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Bioflavonoids and glutamine in diet and the Mg, Ca, Cu, Zn concentrations in heavy metals intoxicated rats' skins

Bioflavonoids (or flavonoids) are poliphenols compounds that occur ubiquitously in food and plant origin (15). They have been shown to exert antimicrobal, antiviral, cytotoxic, antineoplastic, antimutagenic, antiinflamatory, antioxidant, antihepatotoxic, antihypertensive and antiplatelet activities (5). The flavonoids contain several phenolic hydroxyl functions attached to ring structures. Structural variations within the rings subdivide the flavonoids into several families, e.g.: flavonols – quercetin, flavanols – catechin, flavanones – naringenin. Quercetin, catechin and naringenin are believed to have health-giving effects. Catechin and quercetin are found in tea leaves and red/white wine and have been shown to contain anticancer activity (2). Catechin inhibits glutathione reductase (16). Naringenin is present in citrus fruit and causes enhanced tumour necrosis factor alpha (TNF-α) cytotoxicity in tumour cells. Catechin and quercetin promote nitric oxide production and increased cGMP concentrations in vascular endothelial cells (4). Despite the apparently beneficial health effects of bioflavonoids, several studies indicate their mutagenicity, genotoxicity and pro-oxidant activities in animal models (9, 12). Glutamine (Gln) is the most abundant free amino acid that plays an important role in interorgans amino acids metabolism by shuttling nitrogen from skeletal muscle in the intestines (serving as energy substrate), to the liver (in case of starvation and injury), to the kidney (for aminogenesis and acid-base balance), and to proliferating cells of the immune systems (13).

Results of lead and cadmium toxic actions are, among others, disorders of essential bioelements-regulated processes and arising various pathological states. The aim of our studies was to determine influence of some bioflavonoids administered with glutamine on the rats' skins toxic actions of lead and cadmium.

MATERIAL AND METHODS

The experiment was conducted on 2-month-old Wistar male rats, weighting 200–220 g. The animals were divided in random into nine groups, each of 6 rats. Group I obtained in drinking water solution of plumbum nitrate, $Pb(NO_3)_2$ in concentration of 500 mg Pb/dm^3, group II solution of lead ions – 500 mg Pb/dm^3 and quercetin – 200 mg/dm^3 and glutamine 4 g/dm^3, group III – lead ions solution (500 mg Pb/dm^3) catechin – 200 mg/dm^3 and glutamine 4 g/dm^3, group IV received water solution of $Pb(NO_3)_2$ – 500mg Pb/dm^3, naringenin – 200 mg/dm^3 and glutamine 4 g/dm^3, V–VII groups obtained the same that groups I-IV but instead of lead cadmium chloride ($CdCl_2$) in amount of 500 mg Cd/dm^3 was used. Group IX was a control group, and these animals received redistilled water to drink. All the rats' groups were on a normal diet (LSM dry food) and they got solutions and food *ad libitum*.

During 6 weeks long experimental period lower solutions consumption in all cadmium ions receiving rats' groups (av. 18 cm^3/day) than in control group and lead ions receiving groups (average consumption 22 cm^3/day), was observed.

At the end of experiment the animals were anaesthetised with intraperitoneal injections of 0.5 ml of 5% ketamine, and skins (together with furs) as well as other tissues were collected.

Results were made using the Cochran-Cox test or t-Student test and obtained values were considered significant with $p < 0.001$, $p < 0.01$ and $p < 0.05$.

The skins were stored at the temperatures of 0–4°C, and then they were drying at 22°C during 24 hrs. Next stage the skins were rinsed in twice distilled water in Wacker type drums and dried for 24 hrs at 25°C with airflow of 0.2–0.8 m/s. Dried skins were incinerated at 25°C for 2 hrs. Such obtained ash was dissolved in 0.2 mol/dm^3 HCl and the elements were determined by ASA method with usage of atomic absorption spectrophotometer AAS-3. Magnesium and calcium concentrations were measured in the presence of correcting buffer; magnesium at wavelength $\alpha = 285.2$ nm and slit of 0.2 mm, calcium at $\alpha = 422.7$ and slit of 0.5 mm, zinc at $\alpha = 213$ nm and slit of 0.2 mm, copper at $\alpha = 324.8$ and slit of 0.3 mm.

RESULTS

Lead and cadmium intoxication as well as bioflavonoids and glutamine dietary additions influenced magnesium, calcium, zinc and copper concentrations in the examined skins. Changes induced by lead ions and lead ions administered together with bioflavonoids and glutamine are shown in Table 1, whereas those induced by cadmium ions are shown in Table 2. Both lead and cadmium indicate significant decrease in magnesium concentration in the examined skins ($p < 0.001$) and diet enrichment in bioflavonoids and glutamine only to a small extent increased Mg concentration and it was the most significant in case of quercitin and glutamine ($p < 0.01$) administered only with lead ions. Those two heavy metals exert different effect on calcium ions concentration. Lead ions decreased calcium level in the examined skins ($p < 0.001$), and diet bioflavonoid and glutamine enrichment only for quercitin resulted in statistically important increase of Ca concentration ($p < 0.01$). Cadmium ions to a smaller extent influenced ($p < 0.05$) Ca level decrease of animals given bioflavonoids and glutamine, and in all groups $p < 0.001$. Lead intoxication, unlike that with cadmium, led to decrease of Cu ($p < 0.001$) and Zn ($p < 0.01$) concentrations and dietary bioflavonoid and glutamine addition resulted in return of those concentrations to the ones of control group.

Table 1. Lead, bioflavonoids and glutamine influence on certain metals concentration in rats' skin

	Metals concentrations [μg x g^{-1} of tissue]							
	Mg		Ca		Cu		Zn	
	X	SD	X	SD	X	SD	X	SD
Control	520.2	12.8	433.5	24.6	8.6	1.0	180.1	26.4
Pb	209.9***	23.1	213.0***	20.5	4.5***	0.8	81.1***	7.2
Pb + quercetin + glutamine	343.7***	13.1	305.2***	11.2	7.9	0.5	147.2	6.2
Pb + catechin + glutamine	266.4***	20.6	236.0***	8.6	6.8	0.7	189.7	7.5
Pb + naringenin + glutamine	260.0***	11.7	218.7***	11.6	8.9	0.7	150.0	8.0

$^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ statistical significance in comparison to control

Table 2. Cadmium, bioflavonoids and glutamine influence on certain metals concentration in rats' skin

	Metals concentrations [μg x g^{-1} of tissue]							
	Mg		Ca		Cu		Zn	
	X	SD	X	SD	X	SD	X	SD
Control	520.2	12.8	433.5	24.6	8.6	1.0	180.1	26.4
Cd	196.4***	21.7	364.1**	31.3	8.7	0.6	148.4	10.2
Cd + quercetin + glutamine	279.9***	26.9	245.6***	29.5	9.3	0.9	218.5	21.2
Cd + catechin + glutamine	297.8***	19.3	119.9***	14.0	9.0	0.6	214.0	13.3
Cd + naringenin + glutamine	260.0***	8.4	128.0***	15.1	9.9	0.7	228.6	22.0

$^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ statistical significance in comparison to control

DISCUSSION

Available literature data do not contain studies on bioflavonoid and glutamine containing the diet influence on the levels of essential elements in skins of rats exposed to toxic lead and cadmium actions. Toxic metals, regardless of the way of intake, firstly penetrate blood vessels, where coupled with serum proteins they are easily transported to other tissues (3, 11). They also influence proper skin functioning through the accumulation that causes hyperkeratosis, scabbing and erythema. The influence of lead and cadmium actions in all groups diminished the most statistically significant magnesium and calcium concentrations. Under lead and cadmium action the most significantly important decrease in magnesium and calcium concentrations in all animals groups was observed, which can be the result of antagonism between those elements as well as the consequence of metals homeostasis disturbance, i.e. induced normal skin functioning disorders (6, 7).

Cadmium and lead act in a non-specific way and they influence numerous mechanisms of damaging of life cells. That ability of diverse mechanisms usage can increase, depending on conditions, the significance of each mechanism in determination of their toxic action. Despite the fact that lead and cadmium toxic action symptoms differ in many organs, numerous studies suggest that endothelium is the first and the main target of that toxic action. A high rate of glutamine uptake is characteristic of rapidly dividing cells such as enterocytes, macrophages and fibroblasts (13) and that can be the explanation of its supplementation need. Although glutamine diet administration did not influence magnesium and calcium levels in the examined skins, its action may have been seen in other, non-determined in our paper parameters. Lead and cadmium probably do not influence lipid peroxidation indirectly, but they act pro-oxidatively causing free oxygen radicals' generation. Trace elements (Zn, Cu, Se) homeostasis disorders caused indirectly by cadmium and lead can inhibit those element--dependent antioxidant enzymes (glutathione peroxidase – GPx, glutathione reductase, superoxide dismutase – SOD) actions. Lack of cadmium ions influence on zinc and copper levels in skins has been noticed in spite of its frequent appearance in literature (especially related with copper). That fact is not explained till now and is quite controversial. Lead ions caused a decrease in zinc and copper levels are compensated by bioflavonoids and glutamine enriched diet. Glutamine is glutathione precursor. Glutathione can act as metals chelating factor as well as inhibiting lipid peroxidation agent and due to it has an indirect influence on diverse metals detoxification (1, 8). Many pharmacological results of bioflavonoids action are enzymes co-operation effects that can cause free radicals removal. They can also co-work with glutathione. Bioflavonoid antioxidant activity is connected with the number and location of hydroxyl groups (10), which are able to chelate transition metals (Zn, Cu) and magnesium ions (14). The mechanism of bioflavonoids' influence on heavy metals like lead or cadmium is still unknown, and it requires further investigation.

CONCLUSIONS

1. Heavy metals intoxication caused significant decrease in calcium and magnesium levels in the examined skins, and bioflavonoids with glutamine diet addition did not change these two elements concentrations.

2. Lead in contrast to cadmium decreased zinc and copper levels in the examined skins, and bioflavonoids with glutamine caused their return into control group values.

3. Individual bioelements concentrations changes did not depend on the type of administrated bioflavonoid.

4. Interdependence between bioflavonoids supplementation, glutamine administration and essential elements concentration changes in skins of heavy metals intoxicated rats is poorly documented in available literature and still requires further researches.

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SUMMARY

The effect of bioflavonoids on the Mg, Ca, Cu and Zn concentration in rats' skin intoxicated with heavy metals Pb or Cd. The animals were divided at random into nine groups, each of 6 rats. Group I obtained in drinking water the solution of plumbum nitrate, $Pb(NO_3)_2$ in concentration of 500 mg Pb/

dm^3, group II solution of lead ions – 500 mg Pb/dm^3 and quercetin – 200 mg/dm^3 and glutamine 4 g/dm^3, group III – lead ions solution (500 mg Pb/dm^3), catechin – 200 mg/dm^3 and glutamine 4 g/dm^3, group IV received water solution of $Pb(NO_3)_2$ – 500mg Pb/dm^3, naringenin – 200 mg/dm^3 and glutamine 4 g/dm^3, V–VII groups obtained the same that groups I–IV, but instead of lead cadmium chloride ($CdCl_2$) in the amount of 500 mg Cd/dm^3 was used. Group IX was a control group, and these animals received redistilled water to drink. All the groups of rats were on a normal diet (LSM dry food) and they got solutions and food *ad libitum*. After the experiment, the animals were sacrificed under ketamine narcosis and while collecting tissues, the skins (together with furs) used for further studies were also obtained. Heavy metals intoxication caused a significant decrease of magnesium and calcium concentrations in the examined rats' skins, and bioflavonoids with glutamine dietary addition did not improve or improve to a small degree the levels of those elements. Lead, in opposition to cadmium, decreased zinc and copper levels in skins, and bioflavonoids with glutamine caused the return of those elements' concentrations to the values of control group.

Bioflawonoidy i glutamina a stężenia Mg, Ca, Cu, Zn w skórach szczurów intoksykowanych metalami ciężkimi

Przeprowadzono badania wpływu bioflawonoidów na stężenie Mg, Ca, Cu i Zn w skórach szczurów intoksykowanych metalami ciężkimi Pb lub Cd. Do doświadczenia użyto dwumiesięcznych szczurów samców rasy Wistar, podzielonych losowo na 9 grup, każda po 6 szczurów. Grupa I otrzymywała do picia wodny roztwór azotanu ołowiu, $Pb(NO_3)_2$ o stężeniu 500 mg Pb/dm^3, grupa II roztwór jonów ołowiu – 500 mg Pb/dm^3, kwercetynę – 200 mg/dm^3 i glutaminę – 4g/dm^3, grupa III roztwór jonów ołowiu – 500 mg Pb/dm^3, katechinę – 200 mg/dm^3 i glutaminę – 4g/dm^3, grupa IV wodny roztwór $Pb(NO_3)_2$ – 500mg Pb/dm^3, naringeninę – 200mg/dm^3 i glutaminę – 4g/dm^3, grupy V –VIII otrzymywały zamiast ołowiu kadm – roztwór chlorku kadmu, $CdCl_2$ – 500mg Cd/dm^3. Grupa IX była kontrolną, a zwierzęta otrzymywały do picia wodę redestylowaną. Wszystkie zwierzęta karmiono standardową, granulowaną paszą (LSM). Pasza i płyny były podawane zwierzętom *ad libitum*. Po zakończeniu doświadczenia zwierzęta usypiano podawaną dootrzewnowo ketaminą i przy okazji pobierania narządów wewnętrznych uzyskiwano skóry (wraz z sierścią), które stanowiły materiał do dalszych badań. Intoksykacja metalami ciężkimi powodowała istotne obniżenie stężenia jonów magnezu i wapnia w badanych skórach szczurów, a dodatek do diety bioflawonoidów z glutaminą nie poprawiał lub poprawiał tylko nieznacznie stężenie tych biopierwiastków. Jony ołowiu w przeciwieństwie do kadmu obniżały poziom cynku i miedzi w badanych skórach, a bioflawonoidy z glutaminą powodowały powrót stężeń obu pierwiastków do wartości grupy kontrolnej.