http://dx.doi.org/10.1590/1678-4162-9790

Arq. Bras. Med. Vet. Zootec., v.70, n.4, p.1017-1022, 2018

Distribution of selenium in sheep treated with dipheny diselenide

[*Distribuição do selênio em ovinos tratados com disseleneto de difenila*]

M.L.R. *Leal*, J.B.T. *Rocha*, C.L.D. *Corte*, A.R. *Aires*, J.F.X. *Rocha*, R.S. *Zanatta*, J.L.S. *Carpes*, G.A. *Szinwelski*, S. *Stefanello*, F.L. *Pivoto*

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 (6µmol/kg) of Diphenyl Diselenide, intravenously. Plasma and erythrocytes samples were collected at different times. Adipose tissue, muscles (*latissimusdorsi*, *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 (4.8mg/L, six hours post-treatment) when compared with the control sheep. The deposition of selenium occurred in the liver (7.01µg/g), brain (3.53µg/g) and kidney (2.02µg/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

RESUMO

O objetivo do presente estudo foi investigar a distribuição in vivo *do selênio em ovinos. Para isso, os animais foram distribuídos em dois grupos (grupo controle e grupo tratado) e mantidos em gaiolas metabólicas por um período de 37 dias. O grupo tratado recebeu uma dose única (6µmol/kg) de disseleneto de difenila, por via intravenosa. As amostras de plasma e de eritrócitos foram recolhidas em momentos diferentes. Tecido adiposo, músculos (*latissimus dorsi, *semitendinoso e supraescapular) coração, fígado, pulmão, rim, intestino e cérebro foram amostrados aos 30 dias pós-tratamento, a fim de se determinar a concentração de selênio. Os resultados demonstraram que o selênio, do grupo disseleneto de difenila, foi maior em eritrócitos (4,8mg/L, seis horas após o tratamento) quando comparado com o grupo controle. A deposição de selênio ocorreu no fígado (7,01µg/g), cérebro (3,53µg/g) e rim (2,02µg/g). Após 30 dias de uma única injeção intravenosa de disseleneto de difenila, o fígado foi o principal órgão de deposição de selênio.*

Palavras-chave: antioxidante, ovinos, selênio, tecidos

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;

Recebido em 3 de março de 2017
Aceito em 22 de novembro de 2017
E-mail: martalizandra@gmail.com

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). 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 (1000g 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 *latissimusdorsi*, *semitendinosus* and *supra-scapular*, 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 ultra-pure 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).

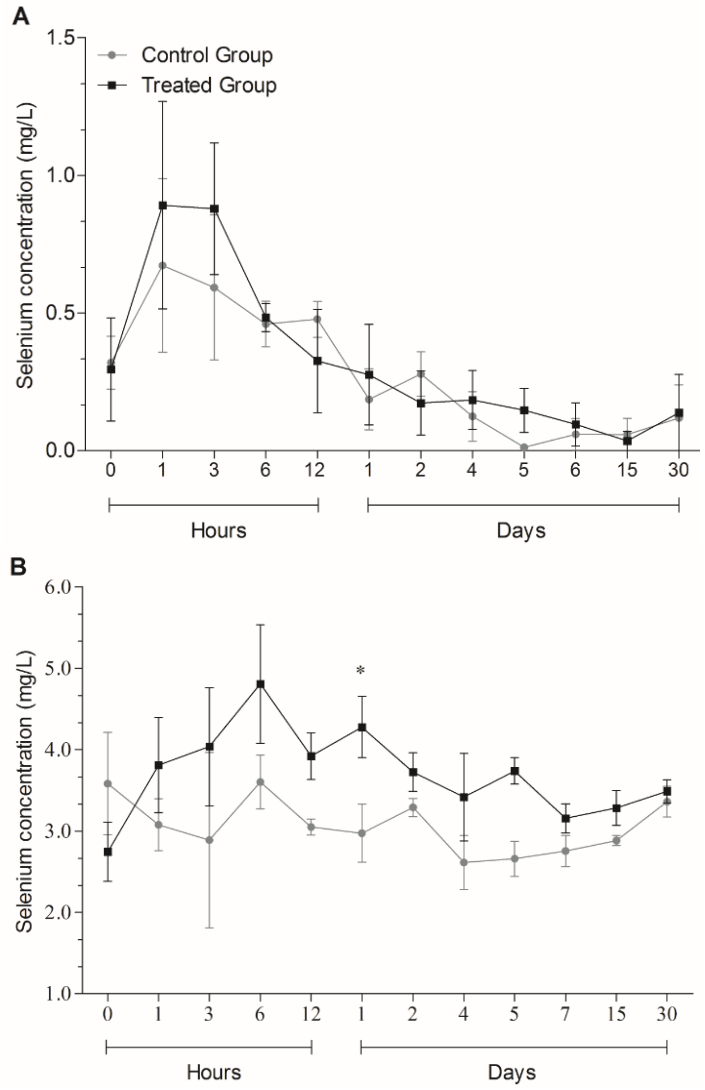

Figure 1. Selenium concentration in the plasma (A) and erythrocyte (B) of sheep treated with 6µmol of Diphenyl Diselenide/kg of weight live (Treated Group) and not treated (Control Group). Each point represents the average and the lines represent the standard error for each experimental point.

Selenium peak concentration in erythrocytes (4.80mg/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 (7.01µg/g), brain (3.53µg/g) and kidney (2.02µg/g). The concentration in the liver of TG animals (7.01µg/g) was twice as high as that found in CG animals (3.96µg/g), but the difference was not significant (P= 0.158). In the kidney, the observed concentration was ten-fold between the TG (2.02µg/g) and CG (0.29µg/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 0.66µg/g, 0.64µg/g, 0.62µg/g, 0.32µg/g and 0.19µg/g, respectively, values which were similar to those detected in the CG. It was not possible detect selenium in the *Latissimusdorsi* muscle, lymphnode, omentum, and adipose tissue of animals TG (Table 1).

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

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

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

DISCUSSION

No signs of over toxicity were observed in TG animals using 70-100mg of DD for animal, as well as, when sheep were treated with sodium selenite (1mg/kg b/w) and Se-methylselenocysteine (4mg/kg b/w) (Davis *et al.*, 2013). But, unlike other dose and sources with sodium selenate at a dose 2 and 4mg/kg body weight (Davis *et al*., 2013) and selenomethionine which was evidenced to be toxic to sheep when in doses of 4, 6, and 8mg/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 (0.29µg/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. 0.29µg/g Se) and treatment groups (3.53 vs. 2.02µg/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

CASEY, C.E.; GUTHRIE, B.E.; FRIEND, G.M.; ROBINSON, M.F. Selenium in human tissues from New Zealand. *Arch. Environ. Health Int. J.*, v.37, p.133-135, 1982.

COSTA, M.D.; FREITAS, M.L.; DALMOLIN, L. *et al*. Diphenyl diselenide prevents hepatic alterations induced by paraquat in rats. *Environ. Toxicol. Pharmacol*., v.36, p.750-758, 2013.

DAVIS, T.Z.; STEGELMEIER, B.L.; WELCH, K.D. *et al*. Comparative oral dose toxicokinetics of selenium compounds commonly found in selenium accumulator plants. *J. Anim. Sci*., v.91, p.4501-4509, 2013.

DIAS, G.R.M.; ALMEIDA, T.M.; SUDATI, J.H. Diphenyl diselenide supplemented diet reduces depressive-like behavior in hypothyroid female rats. *Physiol. Behav*., v.124, p.116-122, 2014.

GARG, A.K.; MUDGAL, V.; DASS, R.S. Effect of organic zinc supplementation on growth, nutrient utilization and mineral profile in lambs. *Anim. Feed Sci. Technol.*, v.144, p.82-96, 2008.

GLASER, V.; MORITZ, B.; SCHMITZ, A. Protective effects of diphenyl diselenide in a mouse model of brain toxicity. *Chemico-Biol. Interact*., v.206, p.18-26, 2013.

HERDT, T.H.; HOFF, B. The use of blood analysis to evaluate trace mineral status in ruminant livestock. *Vet. Clin. Food. Anim*., v.27, p.255-283, 2011.

HIJMANS, B.S.; GREFHORST, A.; OOSTERVEER, M.H.; GROEN, A.K. Zonation of glucose and fatty acid metabolism in the liver: mechanism and metabolic consequences. *Biochimie*, v.96, p.121-129, 2014.

HOSTETLER, C.E.; KINCAID, R.L.; MIRANDO, M.A. The role of essential trace elements in embryonic and fetal development in livestock. *Vet. J*., v.166, p.125-139, 2003.

KENDALL, N.R.; MACKENZIE, A.M.; TELFER, S.B. The trace element and humoral immune response of lambs administered a zinc, cobalt and selenium soluble glass bolus. *Livest. Sci.*, v.148, p.81-86, 2012.

KENDALL, N.R.; MCMULLEN, S.; GREEN, A.; RODWAY, R.G. The effect of a zinc, cobalt and selenium soluble glass bolus on trace element status and semen quality of ram lambs. *Anim. Reprod. Sci*., v.62, p.277-283, 2000.

KRISHNA, D.; TICIANA, N.F.; VIVIAN,A.N.; PEIXOTO, V.P. Enfermidades associadas à intoxicação por selênio em animais. *Pesqui. Vet. Bras.*, v.24, p.125-136, 2007.

LOBANOV, A.V.; HATFIELD, D.L.; GLADYSHEV, V.N. Eukaryotic selenoproteins and selenoproteomes. *Biochim. Biophys. Acta*, v.1790, p.1424-1428, 2009.

LORETO, É.S.; ALVES, S.H.; SANTURIO, J.M. *et al*. Diphenyl diselenide in vitro and in vivo activity against the oomycete pythium insidiosum. *Vet. Microbiol*., v.156, p.222-226, 2012.

MACIEL, E.N.; FLORES, E.M.M.; ROCHA, J.B.T.; FOLMER, V. Comparative deposition of diphenyl diselenide in liver, kidney, and brain of mice. *Bull. Environ. Contam. Toxicol.*, v.70, p.470-476, 2003.

NOGUEIRA, C.; ROCHA, J.T. Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds. *Arch. Toxicol*., v.85, p.1313-1359, 2011.

OSTER, O.; PRELLWITZ, W. The daily dietary selenium intake of west German adults. *Biol. Trace Elem. Res.*, v.20, p.1-14, 1989.

PITTS, M.W.; REEVES, M.A.; HASHIMOTO, A.C. *et al*. Deletion of selenoprotein M leads to obesity without cognitive deficits. *J. Biol. Chem.*, v.288, p.26121-26134, 2013.

PRAUCHNER, C.A.; PRESTES, A.D.S.; ROCHA, J.B.T. Effects of diphenyl diselenide on oxidative stress induced by sepsis in rats. *Pathol. Res. Pract.*, v.207, p.554-558, 2011.

PRIGOL, M.; BRÜNING, C.; MARTINI, F.; NOGUEIRA, C. Comparative excretion and tissue distribution of selenium in mice and rats following treatment with diphenyl diselenide. *Biol. Trace Elem. Res.*, v.150, p.272-277, 2012.

PRIGOL, M.; PINTON, S.; SCHUMACHER, R. *et al*. Convulsant action of diphenyl diselenide in rat pups: measurement and correlation with plasma, liver and brain levels of compound. *Arch. Toxicol.*, v.84, p.373-378, 2010.

PRIGOL, M.; SCHUMACHER, R.F.; WAYNENOGUEIRA, C.; ZENI, G. Convulsant effect of diphenyl diselenide in rats and mice and its relationship to plasma levels. *Toxicol. Lett.*, v.189, p.35-39, 2009.

SCHOMBURG, L.; SCHWEIZER, U.; KÖHRLE, J. Selenium and selenoproteins in mammals: extraordinary, essential, enigmatic. *Cell. Mol. Life Sci.*, v.61, p.1988-1995, 2004.

SHCHEDRINA, V.A.; ZHANG, Y.; LABUNSKYY, V.M. *et al*. Structure-function relations, physiological roles, and evolution of mammalian ER-resident selenoproteins. *Antioxid. Redox Signal.*, v.12, p.839-849, 2010.

STRALIOTTO, M.R.; MANCINI, G.; OLIVEIRA, J. *et al*. Acute exposure of rabbits to diphenyl diselenide: a toxicological evaluation. *J. Appl. Toxicol.*, v.30, p.761-768, 2010.

TIWARY, A.K.; STEGELMEIER, B.L.; PANTER, K.E. *et al*. Comparative toxicosis of sodium selenite and selenomethionine in lambs. *J. Vet. Diag. Invest.*, v.18, p.61-70, 2006.