Distribution of selenium in sheep treated with dipheny diselenide


Universidade Federal de Santa Maria - Santa Maria, RS

ABSTRACT

The aim of the present study was to report the in vivo distribution of selenium in sheep. For this, animals were allocated into two groups (control group and treated group) and kept in metabolic cages for a period of 37 days. The treated group received a single dose (6 µmol/kg) of Diphenyl Diselenide, intravenously. Plasma and erythrocytes samples were collected at different times. Adipose tissue, muscles (latissimus dorsi, semitendinosus, and supra-scapular) heart, liver, lung, kidney, intestine and brain were sampled at 30 days post-treatment, in order to determine the selenium concentration. The results demonstrated that the selenium, from the Diphenyl Diselenide group, was higher in erythrocytes (4.8 mg/L, six hours post-treatment) when compared with the control sheep. The deposition of selenium occurred in the liver (7.01 µg/g), brain (3.53 µg/g) and kidney (2.02 µg/g). After 30 days of a single intravenous injection of Diphenyl Diselenide, liver was the main organ of selenium deposition.

Keywords: antioxidant, ovine, selenium, tissue

INTRODUCTION

Trace elements are essential chemical elements for maintaining organic homeostasis, playing an important role in fetal growth (Hostetler et al., 2003), semen viability (Kendall et al., 2000), immune response (Kendall et al., 2012) and weight gain (Garg et al., 2008). All of these characteristics are dependent on the amount of trace element available to animals and are influenced by the route of administration (oral or injection) and the product chemical form of the element (for instance, organic or inorganic) (Herdt and Hoff, 2011).

Among these elements, attention has been given to selenium, which has been studied in several research groups worldwide. This element is a crucial constituent of about 20-25 selenoproteins in vertebrates (Lobanov et al., 2009) which have important physiological functions in mammals;
for instance, in the metabolism of thyroid hormones, peroxides and other reactive species (Lobanov et al., 2009; Shchedrina et al., 2010). Recently, it was demonstrated that selenoprotein M knock-out mice exhibit obesity, indicating a role for this protein in the regulation of fat deposition in rodents (Pitts et al., 2013).

Diphenyl Diselenide (DD) is a synthetic compound of selenium which has been shown to have hepatic protective effects (Costa et al., 2013), reduce depressive-like behavior (Dias et al., 2014), prevent on oxidative stress induced by septicemia in rats (Prauchner et al., 2011), exhibit neuroprotection against methyl mercury toxicity in mice (Glaser et al., 2013) and fungicide effects in rabbits infected with Pythium insidiosum (Loreto et al., 2012). However, at high doses or concentrations, DD has toxic potential to rodents and rabbit (Nogueira and Rocha, 2011; Straliotto et al., 2010, 2009). In rodents, liver followed by kidney, are the organs that accumulate selenium after acute or chronic exposure to high doses of DD (Maciel et al., 2003; Prigol et al., 2010; Prigol et al., 2009), and at high doses Diphenyl Diselenide can be hepatotoxic (Nogueira and Rocha, 2011).

There is no information available about the toxicity and distribution of Diphenyl Diselenide in sheep. Since this compound has interesting biological properties, its therapeutic use is possible in human and veterinary medicine. Therefore, the aim of the present study was to assess tissue distribution of selenium in animals treated with DD.

MATERIAL AND METHODS

The present study was approved by the Ethics Committee for Animal Experimentation of the Universidade Federal de Santa Maria under protocol number 130/2010.

The experimental design was organized using five-month old sheep, weighing between 37 to 53kg (Texel breed) were separated into a control group (CG; n= 3) and a treated group (TG; n= 3). The animals were maintained in metabolic cages for a period of 37 days (seven days of adaptation and 30 days post-treatment). Throughout the study period, the animals were fed with combined oat hay (Avena sativa) and ryegrasses hay (Lolium multiflorum) twice a day (2% of body weight).

Diphenyl Diselenide (C$_{12}$H$_{10}$Se$_{2}$) (Ref 180629) was presented in a powder formulation with 98% purity and a molecular weight of 312.13g/mol was used. Animals in the CG were treated with 20ml of dimethyl sulfoxide (DMSOL) whereas those in TG received 6μmol of DD/kg of body weight diluted in 20ml of DMSO. In both groups, each animal was treated intravenously (IV) with a total volume of 500mL (v/v, 480mL of physiological solution plus 20mL of DMSOL (4% solution of DMSO) at a flow rate of 8ml / minute.

Blood samples were taken before infusion (H0) and, six (H6) and twelve (H12) hours post infusion, as well as on the following days after diselenide treatment: D1, D2, D3, D4, D5, D7, D15 and D30. Blood sample collected were centrifuged (1000 g for 10 minutes) and the plasma and erythrocytes were separated.

During the experimental period, the animals were submitted to clinical evaluation in order to observe possible signs of acute selenium intoxication in ruminants (Krishina et al., 2007) such as ataxia, anorexia, dyspnoea, tachypnea and diarrhea.

On D30, the animals were euthanized with thiopental 1g/animal followed by 100mL of potassium chloride. Perirenal fat, muscles latissimus dorsi, semitendinosus and suprascapular, along with fragments of heart, liver, lung, kidney (cortex), intestine and brain were collected.

Content of selenium was analyzed in plasma, erythrocytes, and tissues sheep samples. Plasma and erythrocytes (0.5mL) were digested in HNO$_3$ (1:1v/v). Tissue samples were weighed (approximately 0.5g/each) and also digested in HNO$_3$ (1:3w/v). All the processed materials obtained were incubated at 100°C for 12 hours. The samples were, then, diluted (10x) in ultrapure water, and the selenium level was measured by inductively couple plasma atomic emission spectrometry (ICPE- 9000, Shimadzu Scientific Instruments). Calibration standard curves were prepared freshly using selenium stock standard solution, with limit of detection in the 0.1µg/g (Prigol et al., 2012).
Values of selenium in tissue of control and treated groups were calculated by t test, with 5% significance level (P< 0.05). Area under the curve was analyzed for selenium in plasma and erythrocytes. Data were expressed as mean ± standard error of the mean (SEM).

RESULTS

Sheep treated with a single intravenous dose of about 70-100mg of DD (6µmol/kg) or DMSO, did not exhibit any overt sign of toxicity from the time of bolus injection to the end of the experimental observation.

The plasma concentration of selenium in TG was numerically identical when compared with CG (Figure 1A). The analysis of the area under the curve (AUC) for plasma selenium, indicated that the total amount of selenium did not differed between the CG (71.35±51.35) and TG (76.03±54.43) between the first sampling time and the 30th day after injection (P= 0.64).

Figure 1. Selenium concentration in the plasma (A) and erythrocyte (B) of sheep treated with 6µmol of Diphenyl Diselenide/kg of weight live (Treated Group) and not treated (Control Group). Each point represents the average and the lines represent the standard error for each experimental point.
Selenium peak concentration in erythrocytes (4.80 mg/L) was detected six hours post-treatment, which remained high until the last experimental day (Figure 1B). The analysis AUC revealed a significant difference in the total amount of selenium in erythrocytes from 1 hour to 30 days after DD injection. The mean AUC, for treated group, was 2454.0±114.7, whereas in control group it was 2153.0±33.84, but the difference not was significant (P= 0.088).

Thirty days after treatment with DD, the deposition of selenium in the tissues of sheep occurred predominantly in the liver (7.01 µg/g), brain (3.53 µg/g) and kidney (2.02 µg/g). The concentration in the liver of TG animals (7.01 µg/g) was twice as high as that found in CG animals (3.96 µg/g), but the difference was not significant (P= 0.158). In the kidney, the observed concentration was ten-fold between the TG (2.02 µg/g) and CG (0.29 µg/g) (P= 0.037, Table 1). Other tissues, such as the intestine, lung, heart, semitendinosus muscle, and supra-scapular muscle of sheep treated with DD, exhibited selenium concentrations of 0.66 µg/g, 0.64 µg/g, 0.62 µg/g, 0.32 µg/g and 0.19 µg/g, respectively, values which were similar to those detected in the CG. It was not possible detect selenium in the Latissimus dorsi muscle, lymphnode, omentum, and adipose tissue of animals TG (Table 1).

Table 1. Selenium distribution in tissues of sheep treated with 6 µmol of Diphenyl Diselenide/kg of weight live (Treated Group) and not treated (Control Group)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control Group (µg/g selenium)</th>
<th>Treated Group (µg/g selenium)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>3.96±2.57</td>
<td>7.01±1.55</td>
<td>0.158</td>
</tr>
<tr>
<td>Brain</td>
<td>2.55±1.80</td>
<td>3.53±2.42</td>
<td>0.378</td>
</tr>
<tr>
<td>Kidney (Cortex)</td>
<td>0.29±0.16</td>
<td>2.02±0.73</td>
<td>0.037</td>
</tr>
<tr>
<td>Intestine</td>
<td>1.22±0.50</td>
<td>0.66±0.33</td>
<td>0.219</td>
</tr>
<tr>
<td>Lung</td>
<td>0.39±0.23</td>
<td>0.64±0.39</td>
<td>0.330</td>
</tr>
<tr>
<td>Heart</td>
<td>0.36±0.34</td>
<td>0.62±0.50</td>
<td>0.356</td>
</tr>
<tr>
<td>Semitendinosus</td>
<td>0.47±0.27</td>
<td>0.32±0.32</td>
<td>0.358</td>
</tr>
<tr>
<td>Supra-scapular</td>
<td>0.63±0.37</td>
<td>0.19±0.19</td>
<td>0.186</td>
</tr>
<tr>
<td>Latissimusdorsii muscle</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>-</td>
</tr>
<tr>
<td>Lymphnode</td>
<td>0.41±0.26</td>
<td>&lt;0.10</td>
<td>-</td>
</tr>
<tr>
<td>Omentum</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>-</td>
</tr>
<tr>
<td>Perirenal Fat</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>-</td>
</tr>
</tbody>
</table>

P value calculated by t test, with 5% significance level

**DISCUSSION**

No signs of overt toxicity were observed in TG animals using 70-100 mg of DD for animal, as well as, when sheep were treated with sodium selenite (1 mg/kg b.w) and S-methylselenocysteine (4 mg/kg b/w) (Davis et al., 2013). But, unlike other dose and sources with sodium selenate at a dose 2 and 4 mg/kg body weight (Davis et al., 2013) and selenomethionine which was evidenced to be toxic to sheep when in doses of 4, 6, and 8 mg/kg of body weight (Tiwary et al., 2006). It indicates that intoxication by selenium is related not only at a dose, but also with the administration routes.

Several trace elements can exhibit different tissue distribution and excretion, depending on the animal species. In the present study, the administration of DD in sheep resulted in different tissue distribution than those reported previously in mice and rats (Maciel et al., 2003; Prigol et al., 2012). One intriguing observation from the present study was that kidney Se levels (0.29 µg/g) were low in the CG, when compared to the brain and liver. Studies in rodents (Maciel et al., 2003; Prigol et al., 2012; Schomburg et al., 2004) and humans (Casey et al., 1982; Oster and Prellwitz, 1989) have indicated that the organs with the highest selenium content are liver and kidney. Here, Se levels were higher in brain than kidney for both the control (2.55 vs. 0.29 µg/g Se) and treatment groups (3.53 vs. 2.02 µg/g Se). Although we do not have a clear explanation to this fact, the size of the animal, the chemical form and the route of selenium...
administration can also be important factors in determining the preferential deposition of selenium in brain when compared to kidney observed here.

The liver is the most important organ in terms of metabolizing toxic substances (Hijmans et al., 2014) and is an important organ of selenium deposition after Diphenyl Diselenide administration (Maciel et al., 2003; Nogueira and Rocha, 2011; Prigol et al., 2012). Here we observed that the highest concentration of selenium was found in the TG liver sample, even after one month of single administration. However, the difference of was not significantly different from control sheep, which can be attributed to the lag between the injection and selenium determination.

Of particular importance is the high quantity of selenium found in the kidney of TG, when compared with CG. The present results differed from those obtained in mice, where liver was the major site of selenium deposition, after acute or chronic exposure to high doses of DD (Maciel et al., 2003). The deposition of selenium in kidneys may be related to the fact that this compound is mainly excreted via urine (Prigol et al., 2012). The brain was the second organ with the highest concentration of selenium TG and CG (Table 1). Though the differences between groups were not significant, the determination was done 30 days after Diphenyl Diselenide administration, which may stimulate the study of DD in the sheep, especially, in relation to its potential protection and prevention against diseases related to the nervous system. Giving support to this assumption, the concentration of selenium in the brain of sheep was similar to that observed in mice and rats (Prigol et al., 2012), where Diphenyl Diselenide has been shown to exhibit neuroprotective action against different insults (Glaser et al., 2013).

CONCLUSION

The distribution of selenium in the blood of sheep after a single intravenous administration of DD, indicated that erythrocyte may retain selenium better than plasma, may indicate a relatively slow distribution of selenium from plasma to erythrocytes and possibly to other deeper tissues than blood when compared to rodents. One important observation reached here was that Diphenyl Diselenide did not cause any overt sign of toxicity in sheep from the time of bolus injection to the end of the experimental observation (1 month).

REFERENCES


