Evaluation of Isoflurane and Sevoflurane Influence on Rat Liver

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Abstract
The specialty literature contains uneven data regarding the effect of inhaled anesthetics on transaminases’ values. Various studies show that the transaminases’ values range from a small increase up to a massive increase post-anesthesia. In the present study, we utilized 40 rats, divided in 8 groups (n=5). Animals from IsoM and SevoM groups (control) were not anesthetized. Rats from Iso1, Iso2, Iso3 groups were anesthetized with isoflurane, and the ones from Sevo1, Sevo2, Sevo3 groups with sevoflurane (3 times, with 2 days interval between administrations, and the exposure time was 2 hours long every time). After anesthesia, we harvested blood samples at different moments: immediately after the anesthesia (groups IsoM, SevoM, Iso1 and Sevo1), 6 hours post-anesthesia (Iso2 and Sevo2) and 24 hours post-anesthesia (Iso3 and Sevo3) and determined the transaminases. Upon statistical analysis, the results indicated the fact that the two anesthetics taken into study did not significantly modify the ASAT values. Also, isoflurane did not significantly modify ALAT values. On the other hand, after sevoflurane anesthesia, ALAT values registered a statistically significant change. Enzymatic values ranged between normal limits or were slightly increased over the superior limit, which signifies that at the anesthetic dose and duration from our study, none of the tested anesthetics induce hepatic distress.

Keywords: alanine aminotransferase, aspartate aminotransferase, isoflurane, sevoflurane

INTRODUCTION
Aspartate-aminotransferase (ASAT), alanineaminotrasferase (ALAT), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) are among the enzymes most frequently assessed in diagnosing liver diseases (Palmer, 2004). Sometimes, lactate dehydrogenase (LDH) activity is also determined. Among the mentioned enzymes, ASAT and ALAT are most frequently determined to assess the liver status (Palmer, 2004). Transaminases are eliminated from hepatocytes when these cells suffer necrosis and can be determined from serum in order to follow the evolution of the hepatic distress. To all intents and purposes, ALAT and ASAT are markers of the membranar integrity (Robinson and Huxtable, 1988).

The two enzymes are encountered in both cytoplasm and mitochondria. In mammals, the cytoplasmatic ALAT predominates in muscles and myocardium (tissues that catabolise carbohydrates) and mitochondrial ALAT predominates in liver and kidneys (organs with an intense gluconeogenesis) (Ulrich, 1994). Relevant increases of ALAT (10-100 fold) generally signify acute viral hepatitis or toxic necrosis of liver. The moderate changes can be due to a chronic or drug-based hepatitis, cirrhosis, myocardsies or muscular dystrophies (Palmer and Bonner, 2007; Falcă et al., 2011).

ASAT is encountered at a rate of 60% in cytoplasm and 40% in mitochondria. The moderate increase, up to 10 times, of the enzymatic activity indicates the same pathologies as the moderate increase of ALAT. Significant increases of this enzyme occur in myocardial infarction (Palmer and Bonner, 2007; Falcă et al., 2011).
Generally, seric ALAT increases in hepatic diseases, while ASAT increases in myocardial infarction. An increase in the hepatic enzymes does not necessarily indicate hepatotoxicity, however it indicates hepatocytar lesions. Considering that ALAT closely reflects the liver status, it is determined as a marker of liver status (McClatchey, 2002).

The specialty literature abounds in information on the effect of inhaled anesthetics on transaminases values, but the data are uneven and sometimes conflicting. Some authors affirm that the enzymatic values slightly increase (Soubhia et al., 2011), others write that the values massively increase (Son et al., 2006; Park et al., 2009), while others do not register changes (Kharasch et al., 2001) after inhalational anesthesia.

In this context, we considered opportune to investigate the effect of inhaled anesthetics on transaminases values and thus, on the liver. Therefore, 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.

**MATERIALS AND METHODS**

The experimental protocol was approved by the Research Ethics Committee of UASVM Cluj-Napoca. The biological material was represented by 40 Wistar rats (6 weeks old). The animals were divided in 8 groups as follows: 2 control groups (IsoM and SevoM) unsubmitted to anesthesia, 3 groups anesthetized with isoflurane (Iso1, Iso2 and Iso3) and the remaining 3 groups with sevoflurane (Sevo1, Sevo2 and Sevo3). The tested anesthetics were administered 3 times, with 2 days interval between administrations, for 2 hours long each time. After anesthesia, we harvested blood samples from the retro-orbital sinus at different moments: immediately after anesthesia (IsoM, SevoM, Iso1 and Sevo1), 6 hours post-anesthesia (Iso2 and Sevo2) and 24 hours post-anesthesia (Iso3 and Sevo3).

Subsequently, we determined the transaminases through a kinetic method. In order to dose ASAT and ALAT we used kits produced by Hospitex Diagnostics (Italy). The reagents utilized for ASAT determination were: reactive 1 containing: 80 mM TRIS buffer pH 7.8; 240 mM L-Aspartate, >900 U/L LDH (lactate dehydrogenase) and reactive 2 containing: 12 mM α-Oxoglutarate; 0.18 U/l NADH (nicotinamide dehydrogenase); >600 U/l MDH (malate dehydrogenase). When assessing ALAT we utilized reactive 1: TRIS buffer (121 mmol/l), L-Alanine (660 mmol/l), LDH (1650 U/l) and reactive 2: α-Oxoglutarate (176 mmol/l), NADH (≥2.64 U/l).

We read the results using a Jasco V-530 Spectrophotometer, with double beam (200-900 nm) at a 340 nm wavelength (334-365 nm) and the enzymatic activity was expressed in U/l.

The obtained results were statistically assessed. For the statistical analysis we used VassarStats: Website for Statistical computation, applying the One-Way ANOVA test (p<0.05 is considered as significant and p<0.01 very significant). The tables and charts were obtained using Microsoft Office Excel 2007 Program, with data provided by WassarStats.

**RESULTS AND DISCUSSION**

In the case of ASAT values, in isoflurane anesthetized groups, we did not notice any changes in rats taken into study (Fig. 1.). Values ranged between normal limits (45.7-80.8 U/L) reported by Johnson-Delaney (1996) cited by Marcus (2004), which demonstrates the fact that isoflurane did not induce significant changes in liver, heart, skeletal muscles, kidneys, brain or red blood cells. The differences between the values obtained in control group and isoflurane anesthetized groups (Iso1, Iso2 and Iso3) were not statistically significant (p>0.05) (Tab. 1.).

In the case of sevoflurane anesthetized groups, ASAT values also fell between normal ranges (Fig. 2). We registered the highest value in group Sevo1 (immediately after anesthesia), in comparison to the other anesthetized groups, but it did not exceed the normal values. We did not find a statistically significant difference between the values of this enzyme in none of the groups (p>0.05) (Tab. 1.).

In the case of ALAT values, in groups anesthetized with isoflurane, the mean values were situated towards the superior limit for groups Iso1, Iso2. As for groups IsoM and Iso3, they slightly exceeded the superior limit of the normal values (17.5-30.2 U/L) given by Johnson-Delaney (1996), cited by Marcus (2004). The evolution of the mean values of this enzyme, as well as standard deviations are presented in Chart 3. The largest values were registered 24 hours post-anesthesia. Regarding the differences between groups, they
were not statistically significant in none of the groups (p>0.05) (Tab. 2).

In sevoflurane anesthetized groups, the values ranged between normal limits for groups SevoM and Sevo1, and in groups Sevo2 and Sevo3 they slightly exceeded the superior limit of the normal values (17.5-30.2 U/l after Johnson-Delaney, 1996 cited by Marcus, 2004) (Fig. 4.). The highest value was recorded in group Sevo2 (6 hours post-anesthesia), 36.03 U/l, but it did not exceed the normal values by much. Statistically, the recorded increase in ALAT values was very statistically significant (p<0.01) (Tab. 2.).

The value of transaminases’ activity was not modified in the case of isoflurane anesthesia, the differences between groups were not statistically significant. In groups anesthetized with sevoflurane, ASAT did not record statistically significant differences, but ALAT differed statistically significant between groups. We can easily observe that ALAT value is at its peak 6 hours after anesthesia, after which it slightly decreases 24 hours post-anesthesia and probably comes back to normal in the following days. The registered increases in ALAT values slightly exceeded the maximum value. It is stated that only 10-fold or higher increases have a diagnostic significance.

The specialty literature presents controversies regarding the transaminases’ activity after inhaled anesthesia. The almost absent metabolization of isoflurane suggests that it is not nephrotoxic or hepatotoxic. The results of the studies conducted on animals and humans sustain this prediction on isoflurane’s lack of toxicity. Mice, rats and Guinea pigs continuously submitted to subanesthetic

![Fig. 1. Mean values of ASAT in isoflurane anesthetized groups, expressed in U/l and standard deviations](image1)

![Fig. 2. Mean values of ASAT in sevoflurane anesthetized groups, expressed in U/l and standard deviations](image2)
concentrations of isoflurane (up to 0.1 MAC), for 35 days long, did not develop degenerative hepatic lesions (Stevens et al., 1977). Not even pretreatment with phenobarbital (in order to induce the discharge of hepatic enzymes) and subsequent 2 hours exposure to 1 MAC isoflurane induce hepatic necrosis (Harper et al., 1982).

The studies mentioned above indicate the fact that some anesthetics can induce lesions when administered in the presence of severe stress factors, but not isoflurane. Other authors also affirm that study results on both animals and humans support the absence of liver toxicity for isoflurane (Wade and Stevens, 1981).

On the other hand, some authors published an article on fulminant hepatic necrosis, followed by death after isoflurane anesthesia in a 44 year old woman. The second day post-exposure, the woman was lethargic and seric bilirubine, ASAT and lactate dehydrogenase were highly increased. At the time, they could not firmly conclude that isoflurane was the cause for the fulminant hepatic failure which led to death, but mention there were a lot of signs associated with anesthetic-induced hepatitis. The authors said that more similar reports are needed if isoflurane causes hepatic lesions to some of the patients, in order to identify the high risk patients (Carrigan and Straughen, 1987). Others write about a case of sevoflurane-induced hepatitis in an 11 months child, in which ASAT increased to a maximum value 14 days after surgery (Ogawa et al., 1991). Some factors can play a role in the emergence of post-anesthetic liver dysfunctions, enclosing here the decrease of hepatic oxygen supply as a result of hypoxia and hypoperfusion, viral hepatitis, transfusion,

**Fig. 3.** Mean values of ALAT in isoflurane anesthetized groups, expressed in U/l and standard deviations

**Fig. 4.** Mean values of ALAT in sevoflurane anesthetized groups, expressed in U/l and standard deviations
preexistent liver dysfunctions and utilization of hepatotoxic drugs (Ogawa et al., 1991). Authors have eliminated the possible etiopathogenetic agents one by one, sevoflurane remaining the most probable to provoke hepatotoxicity in the presented case.

Other researchers compare the levels of postsurgery hepatic enzymes after propofol, enflurane, sevoflurane and desflurane anesthesia, stating that ALAT, ASAT and alkaline phosphatase were significantly increased after cholecystectomy in all studied anesthetics. The authors write that the transaminases subsequently register a decrease 3 days after the intervention, but remain above the preoperatory levels (p<0.05), mentioning that the anesthetics have a minor effect on the hepatic function (Yoon et al., 2005).

In other studies, a case of acute postoperative hepatic dysfunction is reported, in a woman without previous anesthesias, allergies, hypertension, diabetes or hepatitis. The seric transaminases recorded a significant increase, but the hepatic function improved after conservatory therapy. The authors suspected that sevoflurane would be the probable cause of this hepatic dysfunction (Son et al., 2006). Furthermore, after sevoflurane anesthesia, a pediatric patient developed acute hepatic failure (Song et al., 2007) and a 69 years man, fulminant hepatic necrosis, respectively (Turillazzi et al., 2007).

Although isoflurane is metabolized at a small rate (0.2%), it was incriminated to be the cause of some hepatic lesions which vary from transaminases increase to necrotic hepatitis and death (Turner et al., 2000). Contrary to the studies that incriminate isoflurane, it was shown that it protects liver from ischemia-reperfusion injuries, and some researchers write that sevoflurane seems to be at least as safe as isoflurane (Mohseni et al., 2014).

Isoflurane and sevoflurane are anesthetics frequently used in patients with hepatic issues. It appears isoflurane is more beneficial than sevoflurane because it is metabolized at a smaller rate in the liver. However, the foregoing studies on patients without pre-operative hepatic dysfunctions have shown that the heptic enzymes’ activities increased after isoflurane anesthesia more often than in patients anesthetized with sevoflurane (Nishiyama et al., 2004). In our study, the transaminases’ values were higher in the case of sevoflurane anesthetized rats, but did not exceed...

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**Tab. 1.** Mean values of transaminases in isoflurane anesthetized groups, standard deviation and p-values

<table>
<thead>
<tr>
<th>Group</th>
<th>ASAT</th>
<th>Standard deviation</th>
<th>ALAT</th>
<th>Standard deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IsoM</td>
<td>77.33</td>
<td>23.67</td>
<td>32.85</td>
<td>5.82</td>
<td>0.824659</td>
</tr>
<tr>
<td>Iso1</td>
<td>61.52</td>
<td>60.56</td>
<td>29.82</td>
<td>1.41</td>
<td></td>
</tr>
<tr>
<td>Iso2</td>
<td>57.46</td>
<td>6.71</td>
<td>24.30</td>
<td>9.56</td>
<td></td>
</tr>
<tr>
<td>Iso3</td>
<td>66.55</td>
<td>7.69</td>
<td>37.04</td>
<td>14.29</td>
<td></td>
</tr>
</tbody>
</table>

**Tab. 2.** Mean values of transaminases in sevoflurane anesthetized groups, standard deviation and p-values

<table>
<thead>
<tr>
<th>Group</th>
<th>ASAT</th>
<th>Standard deviation</th>
<th>ALAT</th>
<th>Standard deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SevoM</td>
<td>67.48</td>
<td>11.23</td>
<td>21.35</td>
<td>4.51</td>
<td>0.341531</td>
</tr>
<tr>
<td>Sevo1</td>
<td>74.33</td>
<td>21.10</td>
<td>23.79</td>
<td>3.35</td>
<td></td>
</tr>
<tr>
<td>Sevo2</td>
<td>67.87</td>
<td>2.46</td>
<td>36.03</td>
<td>5.08</td>
<td></td>
</tr>
<tr>
<td>Sevo3</td>
<td>59.19</td>
<td>8.09</td>
<td>33.30</td>
<td>9.95</td>
<td></td>
</tr>
</tbody>
</table>
by much the normal maximum values recorded for this species.

The decreased blood flow during anesthesia, increased calcium concentration in cells (Iaizzo et al., 1990) or anesthetic metabolism products are among the factors that can cause hepatic lesions. Still, some authors think that the hepatic arterial and portal flow are similar during sevoflurane and isoflurane anesthesia (Bernard et al., 1992). Others believe that the prolonged high concentration of intracellular Ca\(^{2+}\) would be involved in hepatotoxicity mechanism and that isoflurane would stimulate the discharge of intracellular calcium, while the effect of sevoflurane is unknown (Iaizzo et al., 1990). Thus, the authors suggest that the different effects of this anesthetics upon Ca\(^{2+}\) could be the cause of the different results obtained when studying the hepatic function after isoflurane or sevoflurane anesthesia.

In a recent study, which aimed to review the hepatotoxicity of inhaled anesthetics (Safari et al., 2014), it is reported that isoflurane induces hepatic lesions similar to halothane, but at a much smaller rate in comparison to halothane (Sinha et al., 1996), while sevoflurane is incriminated in less inhaled anesthetic-induced hepatotoxicity cases (Singhal et al., 2010).

Most of the studies have followed the effect of inhaled anesthetics on liver function (Sahin et al., 2011). Thus, some authors affirm that the transaminases’ values were slightly increased after inhaled anesthesia with sevoflurane (Soubhia et al., 2011). Some of them write that isoflurane and sevoflurane anesthesia induces an increase in the seric concentration of hepatic enzymes, isoflurane inducing a greater increase in comparison to sevoflurane (Nishiyama, 2013), while others write that the seric level of aspartate-aminotransferase (ASAT) slightly increased in the first day after sevoflurane anesthesia (Sun et al., 1997).

In dogs, an increase of transaminases was observed when the hepatic effects of halothane, isoflurane and sevoflurane anesthesia were studied, without clinical evidence of hepatic lesions 14 days after anesthetics (Topal et al., 2003). Some authors investigated the seric activity of aspartate-aminotransferase (ASAT) after isoflurane and halothane anesthesia, observing an increased activity of this enzyme 24 hours post-anesthesia, in isoflurane group (Darling et al., 2000).

Opinions regarding the effect of the two anesthetics on liver in different species are divided according to the consulted authors, but there are authors which sustain that isoflurane and enflurane anesthesia does not produce hepatotoxicity in rats (Harper et al., 1982), aspect which we also recorded in the present study.

**CONCLUSION**

Anesthetics taken into study did not significantly modify aspartate-aminotransferase’s values. Isoflurane did not significantly modify alanine-aminotransferase’s values, while after sevoflurane anesthesia alanine-aminotransferase’s values increased very statistically significant post-anesthesia.

The values of the enzymes ranged between normal limits or were slightly increased over the upper limit, which implies the fact that at the dose and duration of anesthesia in our study, none of the anesthetics induce liver distress.

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**REFERENCES**


