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The influence of ascorbic acid on selected parameters of cell immunity in guinea pigs exposed to cadmium

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Einfluß von Ascorbinsäure auf ausgewählte Parameter der zellvermittelten Immunität bei cadmiumexponierten Meerschweinchen

Summary: The study investigated the possibility of influencing immunotoxic effects of Cd through ascorbic acid. Guinea pigs with high and low intake of ascorbic acid were perorally exposed to cadmium chloride (1 mg Cd/animal/day).

The daily vitamin C intake was 2 and 100 mg per animal, respectively. Phagocytic activity of polymorphonuclear leucocytes and monocytes as well as the percentage of active and total T lymphocytes in peripheral blood of animals were evaluated.

Five- and 12-week experiments showed a mutual potentiation of negative effects of Cd on the immune system by suboptimal intake of ascorbic acid. Toxic effects of Cd on the immune system can be reduced by a sufficient intake of vitamin C.

Zusammenfassung: Es wurde der Einfluß von Ascorbinsäure auf die immunotoxischen Wirkungen von Cadmium untersucht.

Cadmiumchlorid-exponierte Meerschweinchen (1 mg Cadmium/Tier/Tag) mit hoher (100 mg/Tier) und niedriger (2 mg/Tier) Ascorbinsäurezufuhr wurden auf die phagozytäre Aktivität der polymorphkernigen Leukozyten und Monozyten sowie auf die prozentualen Anteile der aktiven und gesamten T-Lymphozyten des peripheren Blutes untersucht.

Die 5 und 12 Wochen durchgeführten Versuche zeigten, daß Cadmium bei niedriger Ascorbinsäurezufuhr eine negative Wirkung auf das Immunitätsystem hat. Diese toxische Wirkung von Cadmium auf das Immunitätsystem kann durch ausreichende Ascorbinsäurezufuhr verringert werden.

Key words: Cadmium – immunotoxicity – ascorbic acid – guinea pig

Schlüsselwörter: Cadmium – Immuntoxizität – Ascorbinsäure – Meerschweinchen

Introduction

Both deteriorating ecological situation and disproportions in nutritional habits which are acquired and stabilized at a very young age contribute to the alarming increase of cardiovascular and oncological diseases. One of the risk factors whose incidence in our environment has dangerously grown is cadmium (Cd).

We chose Cd as the xenobiotic for a laboratory model of a situation in which disproportions in nutrition – an insufficient intake of a protective antioxidant in food – act together with the environment polluted with a toxic xenobiotic. The guinea pig was chosen as an experimental model which allows extrapolation of the experimental results to man. Guinea pigs, like man, do not synthesize endogenous ascorbic acid.

Vitamin C was chosen as a model antioxidant with respect to the contemporary nutritional situation of the Middle and East European population: a chronic latent vitamin C deficiency can be considered the main limiting nutritional factor (7).

Cadmium is a frequent environmental contaminant of food, water and air in industrialized areas and induces a wide variety of toxic manifestations. Cigarette smoke, too, constitutes an important exposure route. The Cd inhaled by smokers exceeds 1 μg/day. According to the WHO, a tolerated weekly intake of Cd is 0.4–0.5 mg/person (23).

Cd metabolism is an example of an extreme accumulation of chemical injurant in the human organism. The highest accumulation occurs in kidneys and liver, the biological half-time of Cd in these organs being approximately 20–30 years. Cd taken up and accumulated in the target organ is mostly bound to an inducible low molecular weight protein, metallothionein (MT), and the metal sequestered as MT is known to be non-toxic in the cell (5). Therefore, Cd not bound to MT in the cell has been implicated as the toxic form and is called free Cd ions (active or toxic form of Cd) (18).

The Cd absorption depends on the composition of the diet. The interaction between Zn and Cd is known. A lower intake of Zn can increase the absorption of Cd.

Materials and methods

Design of experiment

Male guinea pigs (Velaz Praha) with an initial body weight of 350–450 g were housed in polycarbonate cages in groups of four per cage. They were given food and water ad libitum. An ambient temperature of 22±2 °C, relative humidity of 50±5 % and 12-h light/dark cycle were provided. Guinea pigs were allowed to adapt to these conditions for 2 weeks and over this period they were fed standard laboratory diet (MOK, Velaz Praha). The control group continued to receive standard laboratory diet. Four groups were fed an experimental diet (ED). Diet simulating nutritional disorders commonly appearing in economically developed nations (high content of saturated fatty acids and natrium chloride, low content of dietary fiber and antioxidants). The experimental diet lacked vitamin C, but contained higher levels of saturated fatty acids, saccharose and salt (49 % oat flakes, 30 % dried milk, 1 % sodium chloride, 10 % butter, 10 % sugar).

The animals were divided into five groups, each with eight guinea pigs:

1) group – control, the animals were fed a conventional pelletized complete laboratory diet with vegetables;
2) group – experimental diet (ED) + low intake of vitamin C;
3) group – ED + high intake of vitamin C;
4) group – ED + low intake of vitamin C (2 mg/day + cadmium);
5) group – ED + high intake of vitamin C (100 mg/day + cadmium).

Two groups of animals drank water with low or high content of ascorbic acid. An actual intake of ascorbic acid and cadmium was calculated from the volume of drinking water daily consumed and from the decline of ascorbic acid concentration due to oxidation determined by the dinitrophenylhydrazine method (17). The intake of ascorbic acid was 2 mg and 100 mg/animal/day, respectively. Exposed groups drank water with cadmium chloride (10 mg Cd/l). The intake of cadmium was 1 mg/animal/day.

Animals were weighed each week. After 5 and 12 weeks, respectively, they were sacrificed by exsanguination 17 h after the removal of food.

Immune function assays

Phagocytosis: Phagocytic activity of polymorphonuclear cells was assayed by the method of Fornusek (4). We used the uptake of metacrylate beads (particles) by poly-

morphonuclears to monitor their phagocytic activity. Synthetic polymeric hydrophilic particles are based on 2-hydroxyethyl methacrylate (UVVVR Praha, ČSFR).

0.1 ml heparinized peripheral blood (5 units of heparin/ml) of the sacrificed guinea pig was mixed with 0.05 ml solution of particles in phosphate buffered saline and incubated for 60 min at 37 ° C in a water bath. Blood smears were made and the slides were air-dried and stained by panoptic staining of Pappenheim. The number of phagocytic polymorphonuclears and monocytes with 3 and more particles was determined by examining 200 cells.

Active and total T lymphocytes (LY): We used a basic method for staining human lymphocytes (22) and modified this method for guinea pigs. T lymphocytes of these animals could be identified by their ability to form rosettes with rabbit erythrocytes (ERY). Leucocytes were isolated from the peripheral heparinized blood (5 units/ml) diluted by Hanks solution (ICN) and by centrifugation over a Verografin (Spofa, ČSFR) gradient at 400 g for 45 min. Cells were washed twice and resuspended in Hanks solution. Active T lymphocytes have surface receptors with a high avidity for erythrocytes. They form rosettes in the presence of a small quantity of erythrocytes (rate LY:ERY 1:8), in the course of 1 h incubation at 37 ° C. Active T lymphocytes constitute ca. 26–35 % of total lymphocytes. Total T lymphocytes form rosettes with erythrocytes (rate LY:ERY 1:40) in the course of 18 h incubation at 4 ° C. Total T lymphocytes constitute ca. 55–75 % of all lymphocytes.

Statistical analysis: The data are reported as the mean values and standard deviations of the mean. Differences between control and experimental groups were evaluated using Student's *t*-test and Wilcoxon's test.

Results

The weight of animals in the groups not exposed to Cd increased during the whole experiment. In the groups exposed to Cd the weight showed a tendency to increase up to the fifth week. Then, it remained approximately constant or decreased slightly so that at the end of the experiment the weight of animals in the Cd-treated groups was significantly lower than in the control groups (Fig. 1).

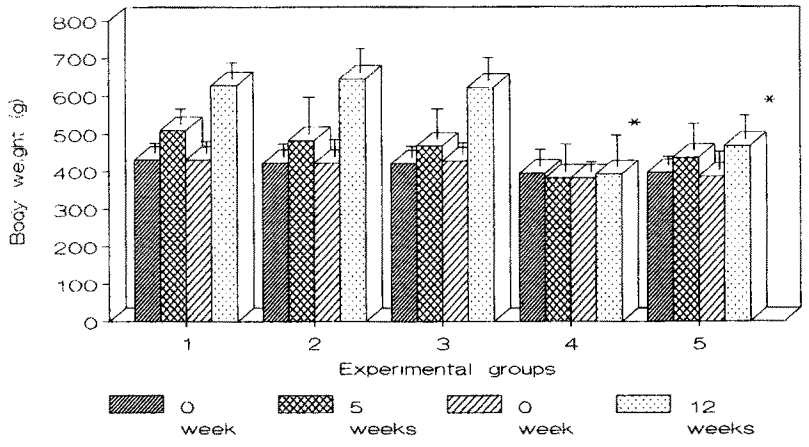

Fig. 1. Body weight of the guinea pigs (Groups 1–5 see footnote in Table 1)
* – $p < 0.05$

Table 1. Relative liver and spleen weight of guinea pigs

Exp. group	Relative liver weight (% of b.w.) 5 weeks mean	SD	12 weeks mean	SD	Relative spleen weight (% of b.w.) 5 weeks mean	SD	12 weeks mean	SD
1	3.35	0.30	3.09	0.40	0.17	0.03	0.16	0.03
2	3.05	0.40	3.29	0.30	0.20	0.05	0.15	0.03
3	3.37	0.30	3.22	0.10	0.20	0.04	0.14	0.02
4	3.72	1.00	5.28*	0.10	0.22	0.10	0.36*	0.15
5	3.15	0.30	3.44	0.30	0.17	0.05	0.26	0.17

Group 1 – complete laboratory diet + vegetables,
Group 2 – experimental diet (ED) + 2 mg vitamin C/animal/day,
Group 3 – ED + 100 mg vitamin C/animal/day,
Group 4 – ED + 2 mg vitamin C + 1 mg $CdCl_2$/animal/day,
Group 5 – ED + 100 mg vitamin C + 1 mg $CdCl_2$/animal/day,
* – $p < 0.05$
b.w. – body weight

Remarkably higher values were observed in relative spleen and liver weight of Cd-exposed guinea pigs on low vitamin C intake (Table 1).

Results of the test of phagocytic activity of polymorphonuclears and monocytes are given in Fig. 2. After a 12-week experiment a statistically significant decrease of phagocytic activity in the groups with low vitamin C intake and without any exposure to Cd (group 2) was observed. The statistically significant suppression of phagocytic activity of polymorphonuclears and monocytes was identified in the groups exposed to Cd at low intake of vitamin C (group 4).

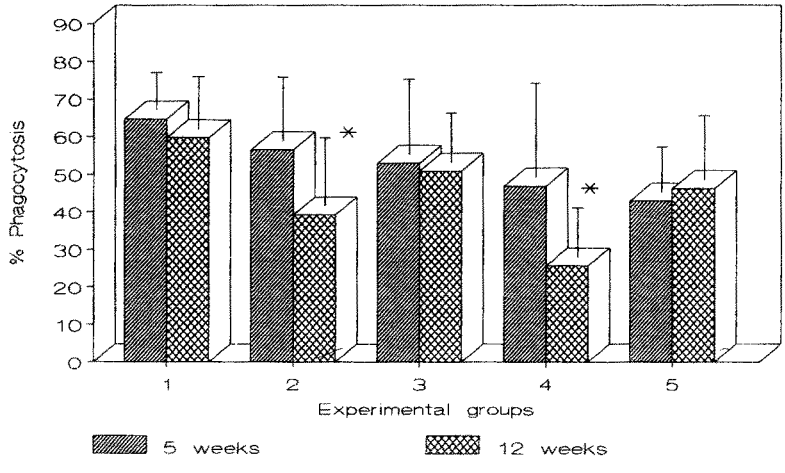

Fig. 2. Phagocytic activity of polymorphonuclears and monocytes (Groups 1–5 see footnote in Table 1)
* – $p < 0.05$

The percentage of total and active T lymphocytes after 5-week exposure to Cd displayed no statistically remarkable changes (Figs. 3 and 4). We observed a higher percentage of active T lymphocytes in the group exposed to Cd with a low vitamin C intake (group 4), but this difference was not statistically significant. A dramatic change occurred after 12-week exposure. In some animals from the groups exposed to Cd we could not identify any formation of rosettes. In the Cd group with high vitamin C intake (group 5) five out of eight guinea pigs were observed to form rosettes, in the Cd group with low vitamin C intake rosettes were formed in three out of eight animals. This decrease in number of animals whose lymphocytes formed rosettes did not permit any statistical evaluation of this functional test, or any demonstration in the figures of groups 4 and 5 after 12 weeks of exposure. A statistically significant lower percentage of total

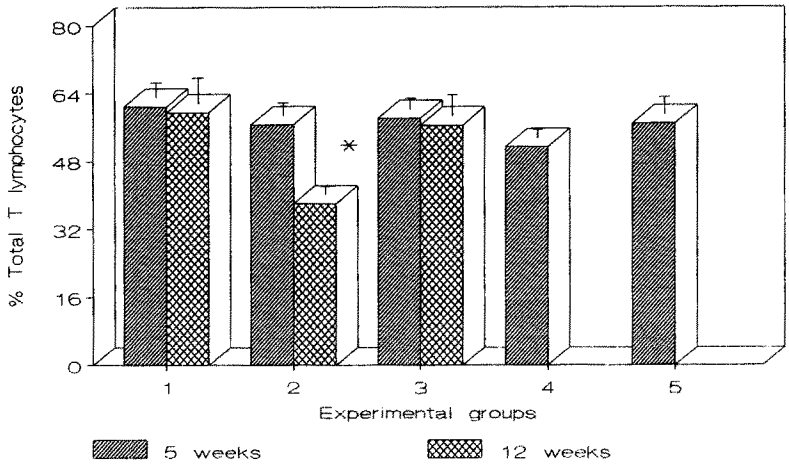

Fig. 3. Percentage of active T lymphocytes of the guinea pigs (Groups 1–5 see footnote in Table 1)
* – $p < 0.05$

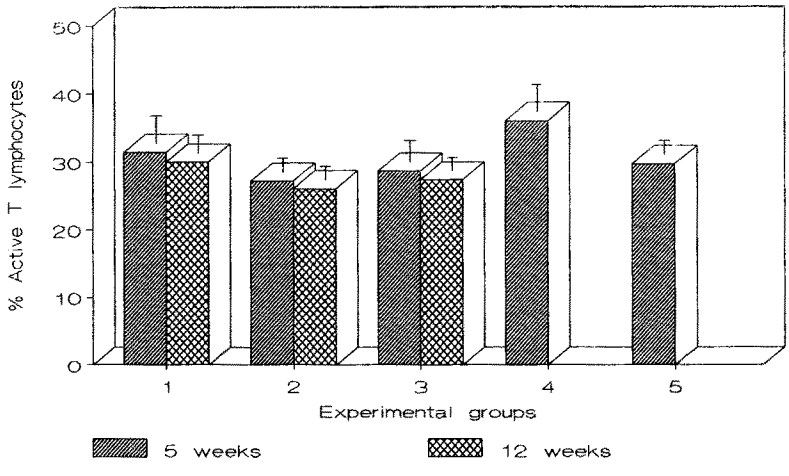

Fig. 4. Percentage of total T lymphocytes of the guinea pigs (Groups 1–5 see footnote in Table 1)
* – $p < 0.05$

lymphocytes was observed in the groups of animals with a low vitamin C intake (group 2). A higher vacuolization of cytoplasmic leucocytes, cell detritus as a cytolytic residue, punctate basophilia of erythrocytes and phagocytic cellular LE-like elements (Fig. 5) were identified in blood smears of animals whose lymphocytes had not formed rosettes.

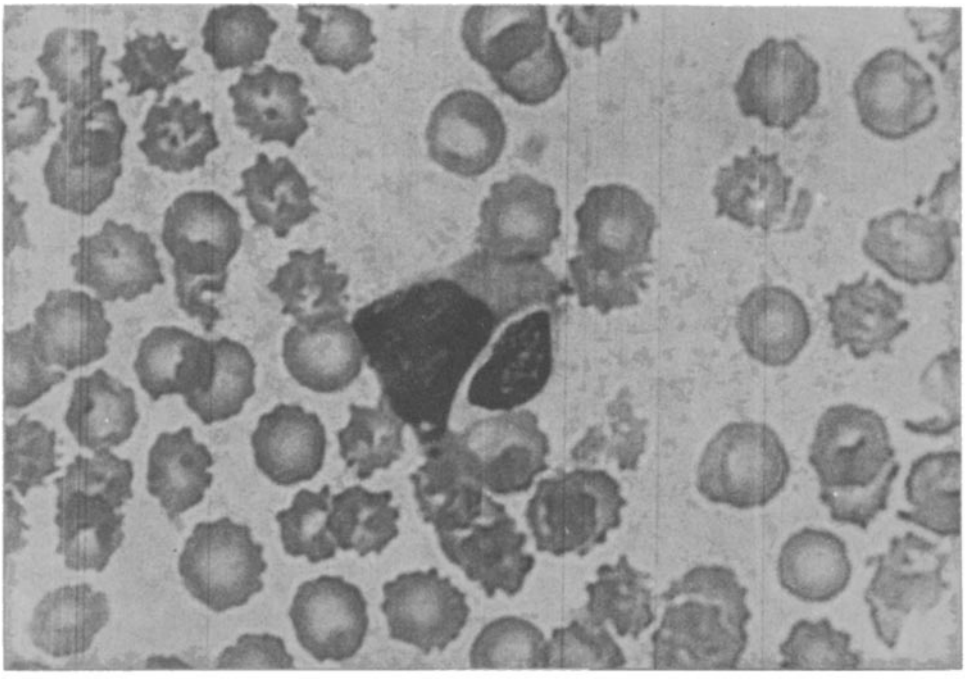

Fig. 5. Phagocytic LE-like cell in blood smears of guinea pigs exposed to with cadmium low intake of vitamin C (Groups 1–5 see footnote in Table 1)
* – $p < 0.05$

Discussion

The aim of this experiment was to evaluate a possibility of influencing the toxic effect of high doses of Cd by means of nutritional factors.

Significantly decreased body weight and increased relative liver weight in Cd-exposed groups resulted from a toxic influence of cadmium. Cd content in the organs of animals in this experiment was determined and has been published (11).

Remarkably higher values of relative spleen weight were probably induced by removal of cell detritus.

The evaluation of parameters of humoral immunity in experiments with animals brought controversial results. Some authors observed a stimulatory effect of Cd on immunity (14), others did not observe any influence (12), while still others determined a humoral immunity suppression (3). This variety of results has sometimes been obtained depending on the dose or duration of exposure, the route of administration, and the strain used.

In order to evaluate the influence of Cd on immunity, we selected parameters of cellular immunity on the basis of data published on immunotoxic influence of Cd. Suppressive effect of Cd on cellular immunity was observed, both after a short-term (6) and long-term (20) oral exposure. A remarkable suppression of parameters of non-specific immunity and NK-cells inhibition was identified in Cd-exposed animals (8, 16, 19). Guillard (9) and Baginski (1) found remarkable suppression of polymorphonuclear leucocytes activity in persons professionally exposed to Cd. In this experiment a statistically significant suppression of phagocytic activity of polymorphonuclear leucocytes was observed in guinea pigs exposed to Cd with a low intake of vitamin C for 12 weeks. An increased intake of ascorbic acid in guinea pigs exposed to Cd prevented a remarkable decrease of phagocytic activity of polymorphonuclears and monocytes. A statistically less significant decrease of phagocytic activity was observed in the group not exposed to Cd with low intake of vitamin C. Vitamin C stabilizes lysosomal membranes of phagocytes. Immunosuppressors, smoking, advanced age, and pregnancy lower the content of vitamin C in phagocytes. Phagocytic activity can be increased by vitamin C intake (13).

The effect of ascorbic acid deficiency on cellular immunity evoked a lowered percentage of total lymphocytes in the group of animals with low intake of vitamin C after 12-week experiments. This result was even more significant because it occurred in the groups with no Cd influence. The increase of active T lymphocytes after a 5-week exposure to Cd observed in our experiments supports the hypothesis on the initial activation of immune system exposed to a xenobiotic (21).

A higher vacuolization of cytoplasmic leucocytes, cell detritus as a cytolytic residue, and phagocytic cellular LE-like elements were identified in the smears of peripheral blood of animals whose lymphocytes had not formed rosettes. This appearance corresponds to the published data on autoimmunity induced by Cd. In mice Cd induced the formation of antinuclear antibody (15). Autoantibodies against laminin and type IV of collagen were formed in Sprague-Dawley rats after intravenous application of Cd (10). A remarkably increased level of antibodies against laminin was found in workers professionally exposed to Cd for about 9 years (2).

Conclusion

The experiments have shown that Cd with low intake of vitamin C caused a mutual potentiation of the influence on immune system of exposed animals. A statistically significant inhibition of non-specific and specific cellular immunity was observed also in the group not exposed to Cd with low intake of vitamin C, which proved the significance of chronic insufficiency of antioxidantly effective substances in food.

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