12±0.15	5.27±0.21	5.38±0.12
2.	5.18±0.2	4.51*±0.12	5.03±0.21
	Blood glucose (mg/dl)		
1.	130.7±8	132.5±7	132.2±6
2.	131±2.5	145.8*±2.3	148.1*±3.2
	Triglyceride (mg/dl)		
1.	71.8±3.1	71.2±7,2	70.5±3.4
2.	72.1±3.1	83.2*±2.8	84.5*±3.1

* p<0,05

The animals used in the experiment were handled and treated in accordance with the rules of good practice and animal welfare. The procedures applied were not painful, and the level of experimental hypovolemia initiated during the experiment did not endanger the lives of the rabbits, which were recovered after the experiment.

Our experiment took place in the biobase of the Faculty of Veterinary Medicine in Bucharest, benefiting from the favorable approval from the Ethics Commission of this institution and being conducted in full compliance with the EU Directive 63/2010.

Results

The study revealed a significant decrease (p<0.05) in the number of red blood cells per unit volume of blood in the case of group 2 at 6 hours after the treatment (Table 1). This decrease was 11.18% at 6 hours and 2.79% at 12 hours from the induction of hypovolemia (Fig.1).

The number of leukocytes per unit volume of blood indicated a significant decrease (p<0.05) in group 2 at 6 hours after the treatment (Table 1). This decrease was 12.93% at 6 hours and 2.89% at 12 hours after induction of hypovolemia (Fig. 2).

Our results indicate a significant increase (P<0.05) in blood glucose in the group of animals in which hypovolemia was induced in a proportion of 12.5% (Table 1). This increase was 11.29% at 6 hours and 13.05% at 12 hours after induction of hypovolemia (Fig. 3).

Our results indicate a significant increase (p<0.05) in blood triglyceride levels in the group of animals in which hypovolemia was induced in a proportion of 12.5% (Table 1). This increase was 15.39% at 6 hours and 17.1% at 12 hours after induction of hypovolemia (Fig. 4).
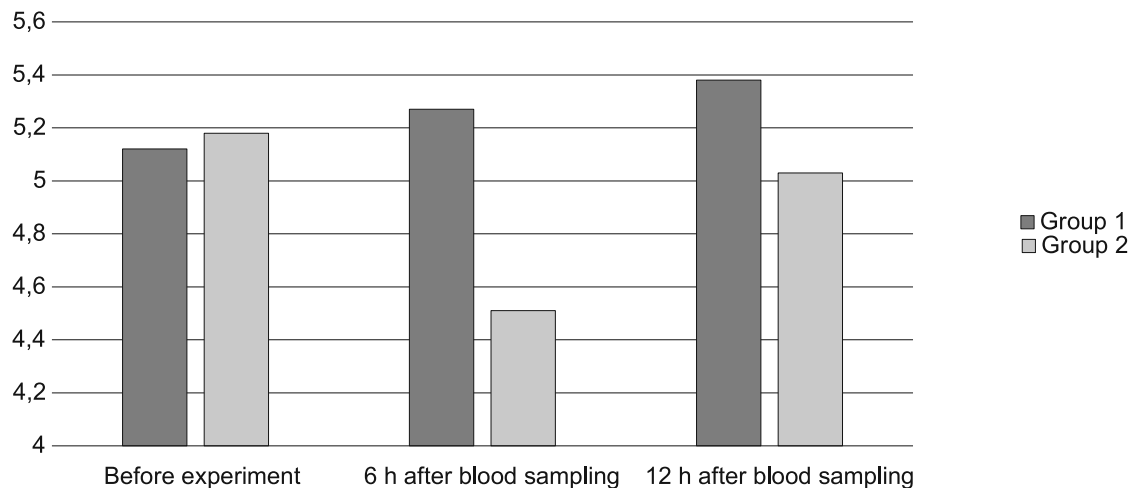
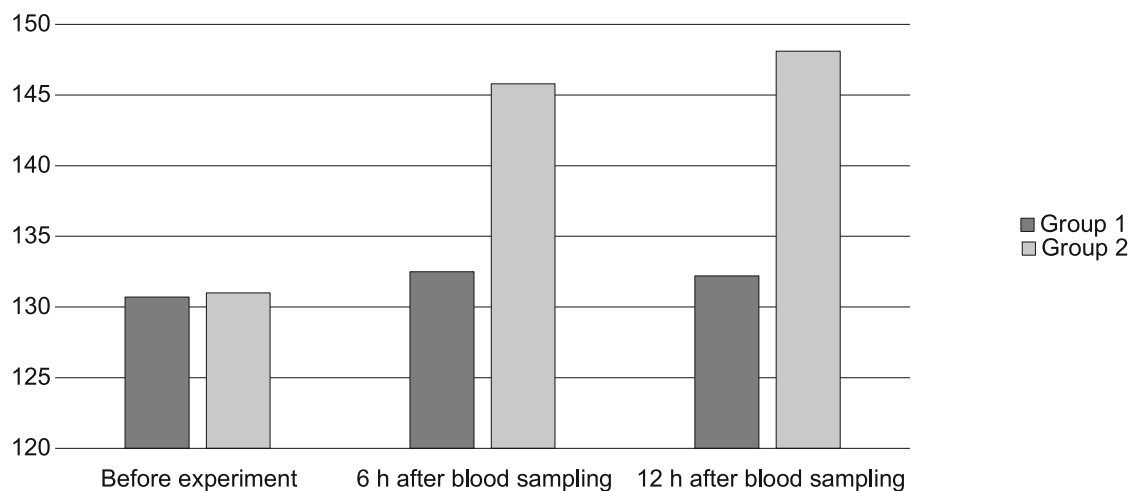Fig. 2. Average leukocyte count/mm³ blood, in rabbits from the two experimental groups.

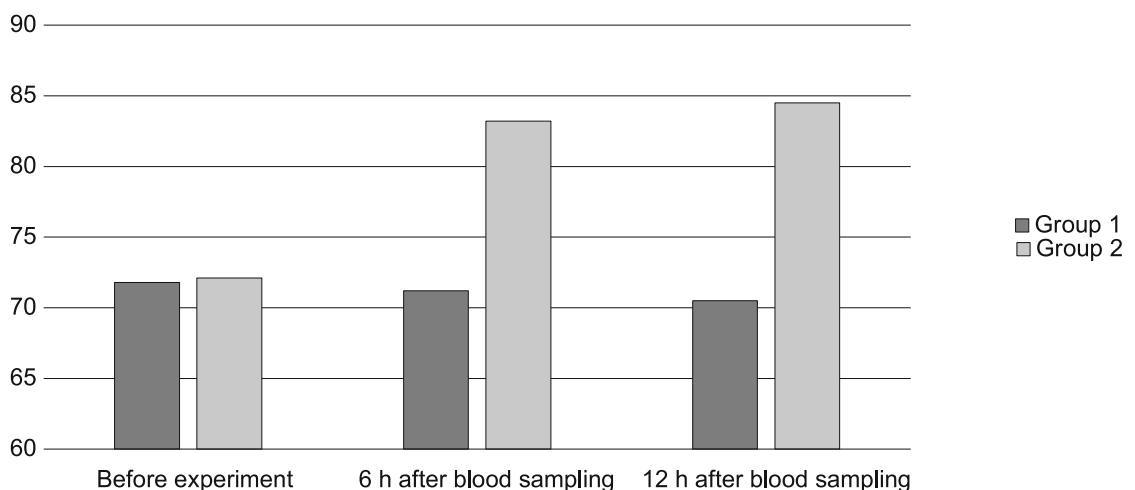Fig. 3. Mean blood glucose, in rabbits from the two experimental groups.

Fig. 4. Average level of blood triglycerides in rabbits from the two experimental groups.

Discussion

The present study has shown a significant posthemorrhagic anemia in rabbits in the experimental group.

This anemic state was the result of the loss of red blood cells, following the applied experimental procedure. Under these conditions, the body tries to compensate for these losses by appealing to the stocks of red blood

cells in the hematopoetic organs (spleen, liver and skin), (Hamar et al. 1979, Gutierrez et al. 2004). However, the compensation is partial, these stocks not being able to cover the erythrocyte losses in full (Porth 2005). This partial compensation is highlighted by the slightly increased values of the number of red blood cells measured 12 hours after the induction of hypovolemia, compared to the values measured 6 hours after this event.

Our results also showed posthemorrhagic leukopenia, a consequence of leukocyte depletion. At 12 hours after hypovolemia induction this parameter tended to return to normal, despite the obvious hypovolemic status. This recovery can be a consequence of hypovolemic and hypoxic stress, which through a reflex mechanism, on the catecholaminergic chain reaction, leads to the centralization of neutrophils (Porth 2005), respective inhibition of their adhesion to the vascular endothelium and delay of diapedesis, which leads to amplification of their number in blood and the level of the parameter called WBC. As expected, the sympathetic nervous system tonus, an implicit evolution of the experimental conditions imagined by us, is accompanied by an amplification of the leukocyte population, thus materializing an intensification of non-specific defense mechanisms of the body, under conditions of aggression (Slauson and Cooper 2002).

Regarding blood glucose levels, our results indicated hyperglycemia, following the posthemorrhagic shock. This evolution can be explained by the specific conditions of hypovolemic status, which induces hypovolemic and hypoxic stress. Stress, regardless of its nature, is always a factor that leads to hyperglycemia, through a mechanism based on a catecholaminergic reflex that aims to increase liver glycogenolysis and, consequently, an increase in blood sugar (Holzrichter et al. 1983, Nunez and Cotton 2009). This evolution emphasizes the body's efforts to cope with post-aggressive circumstances, in which an intense mobilization of energy reserves is necessary in order to recover.

Our results coincide with those published by Holzrichter (1983), who noted the similar evolution of blood glucose in hemorrhagic shock, showing that this parameter remains high for a long time after the shock, the level of this rise being directly correlated with the severity of hemorrhage.

Regarding the last parameter analyzed, the level of blood triglycerides, our experiment showed a significant hypertriglyceridemia, obviously induced by posthemorrhagic shock. This evolution is a consequence of hypovolemic status and hypoxic stress. Stress is always accompanied by an increase in the secretion of corticosteroid hormones, known for their ability to stimulate lipid catabolism, an effect that results in increased plasma triglyceride levels (Nunez and Cotton, 2009,

Ghita et al. 2015). It is clear how the body reacts to this hormonal chain in the direction of intensifying catabolic processes, biochemical processes that provide additional energy resources, which are essential during the post-aggressive systemic reaction, in order to recover.

Conclusions

Experimental hypovolemia induced under the conditions of our experiment determined:

An obvious posthemorrhagic anemia, with predominant manifestation 6 hours after the induction of hypovolemic shock;

Significant leukopenia, especially 6 hours after hypovolemic shock;

A tendency for the leukocyte count to return to normal levels 12 hours after the induction of hypovolemia.

Significant hyperglycemia, manifested mainly at 12 hours after the induction of hypovolemia;

A significant increase in blood triglycerides, especially 12 hours after the induction of hypovolemia.

Acknowledgements

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