EFFECTS OF HAEMOPERFUSION ON SELECTED INDICES OF BLOOD BIOCHEMISTRY IN SHEEP

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Received August 1, 1997
Accepted January 15, 1998

Abstract

Seidel H., P. Bartko, G. Kováč, I. Paulíková, O. Nagy: *Effects of Haemoperfusion on Selected Indices of Blood Biochemistry in Sheep.* Acta vet. Brno 1997, 66: 213-218.

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; Mižík 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 B_1 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; Ništiar 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_2O, 2.48; Al_2O_3, 14.33; Na_2O, 0.97; CaO, 3.40 wt.%; and As and Pb, <0.001 wt.% (Kozáč 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 (Kozáč 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 Kolín, 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, Lc-leukocytes, Hb-haemoglobin, PCV-packed cell volume). Thrombocyte (Tc) counts were determined by routine miscroscopical 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 (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

Time (min)		Er (T/1) animal	Er (T/1) column	Lc (G/1) animal	Lc (G/1) column	Hb (g/dcl) animal	Hb (g/dcl) column	PCV (1/1) animal	PCV (1/1) colum	Tc (G/1) animal	Tc (G/1) colum
0	x	7.45		5.68		8.57		0.34		420.3	
	±s	2.17		1.78		2.80		0.08		100.1	
30	x	5.82	5.88	4.08	3.18	7.98	7.35	0.31	341.0	251.0	
	±s	2.19	2.20	1.01	0.95	1.46	1.09	0.05	0.06	92.5	55.4
60	x	7.47	7.72	3.38	2.92	8.24	8.42	0.31	0.33	260.0	172.0
	±s	1.60	1.59	2.08	1.24	1.46	1.53	0.07	0.07	102.2	34.3
90	x	7.45	7.00	3.04	2.86	7.66	7.32	0.29	0.28	217.2	163.6
	±s	1.63	1.26	1.62	1.03	1.50	1.04	0.06	0.06	70.1	35.8
120	x	6.68	6.41	2.94	3.16	7.46	7.10	0.29	0.28	181.2	139.6
	±s	1.37	1.76	1.24	1.37	1.62	1.81	0.06	0.07	52.4	49.6

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

Time (min)		Er (T/1) animal	Er (T/1) column	Lc (G/1) animal	Lc (G/1) column	Hb (g/dcl) animal	Hb (g/dcl) column	PCV (1/1) animal	PCV (1/1) column	Tc (G/1) animal	Tc (G/1) column
0	x	7.09		6.78		7.88		0.29		409.3	
	±s	1.32		2.47		0.91		0.06		96.2	
30	x	7.66	6.92	4.27	4.15	8.3	7.87	0.32	0.31	273.7	134.3
	±s	1.32	1.32	1.91	1.57	0.85	0.82	0.03	0.03	45.1	28.9
60	x	7.39	7.07	3.20	2.90	8.62	8.52	0.33	0.33	194.0	138.0
	±s	0.87	1.49	1.14	0.77	0.90	1.34	0.04	0.05	72.9	51.8
90	x	7.46	6.76	2.62	2.72	8.23	7.72	0.33	0.32	186.3	115.8
	±s	0.94	1.41	1.62	1.34	0.76	1.26	0.04	0.05	65.9	42.2
120	x	7.03	6.16	2.86	2.66	7.86	7.68	0.30	0.31	154.0	116.4
	±s	1.22	1.63	0.81	1.07	0,07	0.76	0.03	0.04	36.6	69.8

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

Time (min)	animal	Ca (mmol.l^{-1}) column	Ca (mmol.l^{-1}) animal	Mg (mmol. l^{-1}) column	Mg (mmol. l^{-1}) animal	Na (mmol. l^{-1}) column	Na (mmol. l^{-1}) animal	K (mmol. l^{-1}) column	K (mmol. l^{-1}) animal	P (mmol. l^{-1}) column	
0	x	2.073		1.000		153.67		4.748		1.727	
	±s	0.229		0.199		6.35		0.586		0.484	
30	x	2.045	1.720	0.945	0.838	156.33	157.17	4.507	3.987	1.448	1.492
	±s	0.135	0.426	0.238	0.247	8.55	7.36	0.354	0.500	0.434	0.440
60	x	1.980	1.950	0.940	0.872	155.20	153.60	4.168	4.166	1.418	1.474
	±s	0.175	0.275	0.181	0.218	3.56	4.22	0.294	0.397	0.498	0.445
90	x	2.094	1.916	0.924	0.866	153.20	155.80	4.244	3.892	1.416	1.392
	±s	0.396	0.340	0.217	0.191	6.26	5.22	0.397	0.306	0.500	0.471
120	x	1.914	1.880	0.874	0.748	154.20	152.20	3.838	3.634	1.414	1.442
	±s	0.217	0.277	0.161	0.176	4.44	5.93	0.749	0.552	0.555	0.543

Table 4
Average values of plasma macroelement levels after haemoperfusion through columns filled with clinoptilolite-rich tuff

Time (min)		Ca (mmol.l^{-1}) animal	column	Mg (mmol. l^{-1}) animal	column	Na (mmol. l^{-1}) animal	column	K (mmol. l^{-1}) animal	column	P (mmol. l^{-1}) animal	column
0	x	1.933		0.993		151.83		3.947		1.547	
	±s	0.368		0.196		10.07		0.640		0.401	
30	x	1.912	1.430	1.035	1.058	153.00	144.17	4.048	3.542	1.522	1.588
	±s	0.311	0.388	0.197	0.319	11.71	9.13	0.779	2.436	0.301	0.540
60	x	1.855	1.508	0.997	0.992	153.33	145.33	3.843	3.270	1.587	1.613
	±s	0.347	0.491	0.184	0.235	11.98	10.86	0.680	2.153	0.631	0.393
90	x	1.850	1.510	0.978	0.947	153.00	148.93	3.743	2.962	1.572	1.630
	±s	0.361	0.381	0.180	0.221	11.26	12.42	0.588	1.785	0.504	0.350
120	x	1.874	1.646	0.930	0.932	151.40	151.40	3.370	2.508	1.590	1.664
	±s	0.178	0.308	0.173	0.203	10.95	16.68	0.671	1.603	0.355	0.378

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. Juggi (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. Terai 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.

Vplyv hemoperfúzie na niektoré 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 hemoperfúznych kolón. Hemoperfúzii sme podrobili dve skupiny oviec (n = 6) počas 2 hodín. V prvej skupine sme použili komerčné hemoperfúzne kolóny HEMASORB 400 C plnené aktívnym uhlím. 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 opúšťajúcej kolónu zistené signifikantne nižšie hodnoty trombocytov (134.3±28.9 oproti 251.0±55.4 $G.l^{-1}$, $P<0.01$) a koncentrácia sodíka v plazme (144.17±9.13 oproti 157.17±7.36 $mmol.l^{-1}$, $P<0.05$). Napriek tomu, konečné posúdenie biokompatibility zeolitových hemoperfúznych kolón si bude vyžadovať ďalší podrobnejší výskum.

References

BARTKO, P., VRZGULA, L., KOVÁČ,G., BLAŽOVSKÝ, J., PROSBOVÁ, M., REICHEL, P., PAULÍKOVÁ I. 1983: Clinical and laboratory studies of zeolite in pigs (In Slovak). Research Report. Záverečná správa VŠV Košice, 1983, 154 p.

BLANCHARD G.,MAUNAYE M.,MARTIN G. 1984: Removal of heavy metals from waters by means of natural zeolites. Water Res. **18:** 1501-1507

DVOŘÁK, M. 1989: Schopnost bentonitu a přírodního zeolitu adsorbovat aflatoxin z tekutého prostředí. Vet. Med. (Praha). **34:** 307-316

FORBERG, S., JONES, B., WESTERMARK, T. 1989: Can zeolites decrease the uptake and accelerate the excretion of radio-caesium in ruminants? Sci. Total Environ. **79:** 37-41

FRANTA, P., KUCA, L., SANTAROVÁ, M., VACKOVÁ, E., KVASNICOVÁ, M. 1994: Decontamination of accidental wastes from Czech nuclear energy stations (In Czech). Bezpečnost Jaderné Energie. **1:** 343-349

HLAVAY, J., INCZÉDY, J., FOLDI-POLYÁK, K., ZIMONY, M. 1988: Pilot plant study for removal of ammonia from drinking water with natural zeolite. In Occurrence, Properties, and Utilization of Natural Zeolites, D. Kalló and H.S. Sherry (eds.), Akadémiai Kiadó, Budapest, 1988: 483-490

JUGGI, J.S. 1971: Haemoperfusion through cation exchange columns in the treatment of ammonia intoxication in dogs, Proc. Soc. Exp. Biol. Med. **137:** 39-42

KOVÁČ, A. 1993: Hemodialyzačná liečba v praxi. Osveta, Martin, 320 p.

KOVÁČ, G., BARTKO, P., VRZGULA, L., REICHEL, P., NIŠTIAR, F., MOJŽIŠ, J., SEIDEL, H. 1995: Application of clinoptilolite during organophosphate intoxication in sheep. Natural Zeolites'93, Occurrence, Properties, Use, D.W. Ming and F.A. Mumpton (eds.), Comm. Natural Zeolites, Brockport, New York, 1995: 459-466

KOZÁČ, J., OČENÁŠ, D. 1982: Determination of clinoptilolite content in zeolitic tuffits (In Slovak). Mineralia Slov. **14:** 549-552

KOZÁČ, J., OČENÁŠ, D., RUSNAK, D., AND HOPPAN, J. 1982: Mineralogy, characteristic properties and utilization possibilities of zeolitic tuffite from Nižný Hrabovec (East Slovakia) (In Slovak). Mineralia Slov. **14:** 263-275

MIŽÍK, P., HRUŠOVSKÝ, J., TOKOŠOVÁ, M. 1989: Effects of natural zeolite on excretion and distribution of radiocesium in rats (In Slovak). Vet. Med. (Praha) **34:** 467-474

MOJŽIŠ, J., NIŠTIAR, F., KOVÁČ, G., MOJŽIŠOVÁ, G. 1994: Preventive effect of zeolite in intoxication by VX substance in rats (In Slovak). Vet. Med. (Praha) **394:** 443-449

MUMPTON, F.A., FISHMAN P.H. 1977: The application of natural zeolites in animal science and aquaculture. J. Anim. Sci. **45:** 1188-1203

MUMPTON, F.A. 1984: Flammae et fumus proximi sunt: The role of natural zeolites in agriculture and aquaculture. In Zeo-Agriculture: Use of Natural Zeolites in Agriculture and Aquaculture, W.G. Pond, and F.A. Mumpton (eds.), Westview Press, Boulder, Colorado: 3-27

MUSATOVOVA, O., VAVROVÁ, M., MITRO, A., BARTHA, S. 1993: In vivo studies of 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

PAPADOPOULOS, A., KAPETANIOS, E.G., LOIZIDOU, M. 1996: Studies on the use of clinoptilolite for ammonia removal from leachates. J. Environ. Sci. Health A - Sci. **316:** 211-220

POND, W.G., ELLIS, K.J., KROOK, L.P., SCHOKNECHT, P.A.1993: Modulation of dietary lead toxicity in pigs by clinoptilolite. ZEOLITE'93, 4th International Conference on the Occurrence, Properties, and Utilization of Natural Zeolites, Boise-Idaho, June 20-28: 170-172

SEIDEL, H., VRZGULA, L. 1989: Sorption of heavy metals by natural zeolite (clinoptilolite) from water (In Slovak). Poľnohospodárstvo **8:** 729-730

SEIDEL, H., BARTKO, P., PAULÍKOVÁ, I., BENKOVÁ, E., BALDOVIČ, R. 1992: Technical aspects of haemoperfusion in small ruminants (In Slovak). Scientific symposium with international participation „Actual Problems in Diagnosis, Prevention, and Therapy of Internal Diseases in Ruminants", Košice, 3.-4. June: 123-125

SHERRY, H.S. 1993: The cation-exchange properties of zeolites: A review. ZEOLITE'93, 4th International Conference on the Occurrence, Properties, and Utilization of Natural Zeolites, Boise-Idaho, June 20-28, 1993: 189-191

TERAI, C., SHIMIZU, S., MATSUSHIMA, S., TANAKA, I., OKADA, Y., MIYAHARA, T., NAKAJI, S. 1996: Development of a potassium-specific adsorbent for direct hemoperfusion. Artif Organs **20:** 1227-1232

TOMASEVIC-CANOVIC, M., DUMIC, M., VUKICEVIC, O., RADOSEVIC, P., GLISOVIC, L., RAJIC, I., PALIC,T. 1993: Adsorption of aflatoxin B_1 and G_2 on clinoptilolite. ZEOLITE'93, 4th International Conference on the Occurrence, Properties, and Utilization of Natural Zeolites, Boise-Idaho, June 20-28, 1993: 202-204

VAVROVÁ, M., MUSATOVOVA, O., BARTHA, S. 1991: In vitro studies on the ability of natural zeolites to sorb some radionuclides. Isotopenpraxis **17:** 325-327

VRZGULA, L., SEIDEL, H. 1989: Sorption properties of natural zeolite (clinoptilolite) in rumen and abomasal fluid in vitro (In Slovak). Vet. Med. (Praha) **34:** 537-544

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