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Original Research

Effect of Cadmium on the Peripheral Kynurenine Pathway in Rats

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Abstract

This study investigated the kynurenine metabolism in rats treated with cadmium. We used an animal model at the levels of Cd corresponding to human environmental and occupational exposure to this metal which allows the assessment of its early effect on the structure and function of kidneys. We observed significant decrease in the serum concentration of tryptophan (TRP) and its metabolites: kynurenine (KYN), kynurenic acid (KYNA), and 3-hydroxykynurenine (3-HKYN), which was accompanied by a decrease in KYN derivatives in kidney and liver tissues. This effect was dependent on the level of Cd exposure. Regression analysis showed negative correlations between blood concentrations of Cd and TRP derivatives in serum, kidney and liver tissues.

Conversely, the urinary concentration of KYN and KYNA increased. Changes in product degradation of TRP after Cd treatment were proportional to the severity of renal damage and correlated with the concentration of proximal tubular injury marker — urinary isoenzyme B of N-acetyl-D-glucosaminidase (NAG-B).

Our results seem to indicate that intoxication with Cd induced significant disturbances in the peripheral kynurenine pathway.

Keywords: cadmium, kidney, liver, tryptophan, kynurenine, 3-hydroxykynurenine, kynurenic acid

Introduction

Kynurenine (KYN) — the major intermediate product of tryptophan (TRP) metabolism — can be metabolized to a number of substances depending on the extent for KYN pathway enzyme expression in a particular tissue or cell type (Fig. 1). The family of KYN metabolites includes compounds that have been identified as essential cofactors, neurotransmitter agonists or antagonists, neurotoxins, immunomodulators, antioxidants and carcinogens [1-7].

The kidneys have a dual influence on TRP metabolism. Firstly, they constitute a major method for its derivative elimination, most under the form of KYN, 3-hydroxykynurenine (3-HKYN), kynurenic acid (KYNA), xanthurenic acid (XA) and quinolinic acid (QA). Secondly, they possess a complex enzymatic system taking part in KYN metabolism [8, 9].

One of the first health effects of chronic environmental as well as occupational exposure to cadmium (Cd) is an injury of renal proximal tubules, which usually starts insidiously and is irreversible [10, 11]. The kidney has been considered the critical organ for Cd toxicity following long-term exposure in humans or experimental animals [12].

Previously we noted disturbances in tryptophan metabolism under Cd exposure [13, 14]. Moreover, literature data and our observations show the relationship between kidney function and tryptophan (TRP) metabolism [15-17].

In the present study we aimed to evaluate peripheral metabolism of KYN and its derivatives in rats exposed to Cd. The concentration of TRP, KYN, 3-HKYN and KYNA was measured in serum, kidney, liver and urine of animals and correlated with the concentration of renal

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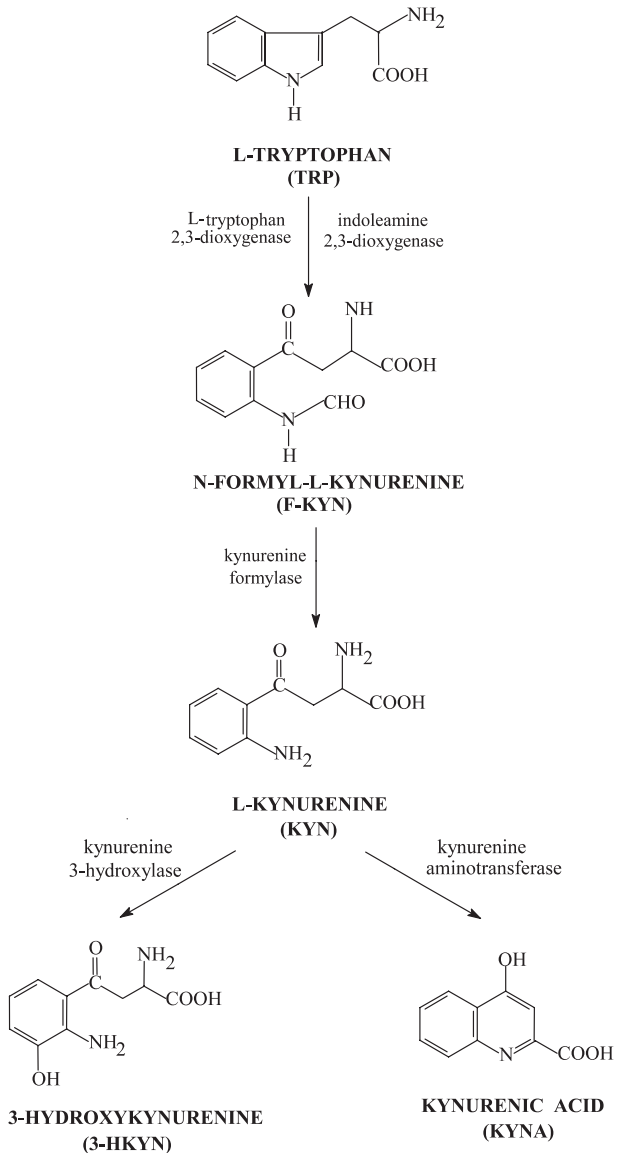

Fig. 1 Scheme of kynurenine pathway.

insufficiency marker — urinary isoenzyme B of N-acetyl-β-D-glucosaminidase (NAG-B).

Materials and Methods

Experimental Protocol

The study was carried out on Wistar rats of initial body weight 180-200 g. All animals were kept under standard laboratory conditions and had unlimited access to standard diet (the LSM diet, Motycz, Poland) and drinking water (redistilled water or water solutions of $CdCl_2$). Two groups received a water solution of $CdCl_2$ at the concentration of 5 or 50 mg Cd/l as the only drink. Control rats drank redistilled water (uncontaminated with Cd). To assess Cd intake, 24-hour consumption of drinking water was measured for the whole course of the experiment. Animals from each group, after 24-hour urine collection in metabolic cages and overnight fasting, were euthanized with Vetbutal (pentobarbitalum 40 mg/kg b. w., i. p.) after 6, 12 and 24 weeks of the experiment. Blood from the heart (in anticoagulant and clotted) and kidneys and liver were collected. The kidneys and liver were washed in cold physiological saline (0.9% NaCl), weighed and cut into a few parts assigned to Cd analysis and biochemical studies (TRP, KYN, KYNA, 3-HKYN).

In the urine the following determinations were done: Cd, NAG-B, KYN and KYNA.

Procedures involving the animals and their care conformed with the institutional guidelines, in compliance with national and international laws and Guidelines for the Use of Animals in Biomedical Research (Giles [18]).

Chemicals

For chemical and biochemical examinations, ultra pure water received from water purification Milli-Q system (Millipore Corporation, USA) was used. All reagents and chemicals were of analytical grade or highest purity.

Measurement of Cd Concentration

Blood collected in heparinized tubes was wet-digested with 5% HNO_3. Slices of kidney and liver were dry mineralized in an electric oven. The ash was dissolved in 1M HNO_3. If necessary, the mineralizates of blood and kidneys were appropriately diluted with 5% and 1M HNO_3, respectively. Cd concentration in urine, after appropriate dilution with ultra pure water, was determined using the flameless atomic absorption spectrometry method (Atomic Absorption Spectrophotometer Z-5000, Hitachi, Japan) with electrothermal atomization in a graphite cuvette. The cathode lamp of Cd (Photron) was operated under standard conditions using its respective resonance line of 228.8 nm. The detection limit was 0.08 μg Cd/l. For Cd calibration a stock atomic absorption standard solution (Sigma, USA) was used.

NAG-B Activity

The activity of NAG-B in urine was determined colorimetrically according to Zwierz et al. [19], using p-nitrophenyl-N-acetyl-β-D-glucosamide as substrate (Sigma, USA).

Determination of TRP and KYNA

Tryptophan and kynurenic acid concentrations were determined according to Herve et al. [20]. The reversed-phase HPLC system consisted of a Waters Sherisorb S3 ODS2 150x2.1 mm column (USA), HP 1050 series pump (Germany), Rheodyne injection valve fitted with

a sample loop (5 µl). The column effluent was monitored by using a programmable fluorescence detector HP 1046A (Germany). The optimized conditions were determined by recording fluorescence spectra with a stop-flow technique. Excitation and emission wavelengths were set at 254/404 nm for TRP and KYNA. The output of the detector was connected to a single LC-2D ChemStation instrument (Germany). The mobile phase was pumped at a flow-rate of 0.25 ml/min consisted of 50 mM acetic acid, 0.25 M zinc acetate (pH-4.9), containing 1.2% of acetonitrile. Chromatography was carried out at 25°C.

Determination of KYN Concentration

KYN concentration was determined by high-performance liquid chromatography (HPLC) according to Holms [21]. The chromatographic system (Hewlett-Packard, Germany) included an HP1050 pump and Rheodyne injection valve fitted with a sample loop (20 µl). Guard column — LiChrospher 100 RP-18. 5 µm, 4x4 mm (Germany) was placed before the C18 reversed-phase column (LiChrospher 100 RP — 18.5 µm, 125x4 mm). The column effluent was monitored with HP 1050 series UV detector (365 nm). The output of the detector was connected to a single instrument LC-2D ChemStation (Germany). The mobile phase was composed of 0.1 M acetic acid, 0.1M ammonium acetate (pH 4.65) containing 2% of acetonitrile and it was pumped at a flow-rate of 1.5 ml/min. Chromatography was carried out at 25°C.

Determination of 3-HKYN

3-hydroxykynurenine was measured using HPLC technique as described by Heyes [22]. The reversed-phase HPLC system consisted of a Waters Sherisorb S3 ODS2 150x2.1 mm column (USA), HP 1050 series pump (Germany), Rheodyne injection valve fitted with a sample loop (5 µl). The column effluent was monitored by using a programmable electrochemical detector HP 1049A (Germany). Potential of the working electrode was 0.6 V. The output of the detector was connected to a single LC-2D ChemStation instrument (Germany). The mobile phase was pumped at a flow-rate of 0.25 ml/min, consisting of 0.1 M triethylamine, 0.1 M phosphoric acid, 0.3 mM EDTA, 8.2 mM heptane-1-sulfonic acid sodium salt, containing 2% of acetonitrile. Chromatography was carried out at 25°C.

Creatinine

The concentration of creatinine in urine was determined colorimetrically using diagnostic laboratory test (POCh, Poland). Urinary creatinine concentration was used to normalize Cd, KYN and KYNA concentrations.

Statistical Analysis

The values are expressed as the mean SD; n — represents the number of results. Multiple groups' comparisons were performed by one-way analysis of variance, and differences between groups were estimated using the Tukey-Kramer test. P value less than 0.05 was considered statistically significant.

Results

Body Weight Gain and Food Consumption

During the experiment an increase in body weight of all the rats was noted. The rats treated with 5 mg Cd/l reached the weights of the control. Exposure to 50 mg Cd/l resulted in a reduction in body weight gain after 6 weeks (by 6.3%, $p<0.05$) and 12 weeks (by 21.4%, $p<0.01$), but the effect was no further observed after 24 weeks of the experiment. In 24 week of study the food intake in control group received 48.9 12.7 g/day and marked decrease to 39.3 16.2 g/day (NS) in rats treated with 5 mg Cd/l or 30.6 9.9 g/day ($p<0.05$) after 50 mg Cd/l.

Effect of Cadmium on Tryptophan and Its Metabolite Concentrations in the Serum

Exposure to 5 and 50 mg Cd/l for 6, 12 and 24 weeks resulted in a marked increase in blood Cd concentration (Table 1). In contrast to Cd, TRP and all its metabolites (KYN, KYNA and 3-HKYN) decreased in serum after Cd exposure. This effect of Cd was dose-dependent. Multiple regression analysis showed negative correlations between blood concentrations of Cd and TRP derivatives in serum: TRP ($r = -0.561$, $p<0.01$), KYN ($r = -0.436$, $p<0.05$), KYNA ($r = -0.557$, $p<0.001$) and 3-HKYN ($r = -0.429$, $p<0.05$).

Effect of Cadmium on Tryptophan and Its Metabolites Concentration in the Kidney

The administration of Cd resulted in a marked dose-dependent increase in renal Cd concentration (Table 2). In turn, TRP, KYN, KYNA and 3-HKYN tissue concentrations decreased significantly in comparison to the control group. Correlation coefficients were noted between Cd concentration in the blood and kidney TRP metabolite concentrations: TRP ($r = -0.632$, $p<0.01$), KYN ($r = -0.421$, $p<0.05$), KYNA ($r = -0.720$, $p<0.001$) and 3-HKYN ($r = -0.483$, $p<0.01$).

Effect of Cadmium on Tryptophan and Its Metabolite Concentrations in the Liver

Exposure to Cd resulted in a marked dose-dependent increase in liver Cd concentration (Table 3). In turn, TRP,

Table 1. Effect of cadmium on tryptophan and its metabolite concentrations in the serum.

	control	Cd 5	Cd 50
6 weeks of Cd exposure			
Cd [μg/l] #	0.61 ± 0.22	2.25 ± 0.65 *	14.42 ± 5.57 *** °°°
TRP[μM]	53.64 ± 8.21	39.24 ±10.34	28.78 ± 9.14 **
KYN [μM]	1.93 ± 0.84	2.06 ± 0.73	1.23 ± 0.55
KYNA [nM]	67.28 ±13.62	43.97 ±16.24 *	45.80 ±11.91 *
3-HKYN [nM]	54.84 ±10.36	58.94 ±11.64	42.14 ± 5.83 *
12 weeks of Cd exposure			
Cd [μg/l] #	0.75 ± 0.32	2.15 ± 0.57 *	15.92 ± 3.97 *** °°°
TRP [μM]	63.74 ±17.2	33.14 ± 11.0 7 **	26.50 ± 9.43 **
KYN [μM]	2.15 ± 0.54	1.71 ± 0.45	1.39 ± 0.58 *
KYNA [nM]	60.81 ± 8.40	51.01 ±13.27	47.64 ± 7.71 **
3-HKYN [nM]	61.58 ±10.24	46.37 ±12.87	38.20 ± 9.83 **
24 weeks of Cd exposure			
Cd [μg/l] #	0.84 ± 0.22	2.08 ± 0.49	16.77 ± 4.15 *** °°°
TRP [μM]	57.24 ±10.60	34.87 ±12.88 **	23.84 ± 7.76 ***
KYN [μM]	2.34 ± 0.75	1.66 ± 0.85	1.44 ± 0.51 *
KYNA [nM]	79.34 ±18.67	55.10 ±14.25 *	42.14 ± 13.61 **
3-HKYN [nM]	53.66 ± 6.37	40.18 ±11.21 *	39.67 ± 8.40 *

Values are presented as mean ±SD of 6 animals. #Cd concentration in the blood. Multiple group comparisons were performed by one-way analysis of variance — ANOVA, for homogeneity of variances Bartelett's test was used, and significant intergroup differences were assessed by Tukey-Kramer test. Comparison with the control group:*p<0.05, **p<0.01, ***p<0.001, comparison with rats exposed to Cd 5 mg/kg: °°°p<0.001

Table 2. Effect of cadmium on tryptophan and its metabolite concentrations in the kidney.

	control	Cd 5	Cd 50
6 weeks of Cd exposure			
Cd [μg/g]	0.06 ± 0.02	2.23 ± 0.61 **	22.83 ± 6.15 *** °°°
TRP [nmol/g]	98.33 ± 27.99	77.83 ±15.04	61.00 ±15.58 *
KYN [nmol/g]	2.87 ± 0.55	2.57 ± 0.65	1.53 ± 0.67 *
KYNA [pmol/g]	562.00 ± 61.03	379.50 ±130.44 *	327.50 ± 81.24 *
3-HKYN [pmol/g]	328.83 ± 71.66	298.50 ± 63.41	336.67 ± 69.47
12 weeks of Cd exposure			
Cd [μg/g]	0.05 ± 0.01	4.01 ± 1.92 ***	39.11 ± 5.52 *** °°°
TRP [nmol/g]	93.17 ± 12.92	72.50 ± 21.23	56.00 ±16.77 *
KYN [nmol/g]	2.68 ± 0.55	2.65 ± 0.40	1.58 ± 0.67
KYNA [pmol/g]	489.17 ± 96.12	347.67 ± 86.83	283.50 ± 64.51 *
3-HKYN [pmol/g]	255.67 ± 41.83	285.00 ± 81.06	177.33 ± 58.20
24 weeks of Cd exposure			
Cd [μg/g]	0.04 ± 0.01	9.82 ± 2.66 ***	60.77 ± 8.43 *** °°°
TRP [nmol/g]	90.50 ± 16.38	50.17 ±15.05 *	53.17 ± 14.33 **
KYN [nmol/g]	3.00 ± 0.49	2.27 ± 0.78	1.62 ± 0.67 **
KYNA [pmol/g]	561.17 ± 119.11	369.00 ±111.27 *	238.33 ± 53.53 **
3-HKYN [pmol/g]	276.00 ± 36.08	181.00 ± 29.83	165.67 ± 40.47 *

Values are presented as means ±SD of 6 animals. Multiple group comparisons were performed by one-way analysis of variance — ANOVA, for homogeneity of variances Bartelett's test was used, and significant intergroup differences were assessed by Tukey-Kramer test. Comparison with the control group:*p<0.05, **p<0.01, ***p<0.001, comparison with rats exposed to Cd 5 mg/kg: °°°p<0.001

Table 3. Effect of cadmium on tryptophan and its metabolite concentrations in the liver.

	control	Cd 5	Cd 50
6 weeks of Cd exposure			
Cd [µg/g]	0.027 ± 0.006	0.432 ± 0.056 **	8.895 ± 0.553 *** °°°
TRP [nmol/g]	40.83 ± 11.08	32.65 ±7.24	24.69 ± 8.25 *
KYN [nmol/g]	2.11 ± 0.35	2.07 ± 0.42	0.99 ± 0.47 * °
KYNA [pmol/g]	45.11 ± 8.26	22.29 ±14.57 *	20.09 ± 9.33 *
3-HKYN [pmol/g]	242.68 ± 28.15	239.86 ± 35.20	209.17 ± 41.29
12 weeks of Cd exposure			
Cd [µg/g]	0.034 ± 0.005	1.080 ± 0.331 **	23.805 ± 2.896 *** °°°
TRP [nmol/g]	42.89 ± 9.11	35.97 ± 7.25	28.14 ± 9.66
KYN [nmol/g]	2.47 ± 0.39	2 .011 ± 0.26	1.53 ± 0.31 **
KYNA [pmol/g]	53.58 ± 9.14	28.91 ± 7.13 *	22.65 ± 6.73 *
3-HKYN [pmol/g]	253.21 ± 38.64	262.94 ± 53.77	255.98 ± 48.34
24 weeks of Cd exposure			
Cd [µg/g]	0.041 ± 0.007	2.457 ± 0.197 **	28.806 ± 2.178 *** °°°
TRP [nmol/g]	39.12 ± 8.56	26.78 ±8.99 *	20.13 ±9.21 **
KYN [nmol/g]	2.06 ± 0.44	1.97 ± 0.56	0.72 ± 0.36 ** °
KYNA [pmol/g]	41.17 ± 7.50	27.24 ±6.95 *	19.14 ± 7.78 ** °
3-HKYN [pmol/g]	237.86 ± 31.42	218.55 ± 41.78	277.66 ± 75.48

Values are presented as means ±SD of 6 animals. Multiple group comparisons were performed by one-way analysis of variance — ANOVA, for homogeneity of variances Bartelett's test was used, and significant intergroup differences were assessed by Tukey-Kramer test. Comparison with the control group:*p<0.05, **p<0.01, ***p<0.001, comparison with rats exposed to Cd 5 mg/kg: °p<0.05, °°°p<0.001

Table 4. Effect of cadmium on urinary excrection of kynurenine, kynurenic acid [mol/mmol creatinine] and NAG-B.

	Control	Cd 5	Cd 50
6 weeks of Cd exposure			
Cd [µg/l]	1.63 ± 0.33	6.22 ± 1.23 ***	17.52 ± 2.11 *** °°°
NAG-B [IU/l]	1.92 ± 0.44	2.70 ± 0.68	6.33 ± 1.01 *** °°°
KYN	6.02 ± 2.61	11.07 ± 3.02	14.84 ± 6.06
KYNA	11.17 ± 3.78	16.08 ± 3.01	22.86 ± 5.17 ** °
12 weeks of Cd exposure			
Cd [µg]	1.50 ± 0.39	5.63 ± 2.03 *	19.50 ± 4.22 *** °°°
NAG-B [IU/l]	1.52 ± 0.36	3.99 ± 0.87 *	6.49 ± 1.24 *** °°
KYN	9.34 ± 4.53	15.72 ± 5.54	23.74 ± 10.18 *
KYNA	13.62 ± 6.45	19.83 ± 6.57	28.31 ± 7.75 *
24 weeks of Cd exposure			
Cd [µg]	1.25 ± 0.37	7.52 ± 2.99 **	20.43 ± 4.20 *** °°°
NAG-B [IU/l]	1.65 ± 0.32	4.27 ± 1.13 ***	6.51 ± 1.74 *** °°
KYN	8.09 ± 2.91	16.01 ± 7.47	18.10 ± 8.27 *
KYNA	14.84 ± 4.81	22.04 ± 6.45 *	25.91 ± 5.23 ** °

Values are presented as means ±SD of 6 animals. Multiple group comparisons were performed by one-way analysis of variance — ANOVA, for homogeneity of variances Bartelett's test was used, and significant intergroup differences were assessed by Tukey-Kramer test. Comparison with the control group: *p<0.05, **p<0.01, ***p<0.001, comparison with rats exposed to Cd 5 mg/kg: °p<0.05, °°p<0.01, °°°p<0.001

KYN and KYNA and tissue concentrations decreased significantly in comparison to the control group. Correlation coefficients were noted between Cd concentrations in blood and liver TRP metabolite concentrations: TRP ($r = -0.496$, $p<0.05$), KYN ($r = -0.423$, $p<0.05$), KYNA ($r = -0.552$, $p<0.01$). 3-HKYN concentrations in liver were not altered in rats exposed to Cd in comparison to the control group.

Effect of Cadmium on Urinary Excrection of Kynurenine, Kynurenic Acid and N-acetyl-β-d-glucosaminidase

In rats exposed to 5 mg Cd/l we observed an increase in urinary Cd excretion and NAG-B activity (Table 3). This effect of Cd was considerably intensified in the following weeks of the exposure. Correlation coefficient was noted between Cd excretion and NAG-B activity in the urine ($r=0.662$, $p<0.001$). We also observed a small increase in the urinary elimination of KYN after exposure to Cd. Regression analysis revealed a correlation between Cd and KYN concentrations in urine ($r=0.662$, $p<0.01$) and between NAG-B and KYN excretion ($r=0.410$, $p<0.01$). The administration of Cd resulted in a marked dose-dependent increase in KYNA urinary elimination. A linear correlation between urinary Cd concentration and KYNA excretion ($r=0.432$, $p<0.01$), as well as between NAG-B activity and KYNA elimination ($r=0.534$, $p<0.01$), was noted.

Discussion

We have to create an experimental model using rats chronically treated with Cd at relatively low and high levels in which we have assessed the effect of this metal on TRP metabolism via kynurenine pathways.

Cd injures the whole kidney but the main tubule (proximal convoluted tubule and straight tubule) is the critical site for its action [10, 11]. We observed a significant decrease in the serum concentration of TRP and its metabolites: KYN, KYNA, 3-HKYN, which was accompanied by a decrease in KYN derivatives in kidney and liver tissues. This effect of Cd was dose-dependent. Statistical analysis showed negative correlations between blood concentrations of Cd and TRP derivatives in serum and kidney or liver tissues (with the exception of 3-HKYN).

Conversely, the urinary concentration of KYN and KYNA increased. The changes of in product degradation of TRP after Cd treatment were proportional to the severity of renal damage and correlated with the concentration of proximal tubular injury marker — urinary isoenzyme B of N-acetyl- -D-glucosaminidase (NAG-B).

KYN is the main product of TRP degradation in peripheral tissues, which is further converted in a series of metabolites, among others to KYNA or 3-HKYN. Renal excretion is the main route of KYN and KYNA elimination [23, 24]. In addition, the kidney is able to uptake KYN from the blood and excretes it in the form of KYNA [23]. Thus, the impairment of kidney function is likely to be associated with changes in the urinary excretion of both metabolites. Abnormalities in KYN and KYNA excretion have been recently reported in humans and rats with chronic renal insufficiency by Pawlak et al. [15, 16, 25, 26].

The plasma concentration of KYN and its metabolites depends on TRP supply with food, the activity of specific enzymes as well as its urinary elimination [24]. The animals had permanent, unlimited access to the granulated food and tap water. In the group exposed to Cd a decrease in the quantity of consumed fodder amounts was observed. According to Holmes et al. [21, 23], the decrease in TRP concentration in renal insufficiency is multifactorial, including diminished absorption of food TRP, transformation of TRP in bowel epithelium to other indoles competing for binding to proteins, glomerular hyperfiltration typical for initial uremia, or diminished reabsorption of the amino acid in renal tubules. It is also possible that, if dietary TRP intake was partially reduced, this case together with the factors mentioned above, would decrease the plasma concentrations of this amino acid and its derivatives. Simultaneously, in rats exposed to Cd we observed an increase in the urinary elimination of KYN. This effect was intensified in the following weeks of exposure. Regression analysis revealed a correlation between Cd and KYN concentration in urine and between NAG-B and KYN excretion. The administration of Cd resulted in a marked dose-dependent increase in KYNA urinary elimination. A linear correlation between urinary Cd concentration and KYNA excretion as well as between NAG-B activity and KYNA elimination was observed.

Thus, it can be concluded that intoxication with Cd induced significant disturbances in peripheral kynurenine pathway, which resulted in the decrease in serum, kidney and liver concentrations of TRP metabolites, and a simultaneous increase in the urinary elimination of KYN and KYNA. Probably, the impairment of kynurenine metabolism is multifactoral, which can be a result of the renal damage induced by the cadmium, partially a consequence of decrease the tryptophan supply with food, and other factors which could influence the activity of kynurenine pathway enzymes. However, further studies involving human subjects exposed to Cd environmentally and occupationally are required to assess the effect of Cd on TRP turnover in organisms.

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