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THE INFLUENCE OF ENTEROSORPTION ON SOME HAEMATOLOGICAL AND BIOCHEMICAL INDICES OF THE NORMAL RATS AFTER SINGLE INJECTION OF MELPHALAN

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Aim: **One of the most prominent side effects of intensive cancer chemotherapy is bone marrow suppression which is an independent negative prognostic factor for the time to tumor progression. The aim of the study was to evaluate the myeloprotective possibilities of carbon enterosorbents in the case of usage of alkilating drug melphalan (L-PAM).** ***Materials and Methods:*** **L-PAM was injected intravenously to healthy inbred rats to cause the myelosuppression. 3 days before and 7 days after this, suspension of two types of carbon granulated enterosorbents were administered *per os* one time per day. On 8th day after L-PAM injection, the rats were weighted and blood and liver tissue were taken under Ketamine general anesthesia for biochemical examination. Peripheral blood smears were made also.** ***Results:*** **Melphalan at a dose of 3 mg/kg causes expressed myelotoxic reaction: leucopenia, decreasing of erythrocytes, hemoglobin and platelets counts. Even on 8th day after single injection of this cytostatic we can detect expressed signs of oxidative stress like increasing of hydroperoxides, TBA-reactive substances, and decreasing of activity and level of main endogenic antioxidants — superoxide dismutase (SOD), catalase and reduced glutathione. L-PAM causes also the violation of kidney function such as increase of urea and creatinine level; and rising of endogenic intoxication with elevation of middle mass molecules level. In a dose of 3 mg/kg melphalan has no negative influence on liver function on 8th day of experiment. Enterosorption with carbon enterosorbents C1 (bulk density $\gamma = 0.28$ g/cm^3, granules diameter 0.15–0.25 mm, BET pore surface 1719 m^2/g, therapeutic dosage 1400 mg/kg) and C2 (bulk density $\gamma = 0.18$ g/cm^3, granules diameter 0.15–0.25 mm, BET pore surface 2162 m^2/g, therapeutic dosage 900 mg/kg) diminishes and mitigates negative side effects caused by single intravenous injection of melphalan. Carbon enterosorbent C2 have rather more expressed positive effect than C1 for practically all indices. The most important curative effect due to C2 administration is prominent myeloprotection of bone marrow of experimental animals.** ***Conclusion:*** **Carbon enterosorbent C2 is promising and perspective sorbent for prophylaxis and treatment of side effects of cytostatic chemotherapy including myelotoxicity, mucositis, kidney injuries, gonadotoxicity, etc.**

Key Words**: L-PAM, myelosuppression, oxidative stress, carbon enterosorbents.**

Enterosorption with carbon sorbents is a well-known method of sorption therapy, which is widely used for combined treatment of exogenic and endogenic intoxications of different origin [1–4] including toxic reaction attributable to intensive cancer therapy.

Melphalan (L-phenylalanine mustard, phenylalanine mustard, L-PAM, or L-sarcolysin) is a phenylalanine derivative of nitrogen mustard. It is a bifunctional alkylating agent and one of the most aggressive antineoplastic drugs [5]. Before their use in chemotherapy, alkylating agents better known for their use as sulfur mustard, "mustard gas" and related chemical weapons in World War I. Its hematological suppression effects are known since sixties [6]. Bone marrow suppression is the most significant toxicity associated with L-PAM, especially in case of intravenous injection. Thrombocytopenia and/or leukopenia are indications to withhold further therapy until the blood counts have sufficiently recovered.

It is known that detoxification procedures with the use of carbon adsorbents have myeloprotective action. The first fact [7] is related to an influence of hemocarboperfusion, i.e. passing blood through column with activated carbon, on survival rate of dogs irradiated with minimal absolutely lethal dose with enhancing its survival rate from 3.2% to 60–70%.

It's is important, too, that enterosorption demonstrates also certain positive effect in severe radiation injuries combined with thermal trauma [8, 9].

The reputed fact — alkylating anticancer preparations have radiomimetic mechanism of their cytostatic action mimicking cytostatic effects of irradiation [10, 11]. Principal action of alkylating drugs, including melphalan that is a "nitrogen mustard" derivative, related to their covalent interaction with bases of DNA chain with generation of adducts that hinder DNA replication and, consequently, stops cell division as it happens during irradiation. At present time, it is possible to study melphalan-DNA-adducts formation on the level of separate haemopoietic cells [12].

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Abbreviations used: ALT – alanine aminotransferase; AST – aspartate aminotransferase; AUC – area under the curve; BET – Brunauer – Emmett – Teller model of pore distribution calculation; BJH – Barrett – Joyner – Halenda model of pore distribution calculation; CP – ceruloplasmin; DR – Dubinin – Radushkevich model of pore distribution calculation; GIT – gastro-intestinal tract; G-SH – reduced glutathione level; HB – hemoglobin level; L-PAM – melphalan, L-phenylalanine mustard or L-sarcolysin; MMM – middle mass molecules; PLt – platelets count; RBC – red blood cell count; SOD – superoxide dismutase; TBARS – thiobarbituric acid-reactive substances; TNF – tumor necrosis factor; WBC – white blood cell count.

The second fact is directly related to myelodepression caused by alkylating preparations. In this study [13] cyclophosphan preparation was administered to rats with transplanted Guerin carcinoma at the dose of 100 mg/kg of body weight on 10th and 13th days after tumor transplantation, while enterosorption with the use of synthetic SCN carbons (bulk density 0.3–0.4 g/cm^3) was initiated from the next day after cyclophosphan injection. In this case enterosorption shows an evident myeloprotective effect toward all main elements of bone marrow.

The presence of myeloprotective effect of carbon enterosorbents was also observed during treatment of patients with lymphogranulomatosis irradiated by radical scheme [14]. Silicon-organic enterosorbents were also used in patients with different tumors of peritoneal cavity who underwent polychemotherapy [15].

Apart of myelotoxicity, melphalan possesses definite hepatotoxicity that is notably manifested in the case of injection into hepatic artery [16]. Upon isolated hepatic perfusion expression of melphalan-dependent tumor necrosis factor (TNF) pathogenic pathways via activation of both of TNF-receptors in Kupffer cells was demonstrated as an important component of melphalan toxicity [17]. These receptors are also a target for bacterial lipopolysaccharide, which intensively enters portal blood flow upon different intestinal injuries. It is known for a long time about gastro-intestinal toxicity (GIT) of melphalan that may result in the development of mucosa injuries (mucositis) and other manifestations of enteropathy, including hemorrhagic diarrhea and penetration of large bowel [18, 19].

At the same time, enterosorption causes an expressed curative effect in the case of different liver pathologies, like viral and toxic hepatitis as well as mechanical jaundice [20–25]. On other side there are multiple demonstrations of favorable action of enterosorption in the treatment of various GIT mucosa injuries, including the ones caused by radiation and severe burn toxicities [8] or enteric disbiosis [26], as well as for prevention of intestinal flora translocation [27]. As was found above, enterosorption mitigates the signs of oxidative stress [28, 29] due the activation of excessive lipid peroxidation which is a stereotype reaction of organism toward administration of alkylating preparations [30]. Enterosorption also corrects "metabolic chaos" that develops in a body upon action of radiation or radiomimetics caused by disturbed coordination in the functioning of various enzymatic systems [31, 32]. It is also important to note that enterosorption diminishes the direct and delayed toxic reactions of patients (nausea, vomiting etc.) after intensive chemotherapy [33, 34].

Thus, it is reasonable to use carbon sorbents for mitigation of symptoms of melphalan polipathic iatrogenic intoxication.

MATERIALS AND METHODS

Animals and experiment design. The studies were carried out on 55 white inbred rats weighting 200 ± 20 g from the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR) vivarium. All animals' procedures had been done according to the rules and requirements of European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and local Ethic Committee of IEPOR.

Evaluation of sorption properties of carbon sorbents was performed by standard methods, an impact of microporous structures was calculated by Dubinin — Radushkevich model, pore distribution by sizes — by Brunauer— Emmett — Teller model, mesopore area — by the method proposed by Barrett, Joyner and Halenda [35].

Animals were randomly distributed into 4 groups: 1 — intact group (n = 25); 2 — rats who had got L-PAM (n = 10) — control group; 3 — L-PAM and carbonic enterosorbent C1(L-PAM + C1) (n = 10); and 4 — L-PAM and carbonic enterosorbent C2 (L-PAM + C2) (n = 10). The absorption of oral melphalan is highly variable due to incomplete intestinal absorption, differences in "first pass" hepatic metabolism, and rapid hydrolysis. Oral administration of melphalan with a high fat meal may reduce melphalan exposure (AUC) by 36 to 54% [35]. That's why we chose intravenous administration of L-PAM (AlkeranTM Injection, GlaxoSmithKline, UK). It has been injected at once in tail vein at the dose of 3.0 mg/1 kg body weight.

We used carbonic granulated enterosorbents C1 of IEPOR production with bulk density γ = 0.28 g/cm^3, with coincides with enterosorbents, used by L.V. Bonatskaya (1985) and highly activated enterosorbents C2 with γ = 0.18 g/cm^3. Both enterosorbents have granules with diameter of 0.15–0.25 mm, and used in the dose of 5 ml per 1 kg of animals body weight or 1400 mg/kg for C1 enterosorbent and 900 mg/kg for C2. A suspension of enterosorbents in appropriate quantity of distilled water was introduced via the tube into rat stomach once a day during 3 days before the day of L-PAM injection and 7 days after injection during 7 days one time per a day. Rats of control (L-PAM) and intact groups were given equivalent quantity of distilled water. Animals of intact group received IV equal quantities of physiologic solution instead of L-PAM.

On 8th day since L-PAM injection, the rats were weighted and blood was taken from the heart under ketamine hydrochloride general anesthesia. Peripheral blood smears were made also. For the staining of cytological smears we used panoptic method of Pappenheim, using May-Grünwald and Giemsa solutions [36]. The main hematologic indices were analyzed on hematology analyzer BC-3000Plus Mindray. Plasma activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase, concentration of total bilirubin, serum total protein, creatinine and urea were analyzed using a standard kits "Lachema" on Humalyzer 2000. Serum level of middle mass molecules (MMM) [37], thiobarbituric acid-reactive substances (TBARS) [38], catalase

activity [39] were studied. Plasma ceruloplasmin (CP) level was studied also [40]. The liver tissue was homogenized by using the homogenizer SilentCrusher S (Heidolph, Germany). In liver homogenates we studied the concentration of lipid hydroperoxides [41], TBARS, superoxide dismutase (SOD) [42], catalase activity and reduced glutathione level (G-SH) [43].

Statistical analysis. The data are expressed as the mean ± standard error of the mean (M ± m). Probability values $p < 0.05$ were considered statistically significant. The statistical significance of the differences between mean values was assessed by the Mann — Whitney-test and ANOVA-test using Microsoft Excel XP (USA) and Origin 7.5 (OriginLab Corporation, USA). The distribution of indices was estimated by using Shapiro — Wilk Normality Test.

RESULTS

On 8th day after single intravenous injection of L-PAM at a dose of 3 mg • kg^{-1} we detected significant decrease of leukocytes count by 72.5%, erythrocytes count — by 12.4%, hemoglobin level (HB) — by 7.7% and platelets — by 18.6% (Table 1). Enterosorbent C1 promoted 2-fold increase of leukocyte count, C2 — 4.5-fold increase. C2 was more effective than C1: increase of leukocyte count was by 117.4% higher than in L-PAM+C1 group. On the base of non-essential changes of erythrocytes count, HB and platelets count after L-PAM injection both enterosorbents C1 and C2 did not alter these indices.

Table 1. Hematological rats' indices, M ± m

Parameter	Animal's groups			
	Intact rats (n = 25)	L-PAM (n = 10)	L-PAM + C1 (n = 10)	L-PAM + C2 (n = 10)
WBC, ×10^9/l	5.24±0.29	1.44±0.14*	2.98±0.24$^{\#}$	6.48±0.37$^{\#\gamma}$
RBC, ×10^{12}/l	7.34±0.12	6.43±0.11*	6.53±0.13	6.71±0.06
HB, g/l	134.32±1.69	124.0±2.37*	122.0±1.53	127.40±3.44
PLt, ×10^9/l	634.84±22.97	517.0±26.54*	471.90±31.17	499.60±16.89

Notes: statistical significance p < 0.05 comparatively with: *intact rats; $^{\#}$L-PAM; $^{\gamma}$L-PAM + C1.

Concerning the leukocytes formula on 8th day after L-PAM injection we can see the significant increase of neutrophils percentage — by 51.1% (Table 2). Also we can observe the appearance of young forms of granulocytes in peripheral blood smears and basophils, especially in group L-PAM + C2. All others sprouts of blood have no statistical significance in percentage data.

Table 2. Leukocytes formula, M ± m, %

Parameter	Animal's groups			
	Intact rats (n = 25)	L-PAM (n = 10)	L-PAM + C1 (n = 10)	L-PAM + C2 (n = 10)
Promyelocytes	–	–	–	0.78±0.57
Myelocytes	–	–	–	1.11±1.11
Metamyelocytes	–	–	2.28±2.14	1.33±1.10
Neutrophils	25.90±1.83	39.14±3.49*	41.43±4.61	32.67±3.18
Eosinophils	1.0±0.45	0.57±0.37	0.57±0.37	0.44±0.29
Basophils	–	–	–	0.22±0.22
Lymphocytes	68.40±3.10	54.71±4.52	50.86±5.50	58.89±3.64
Monocytes	4.70±0.97	5.58±1.51	4.86±1.68	4.56±1.36

Notes: statistical significance p < 0.05 comparatively with: *intact rats; $^{\#}$L-PAM; $^{\gamma}$L-PAM + C1.

Rather more interesting picture give us the changes of absolute count of different types of leucocytes (Table 3). After L-PAM injection the absolute quantity of neutrophils is lower by 58.5%, lymphocytes count — by 78.0% comparing with intact rats. C1 administration caused the rising of neutrophils quantity by 119.0%, lymphocytes — by 92.4% in comparison with untreated control. Enterosorbent C2 had more prominent effects: absolute number of neutrophils was by 275.5% (3.8-fold) higher comparatively with L-PAM group; and by 71.5% higher comparatively with L-PAM + C1 group. At the same time lymphocytes quantity in L-PAM-C2 group is by 384.3% (4.8-fold) higher in L-PAM group and by 151.8% (2.5-fold higher) in comparison with intact control.

Table 3. Leukocytes formula count, M ± m, ×10^9/l

Parameter	Animal's groups			
	Intact rats (n = 25)	L-PAM (n = 10)	L-PAM + C1 (n = 10)	L-PAM + C2 (n = 10)
Promyelocytes, ×10^9/l	–	–	–	0.05±0.04
Myelocytes, ×10^9/l	–	–	–	0.07±0.07
Metamyelocytes, ×10^9/l	–	–	0.07±2.14	0.09±0.07
Neutrophils, ×10^9/l	1.37±0.09	0.56±0.05 *	1.23±0.14$^{\#}$	2.12±0.21$^{\#\gamma}$
Eosinophils, ×10^9/l	0.05±0.02	0.01±0.01	0.02±0.01	0.03±0.02
Basophils, ×10^9/l	–	–	–	0.01±0.01
Lymphocytes, ×10^9/l	3.58±0.16	0.79±0.06*	1.52±0.16$^{\#}$	3.82±0.24$^{\#\gamma}$
Monocytes, ×10^9/l	0.24±0.05	0.08±0.02	0.14±0.05	0.29±0.09

Notes: statistical significance p < 0.05 comparatively with: *intact rats; $^{\#}$L-PAM; $^{\gamma}$L-PAM + C1.

L-PAM in case of single intravenous injection at a dosage of 3 mg • kg^{-1} body weight to healthy inbred white rats didn't have negative influence on liver function (Fig. 1): there were no definite changes in activity of ALT and total bilirubin level. The activities of AST and alkaline phosphatase decreased by 19.2 and 39.3% respectively, maybe due to L-PAM-dependent inhibition of the synthesis of these enzymes.

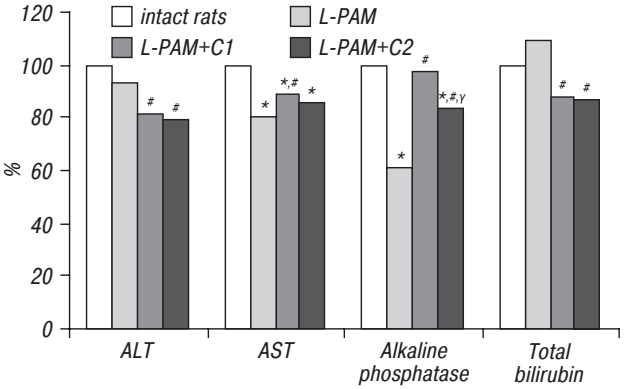

Fig. 1. The liver function indexes in experimental animals treated with enterosorbents and L-PAM.

Notes: statistical significance p ⩽ 0.05 comparatively with: *intact rats; $^{\#}$L-PAM group; $^{\gamma}$L-PAM + C1 group

All changes in hepatic indices due to enterosorption are also located in the borders of its normal value. Still enterosorbents C1 and C2 caused significant decreasing of ALT (by 12.5 and 15.0%) and total bilirubin level (by 19.8 and 20.4%, respectively). Activity of alkaline phosphatase increased by 59.5% under influence of C1, and by 38.0% under the influence of C2 in comparison with the rats of L-PAM-group, but the last index was significantly lower than in intact rats.

L-PAM caused the kidney malfunction: urea and creatinine serum levels were increased by 124.8 and 11.6%, respectively (Table 4). The urea content un-

der the influence of C1 and C2 was lower by 37.2% and 44.8%, respectively, in comparison with L-PAM group. Sorbent C2 caused the decrease of creatinine level by 7.5%, but C1 had no influence on this index. At the same time the signs of activation of synthetic liver function were seen. The total serum protein level in case of enterosorption was higher by 15.2 and 28.2% respectively for C1 and C2, than in untreated and L-PAM groups.

Table 4. The indices of blood serum, M ± m, n = 10

Index	Intact rats	L-PAM	L-PAM + C1	L-PAM + C2
Total serum protein, g/l	51.04±2.53	51.65±1.66	59.50±2.62#	66.20±2.83#
Urea, mmol/l	4.92±0.19	11.06±0.49*	6.94±0.28#	6.10±0.37#
Creatinine, mcmol/l	71.72±1.94	80.06±1.92*	78.42±1.45	74.02±1.16#γ
MMM1, u/l	0.327±0.022	0.606±0.013*	0.456±0.032#	0.429±0.038#
MMM2, u/l	0.448±0.029	0.610±0.022*	0.483±0.020#	0.455±0.029#

Notes: statistical significance p < 0,05 comparatively with: *intact rats; #L-PAM; γL-PAM + C1.

Levels of MMM1 and MMM2 as indices of endogenic intoxication were higher by 85.4 and 36.0% respectively in L-PAM-treated rats comparatively with intact rats. Enterosorption effectively decreased these indexes: in L-PAM + C1 group levels of MMM1 and MMM2 were lower by 24.8 and 20.7% and in L-PAM + C2 group — by 29.2 and 25.4%, respectively, comparing with L-PAM group (see Table 4).

The biochemical sings of intensive oxidative stress were detected after L-PAM administration (Table 5). On 8th day after IV injection of L-PAM the levels of HPL and TBARS in liver tissue were increased by 100.2 and 71.3%, respectively; TBARS in blood serum — by 126.6% in comparison with intact group. The decrease of activity of SOD (on 68.7%) and catalase in liver homogenates and blood serum was by 26.5 and 53.0%, respectively. At the same time the levels of G-SH and CP were lower by 10.7 and 19.8%, respectively, compared to control group.

Table 5. Changes of prooxidant-antioxidant system indices, M ± m, n = 10

Index	Intact rats	L-PAM	L-PAM + C1	L-PAM + C2
HPL, U/kg	2.19±0.05	4.38±0.21*	3.03±0.21#	2.26±0.15#γ
TBARS (liver), mcmol/kg	7.04±0.16	12.07±0.28*	9.08±0.50#	7.26±0.49#γ
TBARS (serum), mcmol/l	0.36±0.04	0.82±0.05*	0.71±0.05	0.58±0.05#
SOD (liver), U/kg	75.96±1.94	23.81±2.50*	24.31±2.55	50.38±2.82#γ
Catalase (liver), cat/kg	65.43±1.27	48.06±3.15*	57.51±1.80#	59.36±3.04#
Catalase (serum), cat/l	6.13±0.33	2.88±0.58*	3.97±0.71	8.50±0.40#γ
G-SH, mmol/kg	2.03±0.05	1.81±0.03*	1.94±0.05	2.07±0.06#
CP, mg/l	322.88±11.62	259.0±12.61*	269.5±16.99	301.0±11.44#

Notes: statistical significance $p < 0.05$ comparatively with: *intact rats; #L-PAM; γL-PAM + C1.

As one can see, enterosorption have significant positive influence on the processes of excessive lipid peroxidation. C1 decreased of HPL and TBARS level in the liver by 30.8 and 24.7%, but there were no significant effects on serum TBARS, catalase, CP and SOD in the liver. At the same time the rise of activity of liver catalase by 19.7% under the influence of C1 enterosorbent was seen. C2 enterosorbent had more prominent effects. The HPL level was by 48.4% less than in L-PAM group and by 25.4% less than in L-PAM + C1 group, TBARS quantity in liver homogenates was by 39.9% lower than in untreated group and 20.1% lower than in rats which got C1. The decrease of TBARS in the serum by 29.1% was also registered.

Enterosorbent C2 promoted more significant restoration of antioxidant system: activity of SOD, and catalase in liver and serum was higher by 111.6; 23.5 and 19.1% respectively than in L-PAM group; G-SH level and CP also increased by 14.5 and 16.2%. Furthermore, liver SOD and serum catalase activities were by 107.3 and 114.1% higher than in L-PAM + C1 group (see Table 5).

So, the difference in efficacy of enterosorbents C1 and C2 is very considerable, that's why we should estimate their structural and sorption parameters more meticulously.

As one can see on porograms (Fig. 2) the pore systems for nitrogen sorption of both samples are similar in the range of 10–500 Å. The main difference between C1 and C2 is that the porous structure of C2 is more developed and shifted toward mesopores, which is confirmed by the ratio of the total surface area of the pore size and surface calculated by BJH model (Table 6).

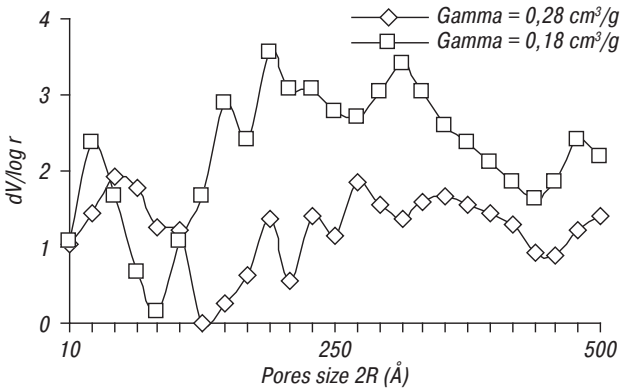

Fig. 2. The pore size distribution of enterosorbents C1 (0.28 g/cm^3) and C2 (0.18 g/cm^3)

Table 6. The specific surface area

Samples	Total surface area, m^2/g		
	BET	DR	BJH
C1	1719	1639	239
C2	2162	2049	565

Notes: pore distribution calculation by: BET – Brunauer – Emmett – Teller model; BJH – Barrett – Joyner – Halenda model; DR – Dubinin – Radushkevich model.

In case of calculating of total surface sorption (BET) taking into account the difference between bulk density (γ, g/cm^3) of samples, the specific surface area of C2 is less than for C1 (1390 *versus* 1719 m^2/g), the assessment of micropore surface (DR) shows the same results C1>C2 (1639 *versus* 1317 m^2/g). At the same time the surface of C2 mesopores is larger than C1 (363 toward 239 m^2/g).

In Table 7 one can see the data of adsorption of freely soluble markers by enterosorbents C1 and C2: the standard dose of C2 is more effective than C1 for sorption of middle molecular weight substances (vitamin B_{12}) and low molecular weight substances (methylene blue) by 2- and 2.6-fold respectively, and is not significantly lower for adsorption of creatinine compared with C1.

From Table 8 one may see that enterosorbent C2 exceeds C1 by the sorption of strongly bound ligands (unconjugated bilirubin) by 29.3%, but it is almost equal by the protein adsorption.

Those data demonstrate that despite of definite range of similarity between porograms of C1 and C2 carbon these enterosorbents have considerable quantitative and qualitative differences in spectra of metabolite adsorption.

Table 7. The adsorption of hydrophilic marker metabolites by enterosorbents C1 and C2

Samples	Y, g/cm^3	Vs, cm^3/g	C residual, mg/ml	Adsorption, mg/g	at C eq. 0.951 mg/ml, mg/g	Recalculation on capacity volume, mg/cm^3
Methylene blue						
C1	0.28	0.87	0.951	218.15	218.15	61.08
C2	0.18	2.02	0.443	420.0	901.62	162.3
Initial concentration 1.5 mg/ml						
Creatinine						
C1	0.28	0.87	0.0968	79.43	79.43	22.24
C2	0.18	2.02	0.0807	86.85	104.18	18.75
Initial concentration 0.3 mg/ml						
B_{12}						
C1	0.28	0.87	0.69	123.6	123.6	34.61
C2	0.18	2.02	0.425	229.24	372.18	67.0
Initial concentration 1.0 mg/ml						

Table 8. The adsorption of unconjugated bilirubin and protein (albumin) by enterosorbents C1 and C2

Samples	Y, g/cm^3	Vs, cm^3/g	C residual, mg%	Adsorption, mg/g	at C eq. 15.75 mg%, mg/g	Recalculation on capacity volume, mg/cm^3
Bilirubin						
C1	0.28	0.87	15.75	8.42	8.42	2.36
C2	0.18	2.02	12.43	14.65	18.56	3.34
Initial concentration 20.0 mg%						
Protein						
C1	0.28	0.87	25.49	0.695	0.695	0.195
C2	0.18	2.02	24.65	0.960	0.960	0.173
Initial concentration 30.0 g/l						

DISCUSSION

Usually adjuvant or neoadjuvant polychemotherapy is too toxic toward healthy cells of the body to be effective. In multivariate analysis, hematological toxicity retained its imperative as an independent negative prognostic factor for time to disease progression [44]. That's why the effective and safe methods which replenish the hematological indices and protect the patient from febrile neutropenia and other bacterial complication during polychemotherapy courses are very important.

As it is follows from experimental data, enterosorption can be one of such methods. In case of C1 and C2 enterosorbents administration prominent myeloprotective effect was observed. Leucocytes count was significantly higher in L-PAM + C2 group: in comparison with L-PAM and L-PAM + C1 groups and even with intact rats — at least by 23.6%. There was the tendency to rising of other hematological indices, too.

Enterosorption also demonstrated prominent potential suppression for oxidative stress caused by L-PAM. For almost all indices of prooxidant-antioxidant system C2 enterosorbent has the definite advantages comparatively with C1 enterosorbent especially for indices of endogenous antioxidant defense. As it is known such enzymes as SOD, catalase and peroxidase act as potent radioprotectors, being the scavengers of free radicals and oxygen metabolites. These enzymes serve as the first line of defense of DNA, membranes, proteins and cell metabolic processes against peroxidation injury [45]. Also irradiation causes the changes of high molecular components of cells resulting which in inactivation of catalase and other antioxidant enzymes, while excessive quantity of peroxides and other highly reactive components leads to their inhibition [46]. Melphalan being one of the most proximate radiomimetic also can exhibit the same mechanism of antioxidant enzymes inactivation.

Endogenic intoxication in oncologic patients could be caused by tumor disposition as well as by curable measures (chemotherapy, surgical treatment and radiotherapy) and results in significantly impaired quality of life of such patients, limited special treatment modalities, renal, hepatic and enteric malfunction, and increased mortality. In turn, prominent syndrome of endogenic intoxication aggravates toxic side effects of aggressive therapy. So, it seems reasonable to use accompanying detoxification therapy [47, 48], in particular, enterosorption.

Enterosorption as a part of efferent therapy besides its local effects in intestine, has distant effects, which are expressed in improvement of the function of the body detoxification systems, enhancement of different metabolic processes [48, 49].

The exact mechanisms of myeloprotection due to enterosorption remain largely unknown. However, it has been shown that they may include the decrease and mitigation of the signs of endogenic intoxication, by suppression of excessive lipid peroxidation. In our study we explain the increase of white blood cell count by the improvement of antioxidant defense of bone marrow.

On the other hand, the processes of bone marrow cell proliferation, migration, and homing are downregulated by many factors including adhesion molecules, cytokines, proteolytic enzymes, stromal cells, and hematopoietic cells [50]. Still, we know that enterosorption influences the level of different types of cytokines [51, 52]. It is quite possible that carbon enterosorbents may bind some toxic substances inhibiting the proliferation of bone marrow cells or oppositely — promote the increased content of factors which stimulate sanguification.

Repeated sessions of chemotherapy inevitably result in cumulative myelosuppression, probably due to chronic stem cell depletion. Protection of the stem cell compartment during each cytotoxic treatment with the use of enterosorption may prevent such events [53]. Our data have demonstrated that enterosorption with carbon sorbents has prominent myeloprotective activity especially expressed in the case of usage of superactivated carbon enterosorbent C2.

In conclusion, melphalan at a dose of 3 mg/kg causes expressed myelotoxic reaction such as leucopenia, decrease of erythrocyte and platelet counts, and hemoglobin concentration. Even on 8th day after single injection of this cytostatic one can detect expressed signs

of oxidative stress like increase of hydroperoxides, TBA-reactive substances, and decrease of activity and level of the main endogenic antioxidants — SOD, catalase and reduced glutathione. L-PAM causes also the violation of kidney function such as increase of urea and creatinine levels and endogenic intoxication with elevation of MMM level. At a dose of 3 mg/kg melphalan has no negative influence on liver function. Enterosorption with carbon enterosorbents C1 (bulk density $\gamma = 0.28$ g/cm^3, granules diameter 0.15–0.25 mm, BET pore surface 1719 m^2/g, therapeutic dosage 1400 mg/kg) and C2 (bulk density $\gamma = 0.18$ g/cm^3, granules diameter 0.15–0.25 mm, BET pore surface 2162 m^2/g, therapeutic dosage 900 mg/kg) diminishes and mitigates negative side effects caused by single intravenous injection of melphalan. Carbon enterosorbent C2 have rather more expressed positive effect than C1 for practically all indices. The most important curative effect due to C2 administration is prominent myeloprotection of bone marrow of experimental animals.

Carbonic enterosorbent C2 is promising and perspective sorbent for prophylaxis and treatment of side effects of cytostatic chemotherapy which include myelotoxicity, mucositis, kidney injuries, gonadotoxicity, etc.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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