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EFFICACY OF DIFFERENT METHODS OF GENERAL ANAESTHESIA IN PIGS AND THEIR INFLUENCE ON HAEMATOLOGICAL PARAMETERS

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ABSTRACT

This study focused on how haematology parameters differ with different types of anaesthesia that is given to piglets. In this study 18 piglets were used divided into three groups. In each of these experimental groups a different combination of anaesthetics was used. The first group received the combination of azaperone, diazepam, and ketamine. In the second group, we used the combination of azaperone, xylazine, and ketamine. In the third group, the anaesthesia was induced by azaperone, diazepam, butorphanol and ketamine. Blood samples were taken from the piglets three times throughout the procedure and the relevant haematological parameters were determined. With respect to haematological values, the combination used in group 1 appeared as the best for the anaesthesia of piglets. The haematological values, such as MCV (mean corpuscular volume), were similar in all groups with the exception of the third group where the MCV and the MCH (mean corpuscular haemoglobin) were significantly lower 24-hour post anaesthesia compared to the other two groups. Lymphocytes in the first two groups showed a steady decrease, while in the third group their levels increased. The opposite trend was observed for the segmented neutrophils that showed a steady increase over the 24-hour period in the 1st and 2nd group while in group 3 their levels decreased. Abolishment of pedal and nasal reflexes to external stimuli applied every 15 min post ketamine administration was observed. The piglets in the 1st group showed no reflexes to external stimuli, while the worst responses were observed in the 2nd group where many of the piglets started to show reflexes very early within the experimental time.

Key words: anaesthesia; haematological parameters; pigs

INTRODUCTION

General anaesthesia is a medically-induced loss of consciousness with concurrent loss of protective reflexes due to anaesthetic agents. During this state, the patient is unarousable to verbal, tactile, and painful stimuli which facilitates the performance of surgical tasks. The purpose of using anaesthesia before surgeries is to minimize the stress and pain with as little side effects as possible to be able to perform various medical procedures. Situations in which the patient is going to experience low or high levels of pain will determine the type of anaesthesia as well as the length of care required after the procedure [10].

Premedication, analgesia and anaesthesia is often used before procedures that require more attention. Popular combined anaesthesia induced by xylazine and ketamine

lasts approximately 20–30 minutes, which is the general length of anaesthesia [5].

When anaesthesia needs to be prolonged, it is possible to administer an additional dose of the same anaesthetic to prolong it. However, the overall length of anaesthesia depends then considerably on the dosage, but giving additional dose will prolong the effect by approximately 10–20 minutes [2].

Premedication is a common practice frequently used before anesthetizing the swine. Premedication assists with putting the swine into a more sedated stage in which it is easier to induce anaesthesia and achieve recovery. One of the most common premedications used in piglets is azaperone. This sedative is fast acting, and when used intramuscularly its effects should appear 5–10 minutes after injection [1].

Anaesthesia puts the swine into an unconscious state; however, many anaesthetics do not have analgesic effects. Therefore, in order to ensure safe surgical procedures, an analgesic is administered together with the anaesthesia. There are groups of drugs that are most commonly used with anaesthetics, such as opioids, non-steroidal anti-inflammatory drugs (NSAIDs), and alpha-2 agonists. All these groups of analgesics are suitable for providing analgesia to swine; however, the opioids have a better analgesic and sedative effect compared to the NSAIDs. After major surgeries, the analgesics such as opioids are administered for several days post-surgery. Butorphanol and buprenorphine that are opioids are commonly used in swine. Butorphanol has an analgesic effect lasting 4–6 hours and has less pronounced side effects, while buprenorphine can be given every 12 hours and the onset of action is within 30–60 minutes [4].

Another group of analgesics known as the non-steroidal anti-inflammatory drugs (NSAIDs) is a group of organic acids involved in reducing the amount of inflammatory mediators. NSAIDs are not generally as effective against high level of pain compared to the opioids. In general, NSAIDs can be combined with opioids to give a more pronounced analgesic effect against the treatment that causes severe pain. Similar to opioids, the NSAIDs can be used post-surgery to ensure analgesia. The common NSAID analgesics include aspirin, meloxicam, and ketorolac [4].

In situations in which visceral analgesia is preferred, alpha-2 agonists may be the analgesics of choice. Alpha-2 agonists include xylazine, detomidine, and medetomidine. The analgesia effects of alpha-2 agonists involve peripheral and central alpha receptors [12]. Alpha-2 receptors are located in the dorsal horn of the spinal cord; stimulation of the alpha-2 receptors is located at the periaqueductal grey regions of the midbrain. This region is important as this is the pain modulation location that releases epinephrine [9].

One of the most popular uses of injectable anaesthesia is ketamine. Ketamine is an injectable anaesthetic with fewer side effects that could cause central nervous system issues. The use of ketamine will allow the eye and laryngeal reflexes during anaesthesia. It should be mentioned that the blocking of NMDA (N-methyl-d-aspartate) receptors are most important for the anaesthetic and analgesic effects. Also the use of ketamine does not suppress the cardiovascular functions but causes increased sympathetic outflow that increases the heart rate and arterial blood pressure. It is important to note that ketamine induces a significant increase in the cerebral blood flow, intracranial pressure and cerebrospinal fluid pressure as a result of cerebral vasodilation. Finally, ketamine will cause increased salivation and secretion of mucus in the respiratory tract that can be reduced by administration of an anticholinergic drug. Piglets can also show signs of tachycardia, hypertension, and poor muscle relaxation. The muscle rigidity can be reduced by administration of acepromazine, diazepam, xylazine, and medetomidine in combination with ketamine. The use of diazepam or xylazine with ketamine induces deep anaesthesia and good muscle relaxation. The combination of butorphanol, medetomidine and ketamine will provide anaesthesia lasting approximately 98 minutes. The combination of medetomidine and ketamine induces longer duration of muscle relaxation than other combinations such as xylazine and ketamine. Medetomidine and ketamine does cause a moderate cardiovascular stimulation with minimal respiratory effect [4].

During the procedure, it is necessary to constantly monitor the piglets and check for any abnormalities every 20–30 minutes. When the piglet is under anaesthesia, there can develop situations when the piglet may have difficulties without realising them. The respiration rate and hearth rhythm should be checked every 20–30 minutes. It is also important to monitor the body temperature. During some procedures, the piglet will rest on warming pads to stabilize its temperature, since anaesthesia also reduces the body temperature [5].

After termination of anaesthesia in swine, it is also im-

portant to consider the postoperative issues that could occur. During recovery from anaesthesia the animals should often be kept in a warmer place. Because of one of the side effects of anaesthesia, the body temperature of the pig can be drastically reduced. The decrease in body temperature as well as the pig's inability to increase it can also contribute to changes in haematological parameters. The reduced body temperature as well as the potential decrease in cardiovascular functions caused by the use of medications will decrease blood circulation in the body. Reduced circulation will result in an overall decrease in blood volume. For any study involving the measurement of blood levels in an animal it would be ideal to withdraw the blood earlier rather than later in the postoperative management [9].

The goal of this study was to contribute to understanding how anaesthesia can affect the haematological values of swine. Changes in haematological parameters in piglets were observed in relation to combinations of anaesthetics and sedatives and the time immediately after anaesthesia and after 24 hours. Evaluated was also effectiveness of individual combinations of drugs used for induction of general anaesthesia in piglets on the basis of measurements of vital functions and the evaluation of reflexes in the duration of anaesthesia.

MATERIALS AND METHODS

The experiment was carried out on 18 piglets, aged 10–20 days. They were divided into three groups, every group consisted of 6 piglets. Piglets in the 1st group were given azaperone (4 mg.kg^{-1} body weight – b. w.), diazepam (1 mg.kg^{-1} b. w.) and ketamine (30 mg.kg^{-1} b.w.).

In the 2nd group the anaesthesia was induced by azaperone (5 mg.kg^{-1} b. w.), xylazine (2 mg.kg^{-1} b. w.), and ketamine (20 mg.kg^{-1} b. w.) and in the 3rd group by azaperone (4 mg.kg^{-1} b. w.), diazepam (1 mg.kg^{-1} b. w.), butorphanol (0.2 mg.kg^{-1} b. w.), and ketamine (20 mg.kg^{-1} b. w.).

The respiratory rate, heart rate, and temperature of piglets were measured prior to the injection of the first anaesthetic dose of azaperone. Also, prior to the injection of azaperone, blood samples were collected for comparison with the following haematological results. After the injection of azaperone the piglets were left undisturbed in a quiet environment for 15 minutes and after administration of diazepam or xylazine there was a pause of 10 minutes before injection of ketamine. After the administration of ketamine, the piglets were checked every 15 minutes for the respiratory rate, heart rate, temperature, and response to stimuli for the next 75 minutes. Blood samples were collected after 60 min and 24 hours post anaesthesia.

In order to compare the haematological profile of the piglets in individual groups, the results were statistically evaluated using the ANOVA test. To detect significant differences between the groups we used the Tukey's test in GraphPad Prism 3.0. The $P<0.05$ was considered significant.

ETHICAL CONSIDERATIONS

The project of the study was approved by the Ethics Committee of the University of Veterinary Medicine and Pharmacy in Košice, permit No. EKVP/2022-23.

RESULTS

Comparison of results obtained in groups anaesthetized with different combination of drugs showed some significant differences. At the beginning of the experiment the counts of leukocytes (Leu) were significantly higher in group 1 in comparison with groups 2 and 3. However, in group 2, their counts increased by 24 hours and the difference compared to group 1 was insignificant. After 24 hours post anaesthesia the counts of erythrocytes (Er) were the highest in group 3 compared to the other two groups. Results of haematocrit at 1 hour and 24 hours post anaesthesia were similar in all three groups (Table 1).

MCV (mean corpuscular volume) at the start of the trial also showed levels very similar in groups 1 and 2. In the 3rd group there was detected a gradual decrease from the initial level to the post 24-hour level significantly different from groups 1 and 2 (Table 1).

As far as MCH (mean corpuscular haemoglobin) was concerned, we observed that 1-hour post ketamine administration there was a significant difference between group 1 and group 3 ($P<0.01$) and 24-post ketamine significant differences, between group 1 and group 3, as well as between group 2 and group 3 ($P<0.01$).

The haemoglobin levels showed no significant differences among groups, all three groups showed a slow in-

Table 1. Comparison of blood parameters between experimental groups

Parameter	Group 1	Group 2	Group 3	Ref. range
Hk, 0 hour [L.L^{-1}]	0.30 ± 0.02^{a}	0.34 ± 0$^{a, c}$	0.30 ± 0.04^{c}	
Hk, 1 hour [L.L^{-1}]	0.28 ± 0.01	0.30 ± 0	0.30 ± 0.01	0.3–0.5
Hk, 24 hours [L.L^{-1}]	0.31 ± 0.02	0.31 ± 0.02	0.33 ± 0.02	
MCV, 0 hour [fl]	65 ± 3.01	65 ± 4.3	59.3 ± 8	
MCV, 1 hour [fl]	65 ± 3.3^{b}	63 ± 4	58 ± 7.8^{b}	50–68
MCV, 24 hours [fl]	64 ± 2.7^{b}	63 ± 3.5^{c}	57 ± 9.2$^{b, c}$	
Er, 0 hour [T.L^{-1}]	5.6 ± 2	5.1 ± 0.5	6 ± 0.1	
Er, 1 hour [T.L^{-1}]	4.3 ± 0.4	4.9 ± 0.5	7.1 ± 3	5–8
Er, 24 hours [T.L^{-1}]	4.8 ± 0.5^{b}	5.2 ± 0.6	7 ± 1.8^{b}	
Leu, 0 hour [G.L^{-1}]	14 ± 2.5$^{a, b}$	10 ± 2.6$^{a, c}$	6.8 ± 2.2$^{b, c}$	
Leu, 1 hour [G.L^{-1}]	13.2 ± 3.2^{b}	11 ± 3.1	7 ± 3^{b}	11–18
Leu, 24 hours [G.L^{-1}]	17 ± 10.1	19 ± 10.2	7 ± 1.8	

Hk – haematocrit; MCV – mean corpuscular volume; Er – erythtrocytes; Leu – leukocytes; L.L^{-1} – litres of cells per litre of blood; fl – femtolitres; T.L^{-1} – Tera.L^{-1} (10^{12}.L^{-1}) ; G.L^{-1} – Giga.L^{-1} (10^{9}.L^{-1}); ^{a} – G1:G2 P < 0.05; ^{b} – G1:G3 P < 0.05; ^{c} – G2:G3 P < 0.05

Table 2. Comparison of leukogram between experimental groups (in percentage)

Parameter	Group 1	Group 2	Group 3	Ref. range
Ly, 0 hour [%]	62 ± 5.2	63 ± 4.1	68 ± 0.7	
Ly, 1 hour [%]	62 ± 2.3	60 ± 4.2	63 ± 2.1	39–62
Ly, 24 hours [%]	60 ± 2.8^{b}	61 ± 7.8^{c}	76 ± 8.5$^{b, c}$	
Neu-S, 0 hour [%]	30.3 ± 7	31.2 ± 4.1	28.2 ± 0.71	
Neu-S, 1 hour [%]	31.2 ± 1.47	33.2 ± 4.3	33.5 ± 0	28–47
Neu-S, 24 hours (%)	33 ± 3.2^{b}	36 ± 10^{c}	20 ± 9.2$^{b, c}$	
Neu-B, 0 hour [%]	4.67 ± 2^{b}	3.7 ± 0.81	2 ± 0^{b}	
Neu-B, 1 hour [%]	3.8 ± 1	4 ± 1.4	2.7 ± 1	0–4
Neu-B, 24 hours [%]	4 ± 1.7	3.5 ± 1	3.2 ± 1	
Eo, 0 hour [%]	3 ± 1.4^{b}	1.7 ± 1.5	0.2 ± 0^{b}	
Eo, 1 hour [%]	2.7 ± 1.5^{b}	1.5 ± 0.83^{c}	0,0$^{b, c}$	0.5–11
Eo, 24 hours [%]	2.2 ± 0.7$^{a, b}$	0.7 ± 0.82^{a}	0.2 ± 0.71^{b}	

Ly – lymphocytes; Neu-S – segmented neutrophils; Neu-B – band, unsegmented neutrophils; Eo – eosinophils; ^{a} – G1:G2 P < 0.05; ^{b} – G1:G3 P < 0.05, ^{c} – G2:G3 P < 0.05

Table 3. Changes in respiration rate (number per minute) in experimental groups post ketamine administration

Groups	0 min	15 min	30 min	45 min	60 min	75 min
Group 1	66.8 ± 2.83	59.3 ± 19.8	72.2 ± 3.5	61.7 ± 14.1	64.3 ± 0	65.3± 28.3
Group 2	66.7 ± 22.6	54.5 ± 9.9	76.3 ± 1.41	97.7 ± 7.07	96.7 ± 5.7	89.8 ± 4.24
Group 3	51.3 ± 5.7	58.7 ± 1.41	48 ± 12.7	56.7 ± 17	51.7 ± 8.5	49.7 ±1.41

0, 15, 30, 45, 60, 75 min – respiration rate at 0, 15, 30, 45, 60 and 75 min post ketamine administration

Table 4. Comparison of pulse (number per minute) among experimental groups

Groups	Pulse 0	Pulse 15	Pulse 30	Pulse 45	Pulse 60	Pulse 75
Group 1	129 ± 9.9	103 ± 24.0	122 ± 10.6	115.7 ± 4.2	116.5 ± 5.7	125.5 ± 7.8
Group 2	132.8 ± 1.4	128 ± 28.3	139.5 ± 23	138 ± 18.4	137 ± 15.6	131 ± 56.6
Group 3	125 ± 7	151.7 ± 7.1	148 ± 1.4	137.3± 26.9	151 ±11.3	126.3 ± 8.5

Pulse 0, 15, 30, 45, 60, 75 – pulse at 0, 15, 30, 45, 60 and 75 min post ketamine administration

Table 5. Comparison of body temperature [°C] among experimental groups

Groups	Temp 0	Temp 15	Temp 30	Temp 45	Temp 60	Temp 75
Group 1	39.4 ± 0.07	37.6 ± 0.92	36.6 ± 1.7	36 ± 2.12	35.6 ± 2.48	35.0 ±2.47
Group 2	39.8 ± 0.14	38.45 ± 0.42	38.9 ± 1.13	38.5 ± 0	37.4 ± 0.85	37.3 ± 0.35
Group 3	38.3 ± 0	39.95 ± 1.56	36.7 ± 1.48	36.45 ± 1.5	36.2 ± 1.41	35.7 ± 0.28

Temp 0, 15, 30, 45, 60, 75 – body temperature at 0, 15, 30, 45, 60 and 75 min post ketamine administration

Table 6. Abolishment of reflexes to stimuli in individual experimental groups post ketamine administration

	P–15 min	N–15 min	P–30 min	N–30 min	P–45 min	N–45 min	P–60 min	N–60 min
Group 1	100 %	100 %	100 %	100 %	66 %	66 %	33 %	33 %
Group 2	83 %	66 %	83 %	16 %	0 %	0 %	0 %	0 %
Group 3	100 %	100 %	83 %	83 %	50 %	50 %	50 %	50 %

E 1 – Experimental group 1; E 2 – Experimental group 2; E 3 – Experimental group 3;
P–15, P–30, P–45, P–60 – pedal reflex at 15, 30, 45, 60 min post ketamine administration;
N–15, N–30, N–45, N–60 – nasal reflex at 15, 30, 45, 60 min post ketamine administration

crease throughout the initial phase up to the last sampling. They ranged from 10.2 $g.dl^{-1}$ to 11 $g.dl^{-1}$ which was still within the normal reference range.

Lymphocyte (Ly) levels in groups 1 and 2 showed a slow decrease, however in group 3 we observed an opposite as there was an increase at 24 hours. Segmented neutrophils (Neu-S) showed an opposite trend, in groups 1 and 2 they steadily increased from the initial levels up to the 24-hour period while in group 3 they drastically decreased within 24 hours far below those in the other two groups. Eosinophil (Eo) counts steadily decreased in groups 1 and 2 while their levels in group 3 were much lower (below the reference range) and differed significantly from those in groups 1 and 2 (Table 2).

Within the experiment it was also important to look at the recordings of respiration, pulse, and temperature among the piglets. For the first 15 minutes the respiration in all 3 groups of piglets gradually decreased. After that the respiration in group 3 continued to decrease while increasing respiration was observed in groups 1 and 2. After 45 minutes of observation, respiration in group 1 was similar until the end of the recording, respiration in group 2 was increased and that in group 3 continued to decrease (Table 3).

The pulse rate of the piglets in group 1 decreased until 60 min post ketamine administration, then returned to the initial level. In group 2 the changes in pulse rate were insignificant while pulse rate in group 3 was increased from 15 min up to 60 min of recording and by 75 min dropped to the initial level (Table 4).

At the beginning of the experiment the body temperature of piglets in all 3 groups were normal. As the time progressed, the temperature of piglets continued to decrease. From the start of the recordings, the decrease was steady and similar in all groups, with the exception of 15 min post ketamine administration in group 3 (Table 5).

The last observation involved pedal and nasal responses to a stimulus. We observed whether the piglets responded to an external stimulus applied every 15 minutes. In the first group none of the piglets showed any signs of reflex for the first 30 minutes, which was assessed as 100 % negative response. At 60 min post ketamine administration, the majority of piglets showed signs of reflex.

In group 2, at first 15 min post ketamine administration, there were some positive responses to the stimuli. As the time continued, piglets showed more positive reflexes and by 45 min post ketamine administration all piglets reacted to stimuli.

In the third group the piglets showed no signs of reflexes within the first 15 min post ketamine administration. After 30 min there were some signs of reflexes and after 45 min until the end of recording (60 min) half of the piglets in this group showed signs of a reflex when tested. (Table 6).

DISCUSSION

Our results of the examination of piglet blood showed that the combinations of drugs used to induce anaesthesia resulted in changes in haematological parameters. The increase in leukocyte count observed in this study was within the reference range so this would not be a cause for concern.

The areas that should be looked at on the basis of our results involved haematocrit and haemoglobin. The reference range of haematocrit for piglets is 0.3–0.5 $L.L^{-1}$ [11]. Results of haematocrit were similar in all three groups and corresponded to the reference range (Table 1). The reference range for haemoglobin in piglets is 9–14.4 $g.dl^{-1}$ [11]. The results obtained in our experiment showed that the haemoglobin in piglets ranged from 10.2 $g.dl^{-1}$ to 11 $g.dl^{-1}$ which was still within the normal reference range for piglets.

The experiments conducted by other authors were similar to ours although they used different anaesthesia combinations to see whether there were haematological differences between the groups. In the study by L e r v i k et al. [8] that was similar to ours, piglets were given combinations propofol-ketamine-dexmedetomidine or propofol-ketamine-fentanyl. The dexmedetomidine group showed a higher haemoglobin level compared to the fentanyl group, but still within the normal range. In the above study both combinations provided stable cardiovascular conditions in normovolaemic, healthy pigs. Based on cardiovascular response and depression of nociceptive withdrawal reflex, dexmedetomidine apparently provided superior analgesia to fentanyl.

Xylazine has the effect of cardiac depression as well as muscle relaxation. However, when given at higher doses, xylazine can cause vomiting and higher cardiac depression [7]. G ó m e z de S e g u r a et al. [3] wrote that xylazine

did not induce adequate sedative or analgesic effects in pigs at any dosage tested. However, cardiovascular effects were considerable. At lower dosages, cardiovascular effects were characterized by bradycardia and biphasic blood pressure response, initial hypertension was followed by hypotension. At higher dosages, severe hypotension with moderate bradycardia was followed by marked bradycardia. The results obtained in our experiment showed that hypothermia was observed in group 2 that was administered combination of ketamine and xylazine and azaperone for sedation. In this group we also observed tachypnoe due to increased stress and short duration of anaesthesia. We can assume that the hypotension induced by xylazine also contributed to a decrease in the body temperature of piglets. In our study the haematocrit and haemoglobin levels in group 2 were similar to those in the other groups that did not receive the combination with xylazine.

One of the important areas is the response to stimulation that the piglets had experienced during the anaesthesia. Our results showed that piglets did not respond to the stimulus applies early after the ketamine administration, however later, after 45 minutes, many of the piglets showed pedal and nasal reflexes to external stimuli. The reaction to stimulation is an important indicator how well the piglets responding to the anaesthesia. This observation is similar to that reported by T a n a k a et al. [13] who observed that the use of anaesthetics such as ketamine combined with other anaesthetic drugs is able to keep the piglets in a deep sedation with adequate muscle relaxation.

From the results of the responses to stimuli it can be concluded that the worst analgesic effect was achieved in the piglets from group 2 anaesthetised by combination with xylazine. In this group some piglets were seen to show a reflex response as early as 15 minutes into the experiment.

In a similar experiment it was revealed that the combination involving xylazine also showed that the animals being tested showed poorer recovery score and quicker time to the end of the anaesthesia. As the piglets anaesthetised with xylazine combination showed the poorest results the authors concluded that xylazine does not give the best analgesia [7].

CONCLUSIONS

The results of this study on piglets showed that the best anaesthetic depth from the point of view of responses to external stimuli was achieved with combination used in group 1. All piglets in this group showed no reflex response up to at least 30 min after administration of ketamine. In groups 1 and 3 where most of the piglets were under anaesthesia for longer time the body temperature decreased after 1 hour of anaesthesia.

Counts of erythrocytes and leukocytes in blood samples from group 3 showed little variations. A decrease in erythrocyte counts was detected in group 1 while leucocytes increased in groups 1 and 2 in comparison with the initial sample. At the last sampling, segmented neutrophils in the third group were significantly lower in comparison with groups 1 and 2 while the lymphocytes in this group were significantly higher compared to the other two groups. With respect to haematological values the combination used in group 1 appeared as the best for anaesthesia of piglets.

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