Efficacy of Different Anaesthetics on Depth of Anaesthesia and Hematological Parameters of Pigs

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ABSTRACT— ***Four groups of piglets were included in the experiment. In each of the experiments, we applied azaperone as the first drug for premedication, in the 2nd group 4 mg/kg body weight, in the other groups 5 mg/kg body weight. In experiment no. 1, we subsequently administered diazepam (1 mg/kg, i.m.) and ketamine (10 mg/kg, i.m.) to the piglets. In experiment no. 2, we applied xylazine (2 mg/kg, i.m.) and ketamine (20 mg/kg, i.m.). In experiment no. 3 we used diazepam (1 mg/kg, i.m.), xylazine (2 mg/kg, i.m.) and butorphanol (0.2 mg/kg, i.m.). In experiment no. 4, we administered xylazine (2 mg/kg, i.m.) and butorphanol (0.2 mg/kg, i.m.) to piglets. We applied the mentioned drug combinations intramuscularly in the area of the neck muscles. Subsequently, in each of the experiments, we performed epidural anesthesia using 2% lidocaine, in a dose of 1 ml/40 cm of body length.***

After putting the animals under anesthesia, we recorded breathing and pulse frequencies, body temperature and checked reflex responses at time intervals. We performed a zero blood collection before the application of the first drug, the first collection after anesthesia and then a second collection the next day, in order to determine the hematological profile. From the conducted experiments, we assessed the relationship of the used combinations of substances to the course and depth of anesthesia and, according to the hematological profile, we evaluated the effect of these substances on specific hematological parameters.

We recorded the longest duration of anesthesia in the first experiment, when one of the piglets had disappeared pedal reflexes even after 90 minutes. On the contrary, the shortest anesthesia was during the third experiment, when all piglets reacted 60 minutes post lidocaine. During experiments No. 1 and 3, we noticed a significant drop in body temperature compared to the reference values. In a short time interval after the lidocaine administration, the reflex of the pelvic limbs completely disappeared in all tested subjects in each of the experiments. In experiment no. 3, at time 5 minutes post lidocaine administration, the nasal reflex and the reflex of the forelegs were also negative in all animals. In all tested subjects, 30 minutes after the epidural anesthesia was performed, we observed a negative reflex of the pelvic legs during second and third trials. During experiment no. 3, we also observed a negative nasal reflex and a reflex of the front legs in the majority of the tested animals in the mentioned time interval.

Keywords— Anesthesia, Body temperature, Haematological indices, Pigs

1. INTRODUCTION

Anesthesia is defined as a total loss of sensitivity of a part of the body or the whole body, induced by substances or combinations of substances that suppress the activity of nervous tissue peripherally (local and regional anesthesia) or centrally (general anesthesia) [8].

In general, due to their size and short reproductive cycles, pigs are suitable experimental animals for many surgical techniques requiring the dimensions of the human body (e.g., liver, kidney, heart, circulation, and intestine). Also, there is a strong morphological and functional similarity of the above organ systems in comparisons between humans and pigs [9].Therefore, many surgical techniques were first used in pigs to verify their safety and efficacy [4].

In the pig with a body weight of 30–40 kg, the relationship between heart size and body size is similar compared to humans, the results of studies can be used better than with other laboratory animals [6].

During the anesthesia of pigs, it is necessary to take into account some anatomical and physiological peculiarities. The pig has few superficial veins that could be used for anesthesia, with the exception of the vessels on the dorsal surface of the auricle. If greater vascular access or collection of multiple blood samples is required, sampling from the jugular vein is recommended [11]. Pigs have a blood volume of 65 ml/kg (6.5% of body weight). Adequate collection of samples for various laboratory examinations before and after anesthesia allows the animal to handle the blood collection without harming the organism. In the case of frequent blood collection, the weekly amount of blood collected should not exceed 7.5% [9].

A large part of the drugs is administered to pigs intramuscularly, the application is most often carried out in the area of the neck muscles. Depression of breathing and changes in body temperature are often associated with sedation and general anesthesia. Respiratory depression can be caused by a combination of the depressant effect of the drug, the limited ability to expand the chest, caused by abnormal body position and body fat. A relatively small body surface compared to body weight, the absence of sweat glands and insufficient thermoregulatory mechanisms can cause an increase in body temperature [12]. Hyperpyrexia and malignant hyperthermia have been described in genetically predisposed pigs and can be induced by various inhalation anesthetics [2].

According to the activity, specificity and intensity of the action of substances on the central nervous system, drugs acting in this direction are classified into the following groups: general anaesthetics, local anaesthetics, neuroleptics, ataxia, hypnotics and sedatives, anticonvulsants, antispasmodics, CNS stimulants and analeptics, opioid analgesics, analgesics. Anesthesia during surgical procedures can be performed by inhalation, intravenous or intramuscular, epidural application. Parenteral general anesthesia is possible with a combination of various substances, for example pentobarbital, thiopental, ketamine, xylazine, azaperone, propofol, midazolam and fentanyl. Isoflurane, enflurane, sevoflurane and halothane can be used for inhalation anesthesia [5].

The youngest phylogenetic structure, the cerebral cortex, usually shows the highest sensitivity with respect to anesthetics. The centers in the medulla oblongata, which contain the respiratory center and the circulatory center, are the least sensitive. The central nervous system is not affected by anesthetics immediately, but gradually. Anesthetics first reach the cortex, where consciousness and perception are situated. The cortex is the organ responsible for the discrimination of pain, touch, temperature, the sense of depth, as well as sight, hearing, smell and taste. In the second step, anesthetics affect the brain stem (interbrain and midbrain). The process of anesthesia is characterized by the fact that it passes to another part of the brain, before the anesthesia of the previous part is completed. The required level of anesthesia depends on the severity and expected pain during the procedure. This depends on the effect of the anesthetic and its dose. According to the above-mentioned parts of the brain, we distinguish four degrees of anesthesia [9].

In our work, we assessed the effectiveness of different combinations of medicines and their influence on the depth of anesthesia and hematological parameters, after their intramuscular application and subsequent epidural application of local anesthetic.

2. MATERIALS AND METHODS

Four groups of piglets were included in the experiment. In each of the experiments, we applied azaperone as the first drug for premedication, in the 2nd group 4 mg/kg body weight, in the other groups 5 mg/kg bw. In experiment no. 1, we subsequently administered diazepam (1 mg/kg i.m.) and ketamine (10 mg/kg i.m.) to the piglets. In experiment no. 2 we applied xylazine (2 mg/kg i.m.) and ketamine (20 mg/kg i.m.), in experiment no. 3 we used diazepam (1 mg/kg i.m.), xylazine (2 mg/kg i.m.) and butorphanol (0.2 mg/kg i.m.). As part of experiment no. 4, we administered xylazine (2 mg/kg i.m.) and butorphanol (0.2 mg/kg i.m.) to piglets. We applied the mentioned drug combinations intramuscularly in the area of the neck muscles. Subsequently, in each of the experiments, after putting the animals under sedation or general anesthesia, we also performed epidural anesthesia using 2% lidocaine, in a dose of 1 ml/40 cm of body length.

After putting the animals under anesthesia, we recorded breathing and pulse frequencies, body temperature and checked reflex responses at time intervals. We collected the zero blood sample before the application of azaperone, the first collection after the lidocaine anesthesia was performed, and then the second collection also the next day, in order to determine the hematological profile. Breathing and pulse frequencies, body temperature was measured before the application of drugs and then after the application of lidocaine after 5, 30 and 60 minutes. Reflexes were evaluated 5, 30 and 60 minutes after lidocaine application.

Blood was collected in tubes with the anticoagulant K_3EDTA, hematological parameters were determined on the Vet ABC Animal Blood Counter hematological analyzer at the UVMPh Košice, Clinic of Swine. The leukogram was done manually, by staining according to Pappenheim.

3. RESULTS

The longest duration of anesthesia, 90 minutes, measured from the application of the last anesthetic, lidocaine, was recorded in experiment no. 1, in which we applied a combination of azaperone, diazepam, ketamine and lidocaine. The shortest anesthesia, lasting approximately 1 hour, was in experiment no. 3, in this case we used azaperone, diazepam, xylazine, butorphanol and lidocaine. Of the hematological parameters, the largest deviations from the norm were related to the hematocrit value, with each sampling of all experiments. We recorded its low value compared to the reference values. During the duration of the anesthesia, we recorded a significant decrease in body temperatures in the pigs in experiments no. 1 and 3, in which the average body temperature compared to the norm was reduced by approximately 3°C. In other experiments, we did not notice such a significant drop in body temperature.

After the application of lidocaine, the pedal reflexes of the pelvic limbs disappeared in all pigs in all experiments. In experiment no. 3, within 5 minutes after the application of lidocaine, the nasal reflex and the pedal reflex of the front legs were also negative in all animals (Tab 1, 2, 3).

Table 1: Percentual assessment of reflexes, 5 minutes post lidocaine administration

	nasal reflex (%)		**pedal reflex of front legs (%)**		**pedal reflex of hindlegs (%)**	
	positive	negative	positive	negative	positive	negative
Experiment no. 1	80	20	60	40	0	100
Experiment no. 2	33,5	66,5	33,5	66,5	0	100
Experiment no. 3	0	100	0	100	0	100
Experiment no. 4	100	0	100	0	0	100

Table 2: Percentual assessment of reflexes, 30 minutes post lidocaine administration

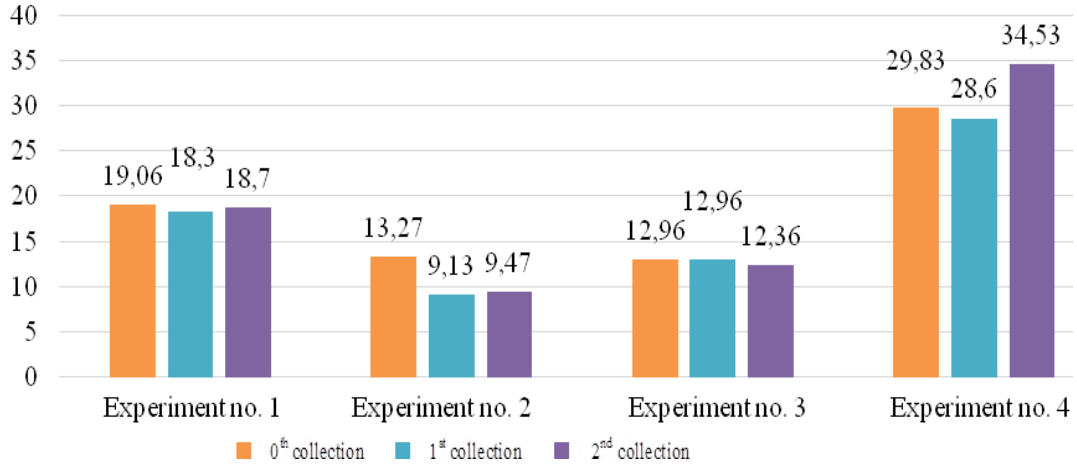
	nasal reflex (%)		**pedal reflex of front legs (%)**		**pedal reflex of hindlegs (%)**	
	positive	negative	positive	negative	positive	negative
Experiment no. 1	40	60	60	40	20	80
Experiment no. 2	33,5	66,5	33,5	66,5	0	100
Experiment no. 3	20	80	20	80	0	100
Experiment no. 4	100	0	100	0	25	75

Table 3: Percentual assessment of reflexes, 60 minutes post lidocaine administration

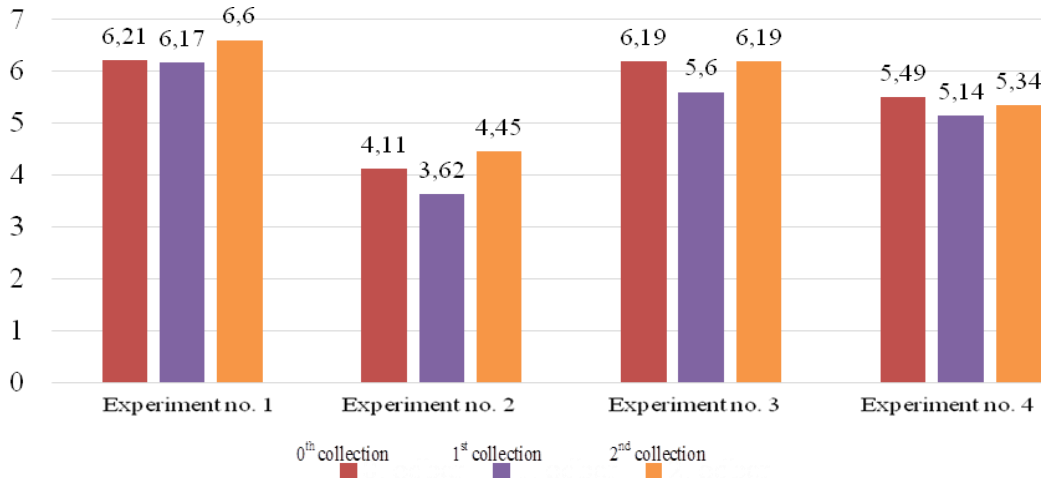
	nasal reflex (%)		**pedal reflex of front legs (%)**		**pedal reflex of hindlegs (%)**	
	positive	negative	positive	negative	positive	negative
Experiment no. 1	100	0	100	0	80	20
Experiment no. 2	66,5	33,5	66,5	33,5	66,5	33,5
Experiment no. 3	100	0	100	0	100	0
Experiment no. 4	100	0	100	0	75	25

From the hematological parameters, based on the Student's t-test, we found in experiment no. 3 significant difference regarding the value of non segmented neutrophils (Ne-non seg) and eosinophils (Eo) between the 0th and 1st collection. In experiment no. 4, we found a significant difference in the value of total leukocytes (Le) between the 0th and the 2nd sampling, as well as in the number of erythrocytes (Er) between the 0th and 1st sampling (Graph 1, 2). Other parameters were without significant changes.

Graph 1: Average number of leukocytes (G/l)



Graph 2: Average number of erytrocytes (T/l)



On the basis of the ANOVA test, in the trial no. 1 was found significant changes in respiratory rate and body temperature values. In trial no. 2, we observed significant changes in respiratory frequency values. In trials no. 3 and 4, we found significant changes regarding the values of pulse frequency and body temperature. In all experiments, there was a drop in body temperature during anesthesia.

4. DISCUSSION

In research comparing different anesthetic protocols, [4] reports that in the group of control animals, which were administered diazepam and ketamine as part of balanced intravenous anesthesia, among other anesthetics, no individual experienced vomiting or nausea during the experiment. We administered diazepam and ketamine during two experiments and also did not observe vomiting or nausea in any of the tested animals.

Sakaguchi [14] states in his study that after the application of xylazine in a dose of 2 mg/kg bw., combined with other anesthetics, he observed weak sedation and also muscle relaxation. In our fourth experiment, in which, among others, xylazine was also applied, we recorded very significant reactions of the pigs to needle injections when lidocaine was applied to the epidural space, which we did not observe in the previous experiments. Thus, we can say that the degree of sedative and analgesic effect of xylazine depends on its combination with other anesthetics used. The author also states that xylazine did not significantly affect respiratory parameters, except for a minimal reduction in breath frequency. During our experiments, in some weanlings, we noticed a significant decrease in respiratory frequency immediately after the application of xylazine, specifically in the second and third trials. He also mentions in his study that mild hypothermia can occur after the application of alpha-2-agonists, we noted a decrease in body temperature in the third experiment.

When ketamine is administered in combination with benzodiazepines or other sedatives, some individuals may have a positive pedal reflex [3]. In our first experiment, shortly after the application of ketamine, we observed a positive pedal reflex in one of the weanlings, in the others the pedal reflex was weakened or completely disappeared. Adetunji and Osunbunmi [1] in their study on the hematological effects of azaperone in pigs reported that the mean values of erythrocytes, hemoglobin and hematocrit were lower compared to accepted reference values for pigs and that they may indicate some degree of anaemia. The average values of leukocytes remained relatively constant and moved at the level of the upper limit of reference values. In our case, azaperone was applied as a premedication in each of the four experiments. Since we did the experiments in small pigs, the values of the red blood component were already at the 0th sampling, before the application of the drugs, at the lower limit of the norm. We did not notice changes in their values at subsequent samplings.

Diazepam was used in our first and third attempts, when after evaluating the hematological profile, we found that there were no significant changes in the level of hemoglobin, hematocrit compared to the 0th collection. In research [15], in which diazepam was applied in combination with propofol, was found that there was a significant decrease in the content of erythrocytes, hematocrit and hemoglobin.

Lugo-Roman *et al.* [13], found in their experiment that after the application of ketamine in experimental animals there was a decrease in the content of erythrocytes, hematocrit and hemoglobin. In our experiments, ketamine was applied twice, and from the results of the hematological profile, we can conclude that a reduced hematocrit value, a slight decrease in hemoglobin content was already detected at the 0th sampling, and there were no changes in the subsequent samplings.

Average duration of surgical anesthesia was approximately 120 minutes using xylazine and ketamine [7]. We applied xylazine together with ketamine during the second experiment, azaperone was applied for premedication as in all of our experiments, and anesthesia was supplemented with epidural application of lidocaine. We monitored the recovery of reflex responses in the majority of the pigs until 90 minutes after the application of xylazine,.

In study on epidural anesthesia using lidocaine, [10] reported that the decrease in hematological profile values including erythrocytes, hemoglobin, hematocrit, neutrophils and platelets after epidural anesthesia may be due to fluid shifting from the extravascular to the intravascular compartment to compensate for normal cardiac output. It also reports an increase in lymphocyte content compared to reference standards. Epidural anesthesia using lidocaine was performed in each trial during our experiments. After evaluating the hematological profile, we did not detect any significant changes in the values of hematocrit, erythrocytes, and hemoglobin in any of the experiments.

5. CONCLUSION

We recorded the longest duration of anesthesia from the application of lidocaine, 90 minutes, in experiment no. 1, in which we applied a combination of azaperone, diazepam, ketamine and lidocaine. The shortest anesthesia, lasting approximately 1 hour, was in experiment no. 3, in this case we used azaperone, diazepam, xylazine, butorphanol and lidocaine.

After the application of lidocaine, the reflexes of the pelvic limbs disappeared in all pigs in all experiments. In trial no. 3, within 5 minutes of the application of lidocaine, the nasal reflex and the pedal reflex of the forelegs were also negative in all animals. After 30 minutes from the application of lidocaine, the reflex of the hindlegs was negative in all the pigs in trials no. 2 and 3, but one hour after the application, all the pigs of experiment 3 had a positive reflex of the pelvic limbs.

Of the hematological parameters, the largest deviations from the norm were related to the hematocrit value, with each sampling of all experiments, we recorded its low value compared to the reference values. In trial no. 4, we found a significant difference in the value of leukocytes (Le) between the 0th and the 2nd collection, also in the number of erythrocytes (Er) between the 0th and the 1st collection. Other parameters were without significant changes.

During the duration of the general anesthesia, we recorded a significant drop in body temperatures in the pigs in experiments no. 1 and 3, in which the average body temperature compared to the norm was reduced by approximately 3°C. In other experiments, we did not notice such a significant drop in body temperature.

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