Effect of Xylazine-Thiopental Anesthesia on Canine Fatty Acid Profiles

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SUMMARY
In this study, the profiles of fatty acid types and concentrations were investigated on 5 control group (CG) and xylazine-thiopental group (XTG) dogs. In CG, after obtaining the blood samples, the dogs were killed with thiopental application and then the tissue sections from their livers and kidneys were harvested. All XTG dogs were anesthetized with i.m. administration of 1.5 mg/kg xylazine hydrochloride and i.v. administration of 20 mg/kg thiopental sodium. After collecting blood samples following full anesthetic recovery they were killed with overdose thiopental application, then tissue sections from their livers and kidneys were taken. All samples were analyzed with a gas chromatograph. During this investigation we found 12, 17 and 18 different types of FAs in serum, liver and kidney tissues of CG and 13, 19 and 17 in serum, liver and kidney tissues of XTG, respectively. When XTG data were compared with those of CG, a significant decrease in palmitic acid (C16:0) and stearic acid (C18:0) data of kidney and linoleic acid (C18:2n-6) data of liver, whereas an increase in eicosatrienoic acid (C20:3n-9) data of the liver were determined. It was also detected that total FA concentrations of serum, liver and kidney in XTG was lower than CG, but this reduction was not statistically significant. In conclusion, the present results suggest that thiopental-xylazine may have some effects on FA profiles at cellular level.

Key Words Dog, Anesthesia, Fatty Acids, Gas Chromatography

ÖZET
Bu çalışmadada 5 kontrol grubu (KG) ve 5 ksilazin-thiopental grubu (KTG) köpekte yağ asitlerinin tip ve konsantrasyon profilı araştırıldı. KG’u köpeklerin kan örnekleri alınmadan sonra thiopental ile ötezani edilip böbrek ve karaciğerlerinden doku örnekleri alındı. KTG’u olgular i.m. 1.5 mg/kg ksilazin hidrochlorid ve i.v. administration of 20 mg/kg thiopental sodium verilerek anestezii edildiler. KTG’u olgular anesteziden tamamen çıkıp kan örnekleri alınmadan sonra thiopental verileri ötezani edilip karaciğer ve böbreklerinden doku parçası alındı. Alınan tüm örnekler gaz kromatografisi kullanılarak analiz edildiler. Analiz sırasında KG’ınun serum, karaciğer ve böbreklerinden sırasıyla 12, 17 ve 18, KTG’ınun serum, karaciğer ve böbreklerden 13, 19 ve 17 farklı tipte yağ asidi saptandı. KTG’ınun verileri KG’nunun kan örnekleri ile karşılaştırıldığında karaciğerdeki palmitik asit (C16:0) ve stearik asit (C18:0) ile karaciğerdeki linoleik asit (C18:2n-6) verilerinde azalma, buna karşın karaciğerdeki eikosatrienik asit (C20:3n-9) miktarında belirgin bir artış saptandı. Ayrıca KTG’ınun serum, karaciğer ve böbreklerindeki yağ asitleri konsantrasyonunun KG’ununun ve KTG’ununun farklı fakat bu yüksekliklerin istatistiksel olarak anımsal olmadığını göstermektedir. Sonuç olarak, mevcut verilerde ksilazin-thiopentalın yağ asitlerini profilinde hücresel düzeyde etki oluşturabiliyor olması düşünülmektedir.

Anahtar Kelimeler Köpek, Anestezi, Yağ Asitleri, Gaz Kromatografi

INTRODUCTION
Thiopental sodium is a short acting barbiturate group anesthetic agent and the first thiobarbiturate to gain popularity as an anesthetic agent for animals ([Lumb and Jones 1984; Hall et al. 2001]. Xylazine, a potent non-narcotic sedative and analgesic as well as a muscle relaxant, was found satisfactory for use in a wide variety of domestic and exotic species ([Lumb and Jones 1984]. The pharmacokinetic and pharmacologic characteristics of thiopental and xylazine, their anesthetic qualities, efficacies in various species, adverse clinical, hematological and biochemical effects on the most commonly affected systems like cardiovascular, respiratory and urinary systems had been widely investigated ([Ko et al. 2000; Lobetti and Lambrechts 2000; Redondo et al. 2000; Hall et al. 2001; Joubert and Lobetti 2002; Kojima et al. 2002; Kabara et al. 2003; Luna et al. 2004].

The fatty acids are the main constituents of lipids which were an important source of oxidative fuel. Lipids serve as thermal insulator in the subcutaneous tissues and around certain organs. They act as mediators of signal...
transduction along myelinated nerves and were also important constituents of cellular membranes (Murray et al. 2000). Knowledge of FA biochemistry is important in understanding many current biochemical areas of interest, e.g., obesity, atherosclerosis, prostate cancer, atopic dermatitis, progressive retinal rod-cone degeneration and role of many polyunsaturated FAs in nutrition and health (Murray et al. 2000, Saevik et al. 2002). Studies investigating direct effect of anesthetic agents on fatty acid (FA) were not well documented. Hence this study was undertaken to investigate the alterations in FA profile during xylazine-thiopental anesthesia used commonly in veterinary practice.

**MATERIALS and METHODS**

Six male and 4 female dogs weighed between 9 - 28 kg and aged between 0.5 - 4.5 years, were divided at random and equally into control group (CG) and xylazine-thiopental group (XTG). XTG dogs were anesthetized with i.m. 1.5 mg/kg xylazine hydrochloride (Rompun, Bayer) and i.v. 20 mg/kg thiopental sodium (Pentothal Sodium, Abbott). Blood samples of CG directly and those of XTG group dogs after full recovery from the surgery were collected from cephalic vein and the serum by separated by usual manner and stored at -20°C until being analyzed. After blood sampling, all dogs were euthanized with lethal dose of thiopental injection and then tissue sections of approximately 2x2x2 cm in size were harvested immediately from liver and kidney, and were processed and analyzed as follows: the samples were homogenized with the mixture of hexane: isopropanol (3:2, v/v), which were centrifuged at 5000 rpm of 4°C and lipid contained hexane: isopropanol phase was stored at -25 °C until the further analysis. Fatty acids were processed and analyzed with the method of Christie (1992).

Experimental results (mean ± SD) were analyzed by ANOVA and independent t-test using SPSS Software package programme. The results were considered as significant at P< 0.05 and 95% confidence interval.

**RESULTS**

During this investigation it was found 12, 17 and 18 different types of FAs in CG serum, liver and kidney tissues and 13, 19 and 17 different types of FAs in XTG serum, liver and kidney tissues, respectively. While some of these FAs existed in all, others presented in some samples usually of negligible in amount. For a particular tissue, the FAs which existed less than 50% of the samples analyzed were not considered for statistical evaluation and they were not included in the Table 1. When the data of CG and XTG were compared for each individual FA, significant decrease in palmitic acid (C16:0) and stearic acid (C18:0) was not significant.

**Table 1. FA concentration (%), Mean ±SD) in the serum, liver and kidney of CG and TXG cases.**

<table>
<thead>
<tr>
<th>FA names</th>
<th>Serum</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CG</td>
<td>TXG</td>
<td>CG</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>20.02±4.41</td>
<td>20.42±8.81</td>
<td>15.64±0.98</td>
</tr>
<tr>
<td>Palmitoleic acid (C16:1 n7)</td>
<td>3.04±1.03</td>
<td>5.16±2.01</td>
<td>0.76±0.36</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>26.74±6.01</td>
<td>20.26±9.44</td>
<td>24.86±0.25</td>
</tr>
<tr>
<td>Oleic acid (C18:1 n9)</td>
<td>10.04±3.28</td>
<td>13.33±4.71</td>
<td>6.70±2.76</td>
</tr>
<tr>
<td>Linoleic acid (C18:2 n6)</td>
<td>19.40±8.08</td>
<td>11.26±6.46</td>
<td>21.23±2.35*</td>
</tr>
<tr>
<td>Eicosatrienoic acid (C20:3 n9)</td>
<td>-</td>
<td>-</td>
<td>0.58±0.27*</td>
</tr>
<tr>
<td>Arachidonic acid (C20:4 n6)</td>
<td>22.56±4.57</td>
<td>16.91±1.09</td>
<td>18.31±1.59</td>
</tr>
<tr>
<td>Eicosateraenoic acid (C20:4 n3)</td>
<td>-</td>
<td>1.27±0.35</td>
<td>0.57±0.02</td>
</tr>
<tr>
<td>Docosapentaenoic acid (C22:5 n3)</td>
<td>-</td>
<td>-</td>
<td>1.15±0.49</td>
</tr>
<tr>
<td>Docosahexaenoic acid (C22:6 n3)</td>
<td>-</td>
<td>-</td>
<td>2.35±0.45</td>
</tr>
</tbody>
</table>

* Significant difference between groups at P< 0.05

**DISCUSSION**

FAs are the main constituents of lipids which were important constituents of cellular membranes (Murray et al. 2000). It is well known that almost all anesthetics cause at different degrees cellular damages in the body. The cellular damage was hypnotized to likely result in an alteration in the fatty acid concentration. This fact encouraged to investigate the alterations in FA profile during TX anesthesia used commonly in veterinary practice as induction anesthesia. When the data of CG and XTG were compared for each individual FA, significant decrease in palmitic acid (C16:0) and stearic acid (C18:0) in data of kidney and linoleic acid (C18:2n-6) in data of liver, and an increase in eicosatrienoic acid (C20:3n-9) in data of the liver were determined after anesthesia. It was also detected that total FA concentrations of serum, liver and kidney in TXG was lower than CG, but this reduction was not significant.

The circulating concentrations of FAs can vary significantly under metabolic conditions, thus, a fast and accurate method for determination of concentrations of individual fatty acids present in serum is essential for studies of in vivo FA metabolism. Traditionally gas chromatography had been used for fatty acid quantification (Vecka et al., 2002). Saevik et al. (2002) investigated FA composition of serum lipids in healthy dogs and found over 14 different types FAs compared to 12 in the present study. In their study, linoleic acid appeared as the FA with highest ratio,
in contrast to stearic acid which consisted of the highest fatty acids concentrations in the present study. The difference between these two studies could be related to factors such as breed, diet, high or low lipolytic activities and exercise (Vecka et al., 2002).

Taugbol et al. (1998) analyzed the FA composition of the total lipid fraction of subcutaneous fat and blood plasma in pruritic dogs and dogs without skin problems using gas chromatography and found that in subcutaneous fat, the concentration of docosatetraenoic acid (C22:4 n6) was lower in the group of pruritic dogs compared to dogs with healthy skin. The amount of eicosatrienoic acid (C20:3 n9) in plasma lipids from pruritic dogs was higher than in dogs without skin problems. Maldonado et al. (2001) studied lipid and FA compositions of canine lipoproteins in comparison with those of humans and found that all canine lipoproteins were relatively richer than those from humans in long-chain (C20-C22) n6 and n3 polyunsaturated fatty acids (PUFA) but had comparable proportions of total saturated and monoenoic fatty acids, with C18:2 n6 (linoleic acid) being the main PUFA in both the mammals. Many studies including the former ones had investigated FA compositions and concentration in a single tissue. However, the present investigations showed the type and concentration profile of FA in serum, liver and kidney and found FAs with different types and concentrations with significant differences between various FA of two groups (Table 1).

When the data of CG and TXG were compared considering data of each individual fatty acids, it was observed just a significant decrease in palmitic and stearic acid data of each individual fatty acids, it was observed just a significant decrease in palmitic and stearic acid data of each individual fatty acids, it was observed just a significant decrease in palmitic and stearic acid of kidney, which indicated that these anesthetic agent combination had some effects on de novo fatty acid synthesis and chain elongation reactions (Gibbons, 2003). On the other hand, a meaningful decrease in linoleic acid (C18:2 n6) concentration but a marked increase in eicosatrienoic acid (C20:3 n9) in the liver tissue may be interpreted as these anesthetic combination likely to produce an inhibitory effect on the delta-6 desaturation pathway catalyzing enzymes (Nakamura and Nara, 2004). Consequently, the present results may suggest that these agents could have some effects on FA profiles at cellular level.

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