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COMPARATIVE EVALUATION OF ISOFLURANE AND SEVOFLURANE INNOCUITY ON SOME ORGANS AND TISSUES IN RAT

(SUMMARY OF Ph.D. THESIS)

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INTRODUCTION

Nowadays the anesthetics are widely used in both human and veterinary medicine. Their effects on the body are beneficial to a certain extent, but they also have a potential aggressive effect on organs and tissues.

The gaseous narcotics induce an inhibition of the following central nervous system segments: cortex, subcortical centres, spinal cord and exceptionally of the pons Varolii. At the same time, they can have certain influences on the tissues and organs they arrive in, through the blood stream. At the present day, isoflurane and sevoflurane are the most frequently used volatile narcotics. By using them, we can obtain a long term or repeated narcosis, which can negatively impact on some morphophysiological structures.

Although the specialty literature contains various studies concerning the effect of isoflurane and sevoflurane on some organs and tissues, there are still unanswered questions, which require further studies. Despite the different metabolic rates of the isoflurane and sevoflurane, it appears like this anesthetics lead to hepatic lesions accompanied by contrilobular necrosis, but the etiology is yet to be discovered. There are few histochemical and immunohistochemical studies on the hepatocellular changes appeared as a result of exposure to isoflurane and sevoflurane.

AIM AND STRUCTURE OF THE THESIS

There is a scarcity concerning the studies on morphological changes in tissues and organs after isoflurane and sevoflurane exposure. Most of the studies concentrate on the physiological changes that take place in organs.

In this context, the objective of this study were focused on:
- comparative clinical evaluation of isoflurane and sevoflurane;
- evaluation of the effects of isoflurane and sevoflurane anesthesia on oxidative status;
- evaluation of isoflurane and sevoflurane toxicity on liver, through immunohistochemical examination and transaminases determination;
- evaluation of the effects of isoflurane and sevoflurane anesthesia on the structure and function of some vital organs.

The thesis is structured in two parts. Part I contains 4 chapters, and Part II extends on 6 chapters. Chapter 1 contains general information on the inhaled anesthetics, Chapter 2
concentrates on the oxidative stress, and transaminases are the main subject of Chapter 3. Chapter 4 represents the Current state of Knowledge.

Part II starts with Motivation and objectives of the study, and Chapter 5 offers information on the material and methods used throughout the study. The original research carried out in order to evaluate the innocuity of isoflurane and sevoflurane in rat is presented in Chapters 6, 7, 8 and 9. Chapter 10 contains the general conclusions of the study.

**MATERIALS AND METHODS**

The anesthetic protocol was identical for all the clinical and paraclinical evaluations carried out in this study. Isoflurane and sevoflurane were the tested inhaled anesthetics and the biologic material was represented by 40 female Wistar rats (6 weeks old). The experimental protocol was approved by the Research Ethics Committee of UASVM Cluj-Napoca and conducted in the Department of Anesthesiology and Surgical Propedeutics, UASVM Cluj-Napoca.

The animals were divided in 4 groups for isoflurane anesthesia (n=5) and 4 other groups for sevoflurane anesthesia (n=5). Rats from control groups (IsoM and SevoM) were not anesthetized. Rats from groups I isoflurane and sevoflurane (Iso1 and Sevo1) were sacrificed immediately after the end of anesthesia, the ones from groups II (Iso2 and Sevo2) 6 hours post-anesthesia, and the ones from groups III (Iso3 and Sevo3) 24 hours post-anesthesia.

The tested anesthetics were administered 3 times, with 2 days interval between administrations, and for 2 hours long each time. In order to achieve an uniform anesthesia we maintained a 1.5% anesthetic concentration and 11 O₂/min., for 2 hours long for isoflurane and 2% anesthetic and 11 O₂/min. for 2 hours long for sevoflurane, respectively.

After anesthesia, we harvested blood samples at different moments, from which we determined the antioxidant enzymes and transaminases. The rats were subsequently sacrificed, organ samples were harvested and submitted to histopathological examination. Among the organs, the liver was also assayed through immunohistochemical examination.

**RESULTS AND DISCUSSIONS**

Chapter 6, entitled “Clinical aspects in isoflurane and sevoflurane anesthesia in rat”, evaluated the induction, maintaining and recovery from anesthesia and compared the anesthetics times between isoflurane and sevoflurane.
The induction of isoflurane and sevoflurane anesthesia took place without excitation signs. The mean times obtained at the first recovery signs of the animals were 6 ± 2.1 minutes for isoflurane and 3.5 ± 1.8 minutes for sevoflurane. The recovery took place at 8.5 ± 2.2 minutes after closing the vaporizer in the case of isoflurane and 5.6 ± 2.4 minutes for sevoflurane, while the mean time until full recovery was 12 ± 3 minutes for isoflurane and 7.8 ± 3 minutes for sevoflurane. The statistical analysis showed that the difference between the mean times of the two groups was statistically significant in the case of full recovery (p<0.05).

Recovery from anesthesia took place gradually, without excitation signs. Animals did not present clinical signs that would suggest irritation of the respiratory airways (cough, nasal discharges) after anesthesia. On the other hand, most of the rats presented horripilation during recovery.

Thus, induction and maintaining of anesthesia unraveled very similar for the two anesthetics taken into study, but the recovery took place faster after sevoflurane exposure, being accompanied by horripilation in the case of both anesthetics.

In Chapter 7, “Evaluation of the isoflurane and sevoflurane anesthesia effects on oxidative status in rat”, we aimed to see if the tested anesthetics trigger oxidative stress and if there are differences between the effect of isoflurane and sevoflurane anesthesia on oxidative status in the organism.

Evolution of mean SOD (superoxid dismutase) values in rats anesthetized with isoflurane and sevoflurane is presented in Chart 1. and Chart 2., respectively. Results obtained after determination of superoxid dismutase were submitted to statistical analysis, using Student’s t-Test.

The value of erythrocyte SOD decreases immediately after anesthesia with isoflurane in comparison to the control group value and then increases 6 hours post-anesthesia exceeding the value from the control group and reaching the highest value registered in the times taken into study. At 24 hours post-anesthesia, the SOD value decreases in comparison to both the value from 6 hours and the one from the control group. The smallest value of the enzyme activity was registered immediately after the isoflurane anesthesia (Chart 1.). The differences between the values obtained after isoflurane anesthesia are not statistically significant (p>0.05).

In sevoflurane anesthetized groups, SOD values gradually decrease immediately after the anesthesia, 6 hours and 24 hours post-anesthesia, respectively (Chart 2). None of the registered changes is statistically significant (p>0.05).
Concerning the erythrocyte glutathione peroxidase (GPx), after isoflurane anesthesia, we registered a decrease in the mean value in comparison to the control group, immediately after the anesthesia, after which the value increased 6 hours post-anesthesia in comparison to group Iso1, without exceeding the GPx value from the control group. 24 hours post-anesthesia, the enzyme value decreases in comparison to the one in the control group, as well as the value from 6 hours post-anesthesia, being the lowest mean value of GPx among the groups taken into study. The GPx values in isoflurane anesthetized groups (Iso1, Iso2 and Iso3) were lower than the ones in the control group (Chart 3). The decrease in the enzyme activity value was statistically significant 24 hours post-anesthesia (p<0.05).

A progressive decrease in the value of this enzyme was registered after sevoflurane anesthesia (Chart 4.). Also, the mean values of the enzyme in anesthetized groups (Sevo1, Sevo2 and Sevo3) were lower than those in the control group. The decrease in the GPx value was very significant 6 hours post-anesthesia (p<0.01), and after 24 hours, the registered decrease was
statistically significant (p<0.05), in comparison to the enzyme activity values from the control group.

The results suggest that isoflurane or sevoflurane anesthesia did not induce significant decreases in the values of superoxid dismutase activity after the utilized doses, number of administrations and interval between them. Isoflurane induced a significant decrease of the values of erythrocyte activity of glutathione peroxidase 24 hours post-exposure, while sevoflurane at 6, 24 hours post-anesthesia, respectively. The inhaled anesthetics reduced the antioxidant defense to a certain extent, thus the organism can be prone to oxidative stress.

Chapter 8 is structured in 2 subchapters. The first subchapter, "8.1. Immunohistochemical evaluation of isoflurane and sevoflurane toxicity on rat liver”, had the following objectives: immunohistochemical testing of isoflurane and sevoflurane toxicity on liver and comparison of the effects of the two anesthetics on this organ.

The immunohistochemical reaction for caspase-3 highlighted a small number of caspase-3-positive cells in the liver of the rats from group IsoM, no more than the normal replacement rate of hepatocytes. Besides being scarce, the caspase-3-positive cells were isolately disposed and did not necessarily appear in a certain area of the hepatic lobule and were not present in all the lobules. On most of the section surface, the hepatic lobules had a normal structure and did not contain apoptotic cells (Fig. 1).

In isoflurane anesthetized group, the liver from rats sacrificed immediately after anesthesia did not present more caspase-3-positive cells in comparison to the control group, thus in the great majority of the lobules these cells did not even appear isolated (Fig. 2.). The situation maintains 6 hours after exposure to the anesthetic, regarding both the general aspect of
the organ and the small number of apoptotic cells, at a comparable state to the control group (Fig. 3).

First notable changes appear 24 hours after the administration of this anesthetic and consist in the presence of caspase-3-positive cells in some lobules. A number of cells in the close vicinity to the centrilobular vein appear affected (Fig. 4.).

We state that the phenomenon is not present in all the hepatic lobules, but only in some of them. The fact that the apoptotic cells are in relatively large numbers in some lobules, while in most of them they are absent is interesting (Fig. 5.).

Livers from rats in group SevoM had a normal aspect from all points of view (Fig. 6.) and the immunohistochemical reaction for caspase-3 highlighted the presence of caspase-3-positive cells in small numbers with an isolated disposition. The number and disposition of these cells suggest that they represent cells at the end of the cell cycle and ensure the normal replacement rate of hepatocytes.
Regarding the liver harvested from the *sevoflurane anesthetized group*, immediately after the anesthetic administration, the situation is highly comparable to the one in the control group, the number of caspase-3-positive cells being very small (Fig. 7.). We did not notice a significant increase in the caspase-3-positive cells 6 hours post-anesthesia (Fig. 8.), nor 24 hours post-exposure (Fig. 9.).

Sevoflurane did not determine the increase of the caspase-3-positive cell number in none of the control moments, which suggests that it did not trigger hepatocyte apoptosis.

Isoflurane induced a slight increase of the caspase-3-positive cells in the centrilobular vein vicinity, in some lobules, which reflects that this anesthetic triggers a discreet hepatocyte apoptosis.

The small number of apoptotic cells suggests that the anesthetics do not present hepatic toxicity, having a mild effect on this organ.
In subchapter 8.2., entitled “Evaluation of the isoflurane and sevoflurane anesthesia influence on transaminases in rat” we set out to evaluate the effect of isoflurane and sevoflurane on transaminases levels and to compare the effects of the two anesthetics on the hepatic enzymes’ values.

Regarding the ASAT values, in isoflurane anesthetized groups, we did not notice any changes in the rats taken into study. The values ranged between normal limits, which demonstrates the fact that isoflurane did not produce significant changes in the liver, heart, skeletal muscles, kidneys or red blood cells. The differences between values obtained in the control group and the ones in groups anesthetized with isoflurane (Iso1, Iso2 and Iso3) were not statistically significant.

In the case of sevoflurane anesthetized groups, ASAT values were also in normal limits. The highest value was registered in group Sevo1 (immediately after the anesthesia), in
comparison to the other anesthetized groups, but it did not exceed the normal values (Chart 6.). There was no statistical significant difference between the enzyme activity values in none of the groups.

Regarding the ALAT values, in isoflurane anesthetized groups, the mean values were situated towards the superior limit in groups Iso1, Iso2 and slightly increased in groups IsoM and Iso3. The evolution of the enzyme activity values is presented in Chart 7. The highest values were registered 24 hours after isoflurane anesthesia. Regarding the differences in the group values, they were not statistically significant in none of the groups.

In sevoflurane anesthetized groups, the values were between normal range in groups SevoM and Sevo1, and in groups Sevo2 and Sevo3 they were slightly above the superior limit of the normal values (Chart 8).

The highest value was registered in group Sevo2 (6 hours post-anesthesia), 36.03 U/l, but it did not exceed by much the normal values. Statistically, the increase of the ALAT values was not significant in group Sevo1 (immediately after the anesthesia), very significant 6 hours post-anesthesia (Sevo2) and significant 24 hours after the anesthesia (Sevo3).

The results show that none of the anesthetics taken into study modified the values of aspartate aminotransferase (ASAT), and isoflurane did not modify the alanin aminotransferase (ALAT) ones. On the other hand, after sevoflurane anesthesia, ALAT values increased very significantly 6 hours post-anesthesia and significantly 24 hours post-anesthesia.

The enzyme values were situated within normal limits or were slightly higher than the superior limit, which indicates the fact that at the dose and duration of anesthesia in our study, none of the tested anesthetics induce hepatic distress.

In Chapter 9, “Evaluation of isoflurane and sevoflurane effects on the structure and function of some vital organs in rat”, we set out to evaluate the effects of the inhaled anesthetics on the target organs and if there are differences between the effects of the two anesthetics on the liver, kidney, lung and brain.

The liver of animals in group IsoM had a normal aspect, with the characteristic lobulation (Fig. 10.) and the disposition of hepatocytes in cords that radially converge towards the centrilobular vein (Fig. 11.).
In the case of isoflurane anesthesia, we did not notice any changes in the liver at light microscopy in neither the central (Fig. 12.) or peripheric (Fig. 13.) area of the lobules, immediately after the anesthesia. Six hours post-anesthesia the general aspect of the liver is conserved (Fig. 14.) and there is a discreet congestion on some sinusoid vessels only in isolated areas (Fig. 15.). The aspect is not present in all the animals taken into study.
At 24 hours post-anesthesia, the liver of the rats taken into study has a comparable histological aspect to the one in the control group.

The **kidney** of the rats in group IsoM has a normal aspect in both cortex (Fig. 16.) and medulla (Fig. 17.).
Immediately after the anesthesia, the kidney does not present notable changes (Fig. 18.), the only aspect that is worth taking into account is the moderate congestion of the vessels in the corticomedullary zone (Fig. 19.).

Six hours post-anesthesia, the congestion in the corticomedullary zone maintains, in fact it seems slightly more intense, but is not marked (Fig. 20.).

Twenty-four hours from the anesthesia, the situation registered at six hours maintains at a great extent (Fig. 21.), but the congestion in the cortico-medullary zone seems a bit more marked (Fig. 22.).

A discreet congestion appears 0-6 hours post-anesthesia in the lungs (Fig. 23.), but diminishes 24 hours post-anesthesia (Fig. 24.).

We did not notice any changes on the histological images in the brain, the aspect is comparable to the one in the control group.

The liver from animals in group SevoM has a normal aspect, without changes on the cellular or vascular components (Fig. 25.).
Immediately after sevoflurane anesthesia, the liver from rats taken into study presented certain changes in comparison to the control group, especially on the vascular component. They consist of zonal vascular congestion in some lobules, usually situated at the periphery (subcapsular area) (Fig. 26.), but sometimes in the depth of the organ (Fig. 27.). The congestion is present in both sinusoid as well as larger vessels (Fig. 28.).

Six hours after the anesthesia, the situation is slightly modified, in the sense that the liver presents a congestive aspect on a smaller number of lobules, disposed at the periphery (Fig. 29.).

Not only the number of lobules with congestion is smaller than the anterior group, but the congestion appears obviously more decreased where present (Fig. 30.).

Twenty-four hours after the anesthesia, the liver has a comparable aspect to the one of the rats from control group (Fig. 31.).

The congestion has significantly reduced and is present in a small number of lobules and at a small intensity (Fig. 32.).
Kidneys from rats in SevoM group present a normal aspect in both cortical (Fig. 33.) and medullary zones.
Fig. 34. Group SevoM - Kidney (Goldner’s trichrome stain)

Fig. 35. Group Sevo1 – Kidney (Goldner’s trichrome stain)

Fig. 36. Group Sevo1 – Kidney (Goldner’s trichrome stain)

Fig. 37. Group Sevo1 – Kidney (Goldner’s trichrome stain)

Fig. 38. Group Sevo2 – Kidney (Goldner’s trichrome stain)

Fig. 39. Group Sevo2 – Kidney (Goldner’s trichrome stain)
We did not notice functional disorders that affect the glomerular ultrafiltration process, Bowman space appears to have a normal size and does not contain material that would suggest changes in the filtration barrier (Fig. 34.).

At the end of the anesthetic period, congestion is present in the cortical zone on both small and large caliber vessels (Fig. 35.), with the highest intensity in the corticomedullary zone (Fig. 36). Vessels from the medulla do not present obvious congestion (Fig. 37.).

Six hours after the anesthetic administration, the congestion maintains in both the cortical area (Fig. 38.), as well as the corticomedullary one (Fig. 39.).

It is not accompanied by functional changes, a statement which is sustained by the fact that the aspects that would suggest functional impairment of the filtration barrier from the renal corpuscles are not present (Fig. 40.). The same statement applies to the cortical tubes in the
medulla (Fig. 41.) (the primary filtrate has a normal aspect and does not contain proteic or sanguine material).

At 24 hours post-anesthesia, the renal congestion maintains in the cortex (Fig. 42.), as well as the passage zone between the cortex and medulla (Fig. 43.). Renal corpuscles present an aspect that does not suggest installation of functional impairment (Fig. 44.).

![Fig. 44. Group Sevo3 – Kidney (Goldner’s trichrome stain)](image1)

![Fig. 45. Group Sevo2 – Lung (Goldner’s trichrome stain)](image2)

In the lungs of sevoflurane anesthetized animals, we noticed a discreet congestion which maintains until 6 hours post-anesthesia (Fig. 45.), but 24 hours after anesthesia it relapses to a very similar aspect to the one from the control group (Fig. 46.).

![Fig. 46. Group Sevo3 – Lung (Goldner’s trichrome stain)](image3)

![Fig. 47. Group SevoM – Brain (Goldner’s trichrome stain)](image4)

In the brain, no aspects suggested unreeling of obvious neuronal apoptosis phenomena (Fig. 47.) after isoflurane and sevoflurane administration.

Isoflurane and sevoflurane determined a moderate hepatic congestion 6 hours post-anesthesia, whose intensity reduced at 24 hours, aspect which suggests that the anesthetics did not have a brutal action on the liver at the utilized dose and exposure time.
In the kidney, both anesthetics determined a slight increase of the sanguine afflux in the corticomedullary zone immediately after the anesthesia, which slightly intensifies after 6 hours and persists until 24 hours after both isoflurane and sevoflurane anesthesia.

In the lung, the anesthetics produced a relatively discreet and passenger congestion of the septal capillaries, in 0-6 hours post-exposure interval (which diminishes until 24 hours).

The increase of the post-anesthetic blood flow in the organs studied was not accompanied by morphological changes detectable in light microscopy which demonstrates that it represents a passenger adaptation to the given situation without any major negative effect.
GENERAL CONCLUSIONS

- Isoflurane, as well as sevoflurane rapidly induced anesthesia, without excitation signs. The recovery was significantly faster after sevoflurane in comparison to isoflurane, being accompanied by horripilation in the case of both anesthetics.

- The anesthetics did not induce significant changes of the superoxid dismutase values, only the decrease of glutathione peroxidase, which suggests the tested anesthetics do not produce marked oxidative stress.

- The tested anesthetics did not determine structural changes in the liver, but isoflurane induced a discreet increase in the apoptotic cell number, without any other noticeable changes in light microscopy.

- The functionality of the liver was not significantly affected by the two anesthetics, the value of transaminases raging between normal limits, even if we registered a slight increase in the alanine aminotransferase values.

- The two anesthetics did not determine structural changes obvious in light microscopy in the other organs taken into study: kidney, lung and brain.

- Excluding brain, all organs taken into study had a discreet to moderate congestion, with the most marked one in the kidney, especially on small vessels from the corticomedullary zone.

- The slightly increased sanguine afflux, unaccompanied by structural or functional changes, can be due to the presence and not necessarily the action of the anesthetics, which can be beneficial because of its participation to a faster elimination of the anesthetics from the organism.

- The results obtained in this study showed a high innocuity of isoflurane and sevoflurane on some organs and tissues, thus suggesting that the anesthetics can be also used in patients with connected pathology without too much risk.