EVALUATION OF RUBIDIUM CONTENTS IN ORGANS OF BITCHES (CANIS LUPUS F. FAMILIARIS)

EWA M. SKIBNIEWSKA, TADEUSZ KOŚLA, AND MICHAŁ SKIBNIEWSKI

Department of Biology of Animal Environment, Faculty of Animal Sciences, Warsaw University of Life Sciences-SGGW, 02-786 Warsaw, Poland,

Abstract

The objective of the presented study was to compare rubidium concentration in different organs of bitches, depending on their health state. Samples of muscles, liver, and kidneys collected post mortem from 45 animals, at the age of 1-8 years, were examined. Chemical analysis was conducted using the inductively coupled plasma atomic emission spectroscopy method. The contents of rubidium in particular organs varied from 2.07 to 4.52, from 1.25 to 3.81, and from 1.32 to 3.78 mg kg−1 of wet weight for muscle, kidney, and liver samples, respectively. The highest values were observed in animals with neoplastic disease and the lowest in healthy animals. These differences were highly statistically significant.

Key words: bitches, kidneys, liver, muscle, rubidium.

Rubidium belongs to the metals from the 1st group of periodic table. In respect of the frequency of its occurrence in the lithosphere, it takes up the 16th place (13, 18). Rubidium minerals do not occur independently in the nature. This element is mostly met as a component of lithium and caesium minerals (1, 10, 12). The investigations on the contents of rubidium in human and animal organisms have been sparse so far and the results of already performed experiments do not provide an explicit answer to the question about its indispensability. Relatively little is known about its biological role. Physiological functions of rubidium and its toxicity were investigated in fragments. It is known that there is a certain synergism in metabolic processes between rubidium and potassium. Both elements are alkali metals with exceptional chemical affinity. Rubidium is able to substitute potassium, which may cause metabolic disturbances. There are many reports on therapeutic action of alkali metals. Experimental administration of rubidium to patients with psychic disturbances (e.g. schizophrenia, manic-depressive disease) showed that it may cause neurochemical and behavioural effects opposite to those obtained by the administration of lithium salts. Its impact on the central nervous system results from the effect of serotonin and γ-aminobutyric acid on the transport of neurotransmitters (3, 21). There are also reports pointing out to the rubidium toxic action. It was observed that rubidium induces the appearance of renal glomerulus’s damages (4). Rubidium toxic action on the organism is particularly visible during a long lasting rubidium therapy. It is connected with the disturbances of potassium distribution in the organism. In patients with periodic affective disturbances treated with rubidium chloride, a decrease in potassium contents in tissues (mainly in the muscular tissue) was observed, as well as extracellular metabolic acidosis of a slight degree (9). Particular interest in rubidium appeared with the publication of results of the research on its role in neoplastic processes. It was observed that this element is closely connected with the breast carcinoma in humans. It was noted that its level in the tumour tissue was significantly higher as compared to the healthy gland tissue. This suggests that it may be connected with the pathogenesis and development of neoplasms (6, 8, 15, 16, 20).

Despite the conducted research, the role of rubidium in the process of neoplasia is still not fully explained. Considering the fact that it is a component of human and animal diet, it is important to monitor its level in the biological samples obtained from healthy animals as well as from those showing various pathologies.

The present study aimed at comparison of the contents of rubidium in muscles, liver, and kidneys of healthy and diseased bitches. The investigations also included the effect of liver and kidney failure as well as neoplastic disease (mammary gland neoplasm) on the concentration of the rubidium in the animal parenchymatous organs and skeletal muscles.
Material and Methods

The research material comprised segments of muscles, kidneys, and liver collected post mortem from 45 bitches, mostly mongrels, with body weight varying from 4 to 40 kg. The material was collected in veterinary clinics in Warsaw and in the suburbs in 2007-2010. The animals were divided into two basic groups. The first group comprised 26 healthy female dogs aged from 1 to 8 years. The death of these animals was caused by extensive injuries resulting from random incidents such as traffic accidents and bites. The bitches in this group were of documented origin and were not treated for any chronic diseases. Information concerning their age, health state, and living conditions were obtained from patient files from particular clinics, which contained medical history of the animal obtained from the animal owner. The files were gradually supplemented at the time of periodical animal vaccinations. The studies excluded the animals, whose death was caused by poisoning resulting from accidental ingestion of various medicines, anticoagulative rodenticides, or other chemical agents, which could potentially affect the distribution of metal ions in the tissues. The second group comprised 19 diseased animals aged 7 to 18 years. This group was divided into two subgroups. The first subgroup comprised nine bitches euthanised because of an advanced neoplastic disease. All cases concerned mammary gland tumours with metastases to the lungs. In the second subgroup, 10 bitches were euthanised due to other diseases, mainly renal or hepatic failure, confirmed by laboratory tests. Animals in this group also were not previously treated. In all cases, the decision concerning euthanasia was taken because of a critical general health stage and after presenting the owners with unfavourable prognosis.

In each case, during the post mortem examinations samples of about 10 g of muscles, kidneys, and liver were collected. Muscle samples comprised segments of the lateral vastus muscle (musculus vastus lateralis), which is a large muscle unit allowing an easy approach and collection of a properly representative sample. The collected renal samples contained both: the renal cortex and renal medulla, and liver samples comprised segments of left lateral lobe. In order to avoid an accidental contamination of the samples with exogenous metals, material was collected with the help of a plastic blade. Until the time of chemical analyses, the samples were frozen and stored in plastic containers at -18°C. Prior to chemical analyses, the tissues were homogenised and then 0.5 g samples were placed in teflon containers and mineralised under pressure in a microwave apparatus (system Milestone MSL 1200) in the presence of 7 ml of the concentrated nitric acid and 1 ml of 30% hydrogen peroxide (Merck, Germany).

The content of rubidium was assessed by the method of inductively coupled plasma atomic emission spectroscopy with the wave length of 780 nm using the Thermo Scientific iCAP 6500 Spectrometer. The accuracy of reading of the analytical method was determined on the basis of Certified Reference Material (NCS ZC 73016 GSB-9). For each sample, two repetitions were performed, and the averaged result was presented as the concentration in milligrams per kilogram of wet weight (mg·kg⁻¹·w.w.).

Statistical calculations were performed using Statistica PI 10 (StatSoft Inc) procedures. The data were presented in the form of arithmetic and geometric means and standard deviation. Calculations also included the median as well as the lower and upper quartile. Before evaluation, data were analysed using the Shapiro-Wilks’ W test to determine their distribution. Since most of them were not normally distributed, nonparametric procedures for analyses were used. The significance of difference between groups was tested with nonparametric Kruskal-Wallis test. Differences were considered as significant at the level of P≤0.05 and P≤0.01. Relationship between the contents of rubidium in the liver, kidneys, and muscles of the animals assigned to particular groups were evaluated calculating the Spearman’s coefficients of correlation. Statistical significance of the correlation coefficients was tested at the level P≤0.05 and P≤0.01.

Results

Mean rubidium content in the organs of all examined animals is presented in Table 1. The highest rubidium content was noted in the muscles of animals with neoplastic disease (4.52 mg·kg⁻¹·w.w.) while in the remaining groups, the concentration of this element had similar values, amounting to not more than 2 mg·kg⁻¹·w.w. Rubidium content in the kidneys was ranked the second. Similarly as in the case of muscles also in the kidneys the highest rubidium concentration (3.81 mg·kg⁻¹·w.w.) was noted in the group of animals with neoplastic disease. In a group of healthy animals its level was the lowest (1.25 mg·kg⁻¹·w.w.). A similar trend was also observed in case of the liver. The highest mean values were obtained in bitches with neoplastic disease and the lowest in the healthy animals. The values obtained in those groups amounted to 3.78 and 1.32 mg·kg⁻¹·w.w., respectively. Statistical analysis of the obtained results showed the presence of highly significant differences (P≤0.01) in rubidium content between the group of animals with neoplastic disease and healthy ones. The statistically significant difference (P≤0.05) was observed between individuals with various pathologies and animals with mammary gland tumour.

Correlations between rubidium content in the organs of animals representing particular groups are presented in Table 2. In the group of healthy animals, a highly significant positive correlation (P≤0.01) was observed between rubidium content in the muscles and kidneys (r=0.59) and between the kidneys and liver (r=0.65). A significant positive correlation (P≤0.05) was noted between the liver and muscles. In the group of animals with neoplastic disease, a significant correlation (P≤0.05) was noted between rubidium content in the kidneys and muscles. In the case of other tissues, no dependencies of such character were noted.
The determination of the content of elements in organs is one of the methods for assessing the state of nutrition and animal exposition to the environment. Rubidium absorption in mammals takes place through the alimentary tract because that element is present in the air, soil, water, plants, and the tissues of living organisms, thus, passing through all links of the food chain (2, 20). Rubidium is assimilated quickly and effectively but its elimination is slow (1). The half time of rubidium elimination from the animal organism lasts from 30 to 134 d (17). In humans, the half time of rubidium presence in the tissues is from 50 to 60 d (21). After absorption, this element is quickly distributed to all organs (17). However, there are only few reports in literature concerning this topic in dogs. Additionally, there are not reference values for the concentration of this metal in dog tissues and organs. The authors observed that the process of neoplasia, taking place in the organism, significantly affects the contents of rubidium in the parenchymatous organs. Rare investigations on that problem point to a certain effectiveness of rubidium as an antineoplastic factor. Such an action was reported by, among others, Monroe (14) and Brewer (5). According to Brewer, the presence of rubidium causes an increase in pH value in the tumour cells up to 8 or higher, which shortens the life time of the tumour cells. Moreover, rubidium salts in the body fluids neutralise acid products of the tumour metabolism, which intoxicate the organism. The investigations concerning rubidium contents in the tumour tissue showed that it reaches significantly higher concentrations as compared to the healthy tissue (6, 7, 8). However, the effect of neoplastic disease on rubidium contents in the parenchymatous organs has not been investigated. Most studies concerned rubidium concentration in body fluids of patients with neoplastic disease. Su et al. (20) observed that the content of this element in urine of patients with neoplastic disease was significantly lower than in people from the control group. Shenberg et al. (19) observed that the level of rubidium in whole blood of patients with the neoplasms of the colon and rectum was lower than in the healthy people. Thus, a theory was put forward that rubidium undergoes a certain sequestration in the tumour cells, which, in turn, explains its low level in patients’ blood and urine. According to the authors of the cited paper, the increase in rubidium level, observed in the course of neoplastic disease, also concerns skeletal muscles, kidneys, and liver. This does not fully support the

Table 1
Rubidium content in the tissues of bitches (mg·kg⁻¹ wet weight) and its dependence on the health state

<table>
<thead>
<tr>
<th>Organ</th>
<th>Group and subgroup</th>
<th>N</th>
<th>AM</th>
<th>SD</th>
<th>Q25</th>
<th>Median</th>
<th>Q75</th>
<th>GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>Healthy animals</td>
<td>26</td>
<td>2.07A</td>
<td>1.78</td>
<td>0.50</td>
<td>1.70</td>
<td>2.60</td>
<td>1.46</td>
</tr>
<tr>
<td></td>
<td>Unhealthy animals</td>
<td>9</td>
<td>4.52B</td>
<td>1.56</td>
<td>3.80</td>
<td>4.50</td>
<td>5.20</td>
<td>4.27</td>
</tr>
<tr>
<td></td>
<td>Liver/kidneys</td>
<td>10</td>
<td>2.19b</td>
<td>1.32</td>
<td>2.20</td>
<td>2.20</td>
<td>2.60</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>45</td>
<td>2.59</td>
<td>1.88</td>
<td>1.20</td>
<td>2.10</td>
<td>4.00</td>
<td>1.89</td>
</tr>
<tr>
<td>Kidney</td>
<td>Healthy animals</td>
<td>26</td>
<td>1.25A</td>
<td>0.78</td>
<td>0.50</td>
<td>1.20</td>
<td>1.80</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>Unhealthy animals</td>
<td>9</td>
<td>3.81B</td>
<td>2.23</td>
<td>2.40</td>
<td>3.60</td>
<td>5.30</td>
<td>3.06</td>
</tr>
<tr>
<td></td>
<td>Liver/kidneys</td>
<td>10</td>
<td>1.44b</td>
<td>1.29</td>
<td>0.50</td>
<td>0.90</td>
<td>2.30</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>45</td>
<td>1.80</td>
<td>1.62</td>
<td>0.50</td>
<td>1.40</td>
<td>2.40</td>
<td>1.27</td>
</tr>
<tr>
<td>Liver</td>
<td>Healthy animals</td>
<td>26</td>
<td>1.32A</td>
<td>1.28</td>
<td>0.50</td>
<td>0.50</td>
<td>1.70</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Unhealthy animals</td>
<td>9</td>
<td>3.78B</td>
<td>1.67</td>
<td>2.40</td>
<td>4.10</td>
<td>5.20</td>
<td>3.33</td>
</tr>
<tr>
<td></td>
<td>Liver/kidneys</td>
<td>10</td>
<td>1.43b</td>
<td>1.80</td>
<td>0.50</td>
<td>0.70</td>
<td>1.30</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>45</td>
<td>1.84</td>
<td>1.75</td>
<td>0.50</td>
<td>0.70</td>
<td>2.70</td>
<td>1.17</td>
</tr>
</tbody>
</table>

Q25 - lower quartile, Q75- upper quartile, N - total animals, AM - arithmetic mean, SD - standard deviation, GM-geometric mean; A, B – the different letters denote statistically significant differences at P≤0.01, b – denotes statistically significant differences at P≤0.05

Table 2
Correlation coefficients between the examined organs of dogs

<table>
<thead>
<tr>
<th>Group and subgroup</th>
<th>N</th>
<th>Value of the coefficient of correlation (r)</th>
<th>between:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver and kidney</td>
</tr>
<tr>
<td>Healthy animals</td>
<td>26</td>
<td>0.65A</td>
<td>0.45a</td>
</tr>
<tr>
<td>Neoplasms</td>
<td>9</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Liver/kidneys</td>
<td>10</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

a - coefficient of correlation significant at P≤0.05, A - coefficient of correlation significant at P≤0.01, ns-non-significant correlation

Discussion
hypothesis of sequestration, assuming the accumulation of rubidium exclusively in the tumour cells. Liver and kidney failures seem to be of no importance in the case of rubidium concentrations in the tissues since its level in animal tissues depends on the nutritional status (1). In the investigations concerning rubidium concentrations in the organs of dairy cows, Kosla et al. (11) demonstrated the concentration of this element within the limits, ranging from 12.88 to 30.59 mg·kg⁻¹ of dry matter of muscles, 25.35–35.67 mg·kg⁻¹ of dry matter of kidneys, and 20.54–37.78 mg·kg⁻¹ of dry matter of the liver. After taking into consideration the degree of tissue hydration, these values for particular organs will amount approximately to 3.22–7.65 for muscles, 5.07–7.13 for kidneys, and 5.14–9.45 mg·kg⁻¹ for liver wet weight. In relation to the results obtained in the present study concerning dogs’ tissues, they are close only in the case of the muscular tissue but in the kidneys and liver, the content of rubidium is decidedly lower. Rubidium contents in the liver of Inuits settling in Greenland amounted, on the average, to 4.5 mg·kg⁻¹ wet weight of the organ while in Danish people that value amounted, on the average, to 3.9 mg·kg⁻¹, wet weight (13). The values are over two fold higher than those observed in the organs of healthy dogs and close to the values obtained in the analysis of material collected from animals with neoplastic disease. Thus, a hypothesis can be formulated that the noted rubidium content in the organs of healthy bitches is a species-specific trait characteristic for the normal supply of animals with rubidium.

Summing up, it should be stated that neoplasms of mammary glands in dogs are accompanied with the increased rubidium concentration in the liver, kidneys, and skeletal muscles and such factors as metabolic hepatic and renal failure seem to have no impact on this parameter. Due to the lack of data concerning rubidium concentration in the organs of dogs, it is difficult to say explicitly whether the values of rubidium contents stayed within a range, which can be considered adequate. It seems necessary to perform further investigations on that subject, analysing the health status and other parameters of dogs, in order to determine the reference values for this species. The studies on establishment of reference values of the concentrations of rubidium in the tissues and organs of healthy and diseased animals will allow determining its role in particular pathologies and, in consequence, they will be helpful in development of effective therapeutic strategies.

References