BENTONITE INFLUENCE ON MANGANESE UPTAKE IN RATS

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Abstract

Bentonite as a 2% additive to a standard laboratory diet was given to rats for 28 d together with traces of manganese chloride (manganese-54). Experimental procedures included the measurement of feed and water intake, body weight gain, organ to body ratio, and determination of manganese radioactivity in the carcass and selected organs during 28 d after the treatment. The results indicated that bentonite produced a moderate but persistent decrease in radiomanganese uptake in the carcass, liver, kidneys, duodenum, and blood. In contrast, no influences of dietary bentonite on the uptake of radiomanganese in the gonads, brain, muscles, and spleen were found. Moreover, bentonite also failed to affect feed and water intake, organs to body ratios, and body weight gains.

Key word: rat, bentonite, manganese-54 uptake.

Bentonite is a clay mineral. Its beneficial effects have received much attention. For many years, bentonite has been used as a binder in the feed industry and pharmaceutical preparations (2). The bentonite used in the feed, slows the passage of the contents in the digestive system and enables the animal to better utilise the feed nutrients. Several studies have shown bentonite as a useful ingredient in the prevention of diarrhoeal diseases and digestion problems in domestic animals (3, 4, 6, 12, 13). Bentonite was also found to influence bacterial composition of the gastrointestinal tract and reabsorption of bacterial products (18). Moreover, it was demonstrated that bentonite may immobilise some heavy metals (8), radiocaesium, and organic toxins (1, 15, 18) or reduce toxic action of bacteria and viruses in the gastrointestinal tract (18). The action of bentonite is rather non-selective. Recent studies have shown that bentonite may react with several indispensable nutrients making them less biologically available from the feed (9-11).

Manganese is an essential trace element for all forms of life. Deficiencies of manganese, which is associated with low manganese-dependent superoxide dismutase activity, may increase cancer susceptibility whereas too high manganese exposure leads to its accumulation in the brain and neurotoxicity (7, 14).

Our objectives for the present studies were to examine if a bentonite supplemented diet affects manganese absorption and distribution in the rat.

Material and Methods

About two-month-old, male Wistar rats from a commercial breeding station (Kozlowska Breeding Station, Warszawa) were used. The rats were kept in groups of five in stainless steel cages. The animals were acclimatised under standard laboratory conditions for one week, and then were randomly assigned into two dietary treatment groups comprising 40 rats each: group 1 (LSM), the controls, fed a standard pellet LSM diet for rodents (Fodder Manufacture, Motycz, Poland), and group 2 (LSM-B) fed the same diet fortified with 2% (w/w) of finely powdered bentonite (Mining and Metal processing Plant, Zębice, Poland). The feed and tap water were offered ad libitum for the whole experimental period. All the rats were given intragastrically daily except weekends for 28 d manganese chloride labelled with manganese-54 (PerkinElmer, USA) in a volume of 0.5 ml comprising about 40 kBq per rat. The daily feed and water consumption was evaluated weekly throughout the experimental period.

All the animals were killed by immersion in gaseous carbon dioxide 3 h, 6 h, 1 d, 2 d, 4 d, 7 d, 14 d, and 28 d postdosing. The following organs were removed: liver, kidneys, small intestine (initial 15 cm), spleen, heart, gonads, brain, and muscles. The content of radiomanganese in the carcass (whole body without the stomach and intestines), and the organs was measured in a whole-body counter ZM 701 (Polon, Poland) and in a well-type scintillation counter ZR 11 (Polon, Poland), respectively. Reference standards for quantification of carcass were prepared by intraperitoneal injection of the appropriate solution of manganese-54 to rats, which were killed 30 min thereafter.
The area under the curves (AUC) of radiomanganese contents versus time points was calculated by the trapezoidal rule.

The data were analysed statistically using the Student’s t-test at P<0.05. The experiments were approved by the Local Ethics Committee for Animal Experiments in Lublin, Poland.

Results

All rats were healthy throughout the experimental period and none of them died. Body weight gain increased steadily in both groups. No significant differences in body weight gains among the rats tested were found, although the rats fed the diet with bentonite showed a lower body weight gain as compared to those fed a standard diet.

The content of manganese-54 in the carcass within a 28-d period after the exposure is illustrated in Fig. 1. The data indicated that the distribution pattern of manganese-54 in the carcass did not vary between the two groups examined; except for a significant decrease on day 28, in rats fed the bentonite enriched diet as compared to that of the controls. Moreover, manganese-54 concentrations in the carcass increased up to day 1 and then continued to decrease until day 28.

The distribution of radiomanganese in the blood, duodenum, liver, kidneys, heart, spleen, muscles, brain, and testicles is shown in Tab. 1. Results indicate that the concentrations of radiomanganese in all organs tested in the two groups of rats, is low within 28 d after treatment except for the liver and kidneys where the highest amounts of radiomanganese were distributed. Significant differences between the two groups tested were found in the blood, duodenum, liver, kidneys, and heart.

Table 1

<table>
<thead>
<tr>
<th>Organ</th>
<th>3 h</th>
<th>1 d</th>
<th>2 d</th>
<th>6 h</th>
<th>28 d</th>
<th>14 d</th>
<th>7 d</th>
<th>4 d</th>
<th>1 d</th>
<th>2 d</th>
<th>6 h</th>
<th>28 d</th>
<th>14 d</th>
<th>7 d</th>
<th>4 d</th>
<th>AUC</th>
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<tr>
<td>Blood</td>
<td>LSM</td>
<td>0.67</td>
<td>± 0.14</td>
<td>3.45</td>
<td>± 0.48</td>
<td>2.33</td>
<td>± 0.29</td>
<td>5.04</td>
<td>± 0.47</td>
<td>5.39</td>
<td>± 0.88</td>
<td>1.61</td>
<td>± 0.67</td>
<td>1.19</td>
<td>± 0.10</td>
<td>0.72</td>
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<td>LSM-B</td>
<td>0.64</td>
<td>± 0.16</td>
<td>1.41</td>
<td>± 0.42</td>
<td>0.97</td>
<td>± 0.26</td>
<td>1.19</td>
<td>± 0.34</td>
<td>1.19</td>
<td>± 0.45</td>
<td>0.99</td>
<td>± 0.19</td>
<td>1.03</td>
<td>± 0.10</td>
<td>0.58</td>
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<td>Duodenum</td>
<td>LSM</td>
<td>2.67</td>
<td>± 0.65</td>
<td>4.80</td>
<td>± 1.01</td>
<td>2.08</td>
<td>± 0.79</td>
<td>3.37</td>
<td>± 0.60</td>
<td>4.81</td>
<td>± 1.23</td>
<td>4.46</td>
<td>± 1.29</td>
<td>3.19</td>
<td>± 0.58</td>
<td>2.17</td>
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<td>2.68</td>
<td>± 0.59</td>
<td>2.64</td>
<td>± 0.81</td>
<td>2.68</td>
<td>± 0.62</td>
<td>4.71</td>
<td>± 0.99</td>
<td>2.84</td>
<td>± 0.73</td>
<td>2.27</td>
<td>± 0.94</td>
<td>1.35</td>
<td>± 0.69</td>
<td>1.04</td>
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<td>Liver</td>
<td>LSM</td>
<td>471</td>
<td>± 89</td>
<td>694</td>
<td>± 132</td>
<td>731</td>
<td>± 126</td>
<td>323</td>
<td>± 57</td>
<td>348</td>
<td>± 59</td>
<td>234</td>
<td>± 33</td>
<td>225</td>
<td>± 57</td>
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<td></td>
<td>LSM-B</td>
<td>415</td>
<td>± 98</td>
<td>413</td>
<td>± 61</td>
<td>440</td>
<td>± 51</td>
<td>356</td>
<td>± 75</td>
<td>232</td>
<td>± 55</td>
<td>129</td>
<td>± 31</td>
<td>112</td>
<td>± 24</td>
<td>104</td>
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<td>Kidneys</td>
<td>LSM</td>
<td>41.5</td>
<td>± 8.5</td>
<td>74.1</td>
<td>± 20.3</td>
<td>64.2</td>
<td>± 23</td>
<td>49.2</td>
<td>± 10.1</td>
<td>47.8</td>
<td>± 10.7</td>
<td>31.7</td>
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<td>17.2</td>
<td>± 2.2</td>
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<td>LSM-B</td>
<td>30.3</td>
<td>± 11.2</td>
<td>26.4*</td>
<td>± 5.4</td>
<td>31.2</td>
<td>± 6.9</td>
<td>31.7</td>
<td>± 7.9</td>
<td>26.9*</td>
<td>± 5.6</td>
<td>17.8*</td>
<td>± 5.7</td>
<td>17.0</td>
<td>± 5.1</td>
<td>7.6*</td>
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<td>Heart</td>
<td>LSM</td>
<td>0.14</td>
<td>± 0.02</td>
<td>0.75</td>
<td>± 0.14</td>
<td>0.76</td>
<td>± 0.12</td>
<td>0.48</td>
<td>± 0.13</td>
<td>0.39</td>
<td>± 0.12</td>
<td>0.51</td>
<td>± 0.13</td>
<td>0.21</td>
<td>± 0.11</td>
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<tr>
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<td>LSM-B</td>
<td>0.14</td>
<td>± 0.03</td>
<td>0.30*</td>
<td>± 0.19</td>
<td>0.28*</td>
<td>± 0.07</td>
<td>0.29</td>
<td>± 0.12</td>
<td>0.29</td>
<td>± 0.10</td>
<td>0.30</td>
<td>± 0.17</td>
<td>0.29</td>
<td>± 0.09</td>
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<tr>
<td>Spleen</td>
<td>LSM</td>
<td>3.25</td>
<td>± 0.19</td>
<td>1.90</td>
<td>± 0.44</td>
<td>2.61</td>
<td>± 10.5</td>
<td>2.02</td>
<td>± 0.75</td>
<td>2.90</td>
<td>± 0.86</td>
<td>2.84</td>
<td>± 0.29</td>
<td>2.43</td>
<td>± 0.53</td>
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<tr>
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<td>2.83</td>
<td>± 0.27</td>
<td>2.15</td>
<td>± 0.34</td>
<td>2.52</td>
<td>± 12.3</td>
<td>2.47</td>
<td>± 0.79</td>
<td>2.06</td>
<td>± 0.63</td>
<td>1.99</td>
<td>± 0.21</td>
<td>1.37</td>
<td>± 0.23</td>
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<tr>
<td>Muscles</td>
<td>LSM</td>
<td>2.07</td>
<td>± 0.90</td>
<td>2.36</td>
<td>± 0.27</td>
<td>2.52</td>
<td>± 1.02</td>
<td>1.71</td>
<td>± 0.20</td>
<td>1.49</td>
<td>± 0.34</td>
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<td>± 0.43</td>
<td>1.54</td>
<td>± 0.34</td>
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<tr>
<td></td>
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<td>1.7</td>
<td>± 0.34</td>
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<td>± 0.32</td>
<td>1.81</td>
<td>± 0.44</td>
<td>1.27</td>
<td>± 0.38</td>
<td>1.07</td>
<td>± 0.42</td>
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<td>± 0.27</td>
<td>0.990</td>
<td>± 0.20</td>
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<tr>
<td>Brain</td>
<td>LSM</td>
<td>2.65</td>
<td>± 0.77</td>
<td>3.38</td>
<td>± 0.65</td>
<td>2.41</td>
<td>± 0.51</td>
<td>3.13</td>
<td>± 0.76</td>
<td>1.04</td>
<td>± 3.14</td>
<td>3.41</td>
<td>± 0.87</td>
<td>3.44</td>
<td>± 0.75</td>
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<tr>
<td></td>
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<td>2.37</td>
<td>± 0.96</td>
<td>1.69</td>
<td>± 0.58</td>
<td>2.35</td>
<td>± 0.71</td>
<td>2.63</td>
<td>± 0.66</td>
<td>2.41</td>
<td>± 0.98</td>
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<td>± 0.93</td>
<td>3.01</td>
<td>± 0.69</td>
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<tr>
<td>Gonads</td>
<td>LSM</td>
<td>3.29</td>
<td>± 0.82</td>
<td>2.50</td>
<td>± 1.06</td>
<td>3.36</td>
<td>± 1.06</td>
<td>1.89</td>
<td>± 0.54</td>
<td>2.43</td>
<td>± 0.34</td>
<td>1.87</td>
<td>± 0.31</td>
<td>2.01</td>
<td>± 0.41</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
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<td>2.13</td>
<td>± 0.85</td>
<td>1.90</td>
<td>± 0.65</td>
<td>2.78</td>
<td>± 0.87</td>
<td>2.09</td>
<td>± 0.43</td>
<td>1.78</td>
<td>± 0.41</td>
<td>1.24</td>
<td>± 0.29</td>
<td>1.10</td>
<td>± 0.36</td>
<td>0.89</td>
</tr>
</tbody>
</table>

* P<0.05, I – control rats, II – rats fed a bentonite fortified diet.

Fig. 1. Radiomanganese content in the carcass (% of total dose).
Hepatic, renal, duodenal, and blood radiomanganese accumulation was about 50% of that in the controls. The differences were especially remarkable in the blood, liver, and kidneys. The remaining organs had comparable manganese-54 incorporation, at levels that remained near similar throughout the time period examined. There was less manganese-54 incorporation into these organs when the rats were fed a bentonite supplemented diet.

Discussion

The lack of significant alterations in organ to body ratios and body weight gains in the rats fed the bentonite enriched diet confirms the results reported in earlier studies (9-11).

In contrast to some other essential elements, manganese level in tissues is relatively low. The only exception seems to be the liver and kidneys (7, 14).

The results of this study demonstrate that manganese-54 given intragastrically to rats fed either a standard laboratory diet or the same diet fortified with bentonite was distributed in the carcass and organs tested in a similar pattern. However, a comparison of manganese-54 uptake between the two groups of rats shows that rats fed a bentonite fortified diet had markedly less manganese-54 uptake than did the controls. The differences in manganese-54 concentration between the two groups tested, indicate that bentonite affected manganese delivery to organs such as the liver and kidneys where this element is mainly distributed after oral intake (4), whereas the accumulation of radiomanganese in the spleen, muscles, brain, and gonads was unaffected by the bentonite diet applied.

To our knowledge, the literature on the bentonite-manganese interference in an animal model is very scarce (15, 18). Moreover, the response of the metal metabolism to a diet supplemented with bentonite seems to be variable with respect to the element studied (9-11, 16). The experimental evidence obtained in this study, showing reduced radiomanganese accumulation, indicates that a diet supplemented with bentonite may decrease this element uptake, at least by the organs with a high metabolic rate. It should also be stressed that considering the present data that manganese uptake by other organs such as brain, spleen, and gonads was not affected in rats fed a bentonite supplemented diet apart from a significant role of these organs in manganese metabolism. However, these organs contain small amounts of manganese and the element retention time is rather long.

The finding that bentonite reduced manganese uptake suggests that this preparation may also cause side effects in addition to its known beneficial effects in animal nutrition. In fact, the results presented here indicated that the reduction of manganese uptake was significant but moderate, and several organs failed to alter their manganese uptake under the experimental conditions involved. However, it should be stressed that any changes in the manganese uptake by the brain, spleen, testicle, and muscles could not be found during the observation time applied in these studies because the rate of manganese metabolism in these organs is very low. For example, the half time of manganese elimination in whole body was estimated to be 95 days, whereas the half time for the cerebrum may be longer than a few hundred days.

The intake of manganese in several parts of Poland may be marginal or not adequate (13). Thus, consideration should be given to translating the presented experimental finding into animal health practice.

References

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