EFFECTS OF HAEMOPERFUSION ON SELECTED INDICES OF BLOOD BIOCHEMISTRY IN SHEEP

H. SEIDEL, P. BARTKO, G. KOVÁČ, I. PAULÍKOVÁ, O. NAGY
Department of Internal Diseases of Ruminants and Swine, University of Veterinary Medicine, Košice, Slovak Republic

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Abstract


The aim of this work was to evaluate the clinoptilolite as a cartridge for haemoperfusion columns. Changes in haematological indices and blood plasma macro-elements were evaluated during haemoperfusion in sheep with the use of zeolitic and commercial haemoperfusion columns. Haemoperfusions were done in two groups of sheep (n=6) for 2 hours. In the first group, commercial haemoperfusion columns HEMASORB 400 C filled with active charcoal were used. In the second group, the columns used were filled with sodium form of natural zeolite-clinoptilolite partially saturated with potassium chloride. During 2 hours of haemoperfusion significant decrease in numbers of leukocytes and thrombocytes (P<0.01) were found in both types of columns. There were no significant differences between the groups, except for the number of thrombocytes and plasma sodium concentration in the blood leaving the zeolitic columns with significantly lower values after 30 min (134.3±28.9 vs. 251.0±55.4 G.l⁻¹, P<0.01; and 144.17±9.13 vs. 157.17±7.36 mmol.l⁻¹, P<0.05; respectively) compared with commercial HEMASORB columns. However, final evaluation of biocompatibility of zeolitic haemoperfusion columns will require more detailed research.

Haemoperfusion, active charcoal, clinoptilolite, blood indices, calcium, magnesium, sodium, potassium, phosphorus, sheep

Haemoperfusion may be the method of choice in therapy of various intoxications. It is based on sorption of toxic substances during extracorporeal blood flow through detoxifying columns. The columns are filled with different sorbents such as active charcoal or synthetic resins. For the haemoperfusion purposes neutral resins are used with the sorption capability depending upon the sorbent’s surface and physical properties. Amberlites (copolystyren-divinylbenzenes) are the most frequent resins used for haemoperfusion (Kováč 1993). In intoxications, the toxic substances present in the blood are retained by these sorbents during haemoperfusion.

Zeolites are crystalline aluminosilicates with tetrahedral framework structure enclosing cavities occupied by cations and water molecules, both of which have enough freedom of movement to permit cation exchange and reversible dehydration (Sherry 1993). Ion-exchange capability of zeolites is the primary function of substitution of four-valent silicon by three-valent aluminium. The higher degree of substitution, the higher deficiency of positive charge, and the higher number of cations needed for the maintenance of electric neutrality (Mumpton and Fishman 1977; Mumpton 1984).

The use of natural zeolites was studied in various areas of human activities. Sorption of radioisotopes by zeolites, particularly of Cs and Sr, was reported by several authors. This property is employed in purification of waste waters from nuclear power stations (Franta et al. 1994) as well as in prevention or therapy of experimental contamination of animals (Forberg et al. 1989; Míčík et al. 1989; Vavrová et al. 1991; Musatovova et al. 1993; and others).
Natural clinoptilolite has also high selective affinity to ammonium (Bartko et al. 1983; Hlavay et al. 1988; Papadopoulos et al. 1996) and some heavy metals cations such as Pb, and Cd (Blanchard et al. 1984; Seidel and Vrzgula 1989; Vrzgula and Seidel 1989; Pond et al. 1993). There are some indications that natural clinoptilolite is able to absorb aflatoxin B1. Dvořák (1989) reported 60% sorption of aflatoxin B1 by natural zeolite in vitro. Similar results were recorded by Tomasevic-Canovic et al. (1993). Clinoptilolite was also shown to have partial protective effect in organophosphate intoxication in rats (Mojžiš et al. 1994; Ništiař et al. 1984) and sheep (Kováč et al. 1995).

Because of the aforementioned sorption and ion-exchanging capabilities of zeolite, the aim of this work was to evaluate the clinoptilolite as a cartridge for haemoperfusion columns.

Materials and Methods

In the experiment we used clinoptilolite-rich tuff from the zeolite deposit at Nižný Hrabovec, Slovakia. Chemical composition (as determined by X-ray spectrophotometry) of the zeolitic tuff was 
SiO$_2$, 69.22; MgO, 0.75; TiO$_2$, 0.18; K$_2$O, 14.33; Na$_2$O, 9.7; CaO, 3.40 wt.%; and As and Pb, <0.001 wt.% (Kozác et al. 1982). The tuff contained about 60-65 wt.% clinoptilolite (as calculated from its cation-exchange capacity) and lesser amounts of cristobalite, plagioclase, quartz, and volcanic glass (X-ray diffraction analysis). The clinoptilolite-rich tuff had a cation-exchange capacity of 0.86 meq/g (Kozác and Očenáš 1982).

Haemoperfusions were carried out in 12 Merino sheep after preceding insertion of intravenous catheters (1.5x2.0 mm) into both jugular veins, and intravenous administration of heparin (250 U/kg b.m.) for 2 h (Seidel et al. 1992). Approximately 5 min before haemoperfusion the animals were placed in a special restraining equipment. Dialyzation monitor CHIRADIS A (Chirana Stará Turá, Slovak Republic) was used with commercial columns HEMASORB 400 C (OPS Kolin, Czech Republic) filled with active charcoal (1st group, n=6), and columns filled with zeolitic tuff (2nd group, n=6). Following previous experiments in vitro (non-published), sodium form of zeolite (particle size 2-4 mm) was used, partially saturated with potassium chloride because of great affinity of the zeolite to potassium. Parallel blood samples (leaving both the animal and the column) were taken at 0, 30, 60, 90, and 120 min of the perfusion. At the same intervals body temperature, pulse and respiratory rates, as well as general state of the animals were checked. Samples of blood were analyzed by blood analyzer SERONO PLUS for haematological variables (Er-erythrocytes, Le-leukocytes, Hb-haemoglobin, PCV-packed cell volume). Thrombocyte (Tc) counts were determined by routine microscopical method. Plasma concentrations of Ca, Mg, Na, and K were determined by AAS (Perkin Elmer, 4100 ZL). Plasma concentrations of inorganic P were determined by colorimetric method (Bio-La, Lachema Brno). The results are expressed as an average ($\bar{x}$) and standard deviation (±s). Significance of the differences between the two groups was assessed by Student's t-test.

Results

Clinical examination of experimental animals revealed no changes in body temperature. However, an increased pulse rate (up to 122/min) was found. At the beginning of haemoperfusion respiratory rate about 45/min was recorded. It later decreased to physiological values (24/min at 120 min). In some animals of both groups, during the haemoperfusion moderate diarrhoea occurred.

Average values of the haematological indices are shown in Tables 1 and 2. In both types of columns the decreasing numbers of leukocytes and thrombocytes were observed (P<0.01).
### Table 1
Average values of haematological indices after haemoperfusion through commercial columns HEMASORB 400 C

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Er (T/1)</th>
<th>Lc (G/1)</th>
<th>Hb (g/dcl)</th>
<th>PCV (1/1)</th>
<th>Tc (G/1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>animal column</td>
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<tr>
<td>0</td>
<td>7.45 ± 2.17</td>
<td>5.68 ± 1.78</td>
<td>8.57 ± 2.80</td>
<td>0.34 ± 0.08</td>
<td>420.3 ± 100.1</td>
</tr>
<tr>
<td>30</td>
<td>5.82 ± 2.19</td>
<td>4.08 ± 1.01</td>
<td>7.98 ± 1.46</td>
<td>0.31 ± 0.05</td>
<td>251.0 ± 92.5</td>
</tr>
<tr>
<td>60</td>
<td>7.47 ± 1.60</td>
<td>3.38 ± 2.08</td>
<td>8.24 ± 1.46</td>
<td>0.31 ± 0.07</td>
<td>260.0 ± 102.2</td>
</tr>
<tr>
<td>90</td>
<td>7.45 ± 1.63</td>
<td>3.04 ± 1.62</td>
<td>7.66 ± 1.50</td>
<td>0.29 ± 0.06</td>
<td>217.2 ± 70.1</td>
</tr>
<tr>
<td>120</td>
<td>6.68 ± 1.37</td>
<td>2.94 ± 1.24</td>
<td>7.46 ± 1.62</td>
<td>0.29 ± 0.07</td>
<td>181.2 ± 52.4</td>
</tr>
</tbody>
</table>

### Table 2
Average values of haematological indices after haemoperfusion through columns filled with clinoptilolite-rich tuff

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Er (T/1)</th>
<th>Lc (G/1)</th>
<th>Hb (g/dcl)</th>
<th>PCV (1/1)</th>
<th>Tc (G/1)</th>
</tr>
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<tr>
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<tr>
<td>0</td>
<td>7.09 ± 1.32</td>
<td>6.78 ± 2.47</td>
<td>7.88 ± 0.91</td>
<td>0.29 ± 0.06</td>
<td>409.3 ± 96.2</td>
</tr>
<tr>
<td>30</td>
<td>7.66 ± 1.32</td>
<td>4.27 ± 1.91</td>
<td>8.3 ± 0.85</td>
<td>0.32 ± 0.03</td>
<td>273.7 ± 45.1</td>
</tr>
<tr>
<td>60</td>
<td>7.39 ± 0.87</td>
<td>3.20 ± 1.14</td>
<td>8.62 ± 0.90</td>
<td>0.33 ± 0.04</td>
<td>194.0 ± 72.9</td>
</tr>
<tr>
<td>90</td>
<td>7.46 ± 0.94</td>
<td>2.62 ± 1.62</td>
<td>8.23 ± 0.76</td>
<td>0.33 ± 0.04</td>
<td>186.3 ± 65.9</td>
</tr>
<tr>
<td>120</td>
<td>7.03 ± 1.22</td>
<td>2.86 ± 0.81</td>
<td>7.86 ± 0.76</td>
<td>0.30 ± 0.04</td>
<td>154.0 ± 36.6</td>
</tr>
</tbody>
</table>

However, there were no significant differences between the groups, except the number of thrombocytes in the blood leaving the zeolitic column with significantly lower values after 30 min (P<0.01) compared with commercial HEMASORB columns.

Mean values of macroelements are presented in Tables 3 and 4.

### Table 3
Average values of plasma macroelement levels after haemoperfusion through commercial columns HEMASORB 400 C

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Ca (mmol.1⁻¹)</th>
<th>Mg (mmol.1⁻¹)</th>
<th>Na (mmol.1⁻¹)</th>
<th>K (mmol.1⁻¹)</th>
<th>P (mmol.1⁻¹)</th>
</tr>
</thead>
<tbody>
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<td>animal column</td>
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<td>animal column</td>
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</tr>
<tr>
<td>0</td>
<td>2.073 ± 0.229</td>
<td>1.000 ± 0.199</td>
<td>153.67 ± 6.35</td>
<td>4.748 ± 0.586</td>
<td>1.727 ± 0.484</td>
</tr>
<tr>
<td>30</td>
<td>2.045 ± 0.135</td>
<td>0.945 ± 0.238</td>
<td>156.33 ± 8.55</td>
<td>4.507 ± 0.354</td>
<td>1.448 ± 0.434</td>
</tr>
<tr>
<td>60</td>
<td>1.980 ± 0.175</td>
<td>0.940 ± 0.181</td>
<td>155.20 ± 3.56</td>
<td>4.168 ± 0.294</td>
<td>1.418 ± 0.498</td>
</tr>
<tr>
<td>90</td>
<td>2.094 ± 0.396</td>
<td>0.924 ± 0.217</td>
<td>153.20 ± 6.26</td>
<td>4.244 ± 0.397</td>
<td>1.416 ± 0.500</td>
</tr>
<tr>
<td>120</td>
<td>1.914 ± 0.217</td>
<td>0.874 ± 0.161</td>
<td>154.20 ± 4.44</td>
<td>3.838 ± 0.749</td>
<td>1.414 ± 0.555</td>
</tr>
</tbody>
</table>

**Notes:**
- ±s indicates standard deviation.
- Ca, Mg, Na, K, P denote calcium, magnesium, sodium, potassium, and phosphate, respectively.
- All values are presented in mmol/l.
- P<0.01 indicates significant differences compared to the control group.

**References:**
- The full reference list is available in the original document.
Table 4

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Ca (mmol. l⁻¹)</th>
<th>Mg (mmol. l⁻¹)</th>
<th>Na (mmol. l⁻¹)</th>
<th>K (mmol. l⁻¹)</th>
<th>P (mmol. l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>±s</td>
<td>±s</td>
<td>±s</td>
<td>±s</td>
<td>±s</td>
</tr>
<tr>
<td>0</td>
<td>1.933 ± 0.368</td>
<td>0.993 ± 0.196</td>
<td>151.83 ± 10.07</td>
<td>3.947 ± 0.640</td>
<td>1.547 ± 0.401</td>
</tr>
<tr>
<td>30</td>
<td>1.912 ± 0.311</td>
<td>1.430 ± 0.888</td>
<td>1.035 ± 0.197</td>
<td>1.058 ± 0.319</td>
<td>153.00 ± 14.17</td>
</tr>
<tr>
<td>40</td>
<td>1.533 ± 0.736</td>
<td>1.547 ± 0.401</td>
<td>1.500 ± 1.057</td>
<td>1.447 ± 0.685</td>
<td>1.547 ± 0.401</td>
</tr>
<tr>
<td>50</td>
<td>1.500 ± 0.736</td>
<td>1.447 ± 0.685</td>
<td>1.500 ± 1.057</td>
<td>1.447 ± 0.685</td>
<td>1.547 ± 0.401</td>
</tr>
</tbody>
</table>

Similarly, we found no significant differences between the groups, except the initial concentration of potassium and plasma concentration of sodium in the blood leaving the zeolitic column with significantly lower values after 30 min (P<0.01) compared with commercial HEMASORB columns.

**Discussion**

To our knowledge, there is no literary information on the use of natural zeolite for haemoperfusion. Therefore we cannot compare our results with those of other authors.

During the haemoperfusion we observed some problems. We suggest that changes in pulse and respiratory rates as well as occurrence of moderate diarrhoea may be related to the stress of the animals restrained in a special equipment.

Another problem was that with decreased blood flow or in case of interruption of haemoperfusion, blood coagulation occurred, being more pronounced with zeolitic columns. In human medicine, for the commercial columns HEMASORB blood flow rate 150-200 ml/min is recommended (critical value 50 ml/min). Because of the capacity of jugular vein, the maximum blood flow rate 70-80 ml/min achieved in our experiment may have contributed to increased blood clotting. Other possible factor involved in blood coagulation is the surface of natural zeolite. In the commercial columns sorbent particles are coated with hydrophilic polymer (poly/2-hydroxyethyl methacrylate). This polymer is permeable for substances of molecular weight up to 6000, and prevents direct contact between blood elements, plasma proteins, and the sorbent.

For biochemical analyses we chose haematological indices and plasma concentrations of macroelements since blood counts are probably among the most important factors for general assessment of the column biocompatibility; and, on the other hand, in macroelements marked changes might be expected because of strong affinity of clinoptilolite to large cations. In this experiment we did not observe marked differences in haematological variables between commercial columns and columns filled with non-coated natural zeolite.

Similarly, no marked changes were observed in plasma concentrations of macroelements (Ca, Mg, Na, K, P). However, because of great affinity of clinoptilolite to potassium (in vitro experiments - not published) sodium form of clinoptilolite was partially saturated with potassium (KCl) before the use for haemoperfusion. Jüggli (1971) reported similar problem with the use of cation exchange columns in the treatment of ammonia intoxication in dogs.
They used sodium form of cation exchange Amberlite. Because this resin caused severe disturbances in blood electrolytes, they developed a mixture of sodium, potassium, calcium, and magnesium forms of the resin. This resin mixture has been shown to be effective in correcting ammonium chloride-induced hyperammonaemia in dogs without any disturbances in other cationic substances of the blood. Tera i et al. (1996) reported the same situation with an exchange resin for sodium, which is capable of rapidly decreasing an elevated serum potassium level, but has not been used clinically because of subsequent electrolyte abnormalities. The authors prepared the sodium/calcium/calcium/magnesium exchange resin mixture specifically to remove potassium from the blood. Haemoperfusion through this resin mixture column for 2 hours reduced elevated plasma potassium concentrations (from 6.7±1.1 to 3.5±0.6 mmol.l⁻¹) in anephric dogs without any side effects on other blood electrolytes.

From the practical point of view, the clinoptilolite (or the fraction of its exchangeable cations) could be specifically adjusted, or a mixture of various cationic forms of the clinoptilolite prepared.

In summary, comparison of a commercial haemoperfusion column filled with active charcoal and a column filled with clinoptilolite showed no marked differences in haematological and macro-mineral profiles during the haemoperfusion in sheep. However, the final evaluation of biocompatibility of zeolitic column will require more detailed research. Moreover, suitable therapeutic indications need to be selected. There is also a need to consider the use of synthetic precisely characterized zeolite instead of natural one.

References


Vplyv hemoperfúzie na niektóre biochemické ukazovatele v krvi oviec

V práci sme porovnávali zmeny hematologických ukazovateľov a makroelementov krvnej plazmy u oviec počas hemoperfúzie s použitím zeolitových a komerčných hemoperfúzných kolón. Hemoperfúzia sme podrobili dve skupiny oviec (n = 6) počas 2 hodín. V prvej skupine sme použili komerčné hemoperfúzné kolóny HEMASORB 400 C plnené aktívnym uhliom. V druhej skupine boli použité kolóny naplnené sodnou formou prírodného zeolitu – klinoptilolitu čiastočne saturovaného chloridom draselným. Počas dvojhodinovej hemoperfúzie sme v oboch skupinách zaznamenali signifikantný pokles počtu leukocytov a trombocytov (P<0.01). Medzi skupinami sme nezistili signifikantné rozdiely sledovaných ukazovateľov okrem 30. minúty hemoperfúzie, kedy boli v skupine so zeolitom v krvi opúštajúcej kolónu zistené signifikantne nižšie hodnoty trombocytov (134.3±28.9 oproti 251.0±55.4 G.l⁻¹, P<0.01) a koncentrácia sodika v plazme (144.17±9.13 oproti 157.17±7.36 mmol.l⁻¹, P<0.05). Napriek tomu, konečné posúdenie biokompatibility zeolitových hemoperfúzných kolón si bude vyžadovať ďalší podrobnejšiu výskum.


Musatovo, O., Vavrova, M., Mitro, A., Barth, S. 1993: In vitro studies on the ability of clinoptilolite to prevent the transfer of environmental Sr and Cs into sheep’s milk. ZEOLITE’93, 4th International Conference on the Occurrence, Properties, and Utilization of Natural Zeolites, Boise-Idaho, June 20-28, 1993: 145-146


Vavrova, M., Musatovo, O., Barth, S. 1991: In vitro studies on the ability of natural zeolites to sorb some radionuclides. Isotopenpraxis 17: 525-537


Address for correspondence:
MVD Dr. Herbert Seidel
University of Veterinary Medicine
Komenského 73
80-41 81 Košice
Slovak Republic

Phone: 421 95 633 211
E-mail: seidel@vsunov.uvm.sk