EFFECT OF BIOFLAVONOID LESPEFLAN ON XANTHINE OXIDASE ACTIVITY IN MERCURY CHLORIDE TOXICITY

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Summary

Free radical production and oxidative stress is one of the mechanisms of tissue damage in heavy metal toxicity. Xanthine oxidase is an enzyme involved in catabolism of purine bases, adenine and guanine. Enzyme exists in two interconvertible forms, as xanthine oxidase and xanthine dehydrogenase. Higher level of xanthine oxidase activity against xanthine dehydrogenase increases production of free radicals and lipid peroxidation level. Because of lot of information in literature about benefits of medical plants in health prevention the aim of the study was evaluation of the role of bioflavonoid Lespeflan on xanthine oxidase activity in the mechanisms of neurotoxicity of mercury chloride. We have studied the possible beneficial effects of bioflavonoid Lespeflan on free radical production and xanthine oxidase activity in acute mercury chloride toxicity. The experiment was performed on male Sprague Dawley rats. Results of the study show increased activity of xanthine oxidase and lipid peroxidation level in brain homogenate 24 hours after mercury chloride administration, ($p<0.05$). Pretreatment of animals by bioflavonoid Lespeflan significantly decreases enzyme activity, as well as lipid peroxidation level compared to control group. Obtained results indicate that increase of xanthine oxidase activity and free radical production may be a mechanism of mercury chloride toxicity. Bioflavonoid Lespeflan decreases xanthine oxidase activity and lipid preroxidation level. Therefore, we may conclude that bioflavonoid Lespeflan has beneficial effect in mercury chloride –induced neurotoxicity.

Key words: xanthine oxidase, lipid peroxidation, mercury chloride, Bioflavonoid Lespeflan, brain, rats.

Introduction

Mercury exists in three forms: elemental mercury, inorganic mercury compounds (primarily mercuric chloride), and organic mercury compounds (primarily methyl mercury). All forms of mercury have toxic effects. Humans and animals are exposed to all of chemical forms of mercury. Mercury chloride ($HgCl_2$) is inorganic mercury compound with ionic mercury. Many inorganic compounds of mercury are used in agriculture (pesticides, fungicides), medicine (as a disinfectants, dental amalgam fillings, vaccines) industrial manufacture (fluorescent lamps, batteries, thermostats, thermometers). Intoxication with mercury occurs through environmental, occupational or accidental exposure {1).

Acute exposure to mercury may include damage of the kidney, gastrointestinal, nervous and cardiovascular system. Chronic exposure leads to neurodegenerative disorders likes Alzheimer's diseases, Parkinson's diseases, autism, immunomodulation, acrodinia, dermatitis, reproductive failure, chronic renal failure, cardiovascular and liver damage and carcinogenesis (2,3).

A mechanism of tissues damage by mercury is not completely clarified, but many studies confirm oxidative stress as important factor in tissues damage. Dietary factors such as nutritional supplements by medicinal plants and herbal extracts can modulate physiological functions of many organs including their beneficial effect in different diseases. Flavonoids are a group of naturally occurring compounds, which are widely distributed in nature. Flavonoids have been reported to have antiviral, antiallergic, antiplatelet, antiinflammatory and antitumoral activities (4). The pharmacological properties of bioflavonoids occur through inhibition of enzymes involved in the production of free radicals and to their free-radical scavenging capacity. They may also function indirectly as antioxidants through: inhibition of "pro-oxidant" enzymes, such as inducible nitric oxide synthase, cyclooxygenases lipoxygenases, and xanthine oxidase (5,6,7).

The xanthine oxidoreductase catalysis the oxidation of hypoxanthine and xanthine in the process of purine catabolism forming uric acid. Xanthine oxido-reductase can exist in two interconvertible forms, either as xanthine dehydrogenase (XD) or xanthine oxidase (XO) and intermediate form. Xanthine oxidase produces uric acid and reactive oxygen species during the catabolism of purines (8).

The aim of this study was the study of the effect of bioflavonoid Lespeflan on lipid peroxidation and xanthine oxidase activity in acute mercury chloride toxicity in brain tissue.

Material and Methods

Male Sprague Dawley rats weighing about 250 g. were used in the experiment. Mercury chloride was given in a dose of 3mg/kg intraperitonaly. Control group of animals was treated with equal volume of saline. Lespeflan (DALJHIMFARM, Heberovsk, Russia) was given one our before mercury chloride administration in a dose 0.3 ml/kg. The animals were killed after 24 hours. The brains of experimental animals were used for research sudy. Tissues were quickly removed, washed and stored at -70 ^{0}C. We have measured the levels of brain lipid peroxidation level, xanthine oxidase activity and proteins in brain homogenates. Tissue levels of free radical production were measured as malondialdehyde level (MDA) by thiobarbituric acid reaction (9). Xanthine oxidase activity in brain tissue was measured according to the method of Kizaki and Sakurachi on the amount of formed uric acid level (10). Enzyme activity was expressed in units on mg of tissue proteins (U/mg.prot.). Tissue proteins were determined by the method of Lowry et al. (11).
Statistical significance between experimental groups was evaluated by student's t-test. $p<0.05$ was used as statistical significance.

Results

The lipid peroxidation level in brain homogenate is expressed as malondialdehyde levels (MDA). As shown, mercury chloride administration significantly increase tissue level of malondialdehyde compared to control group, $p<0.05$. Pretreatment of animals with Lesplefan decreases tissue level of MDA compared to mercury chloride treated group, (Figure 1).

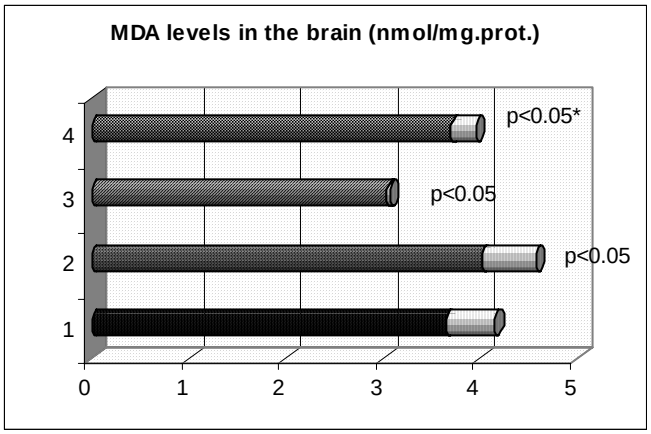

Figure 1. Effect of mercury chloride on free radical production in the brain
1-control, 2- $HgCl_2$, 3-Lespeflan, 4- $HgCl_2$ +Lespeflan
$p<0.05$, vs. control
*$p<0.05$, vs. $HgCl_2$

Results relating to brain xanthine oxidase activity show that mercury chloride leads to increase of xanthine oxidase activity compared to control group, $p<0.05$). Lespeflan pretreatment decreases xanthine oxidase activity compared to mercury chloride treated group of animals (Figure 2). Bioflavonoid Lespeflan itself decreases MDA level and XO activity (Figure 1 and Figure 2.).

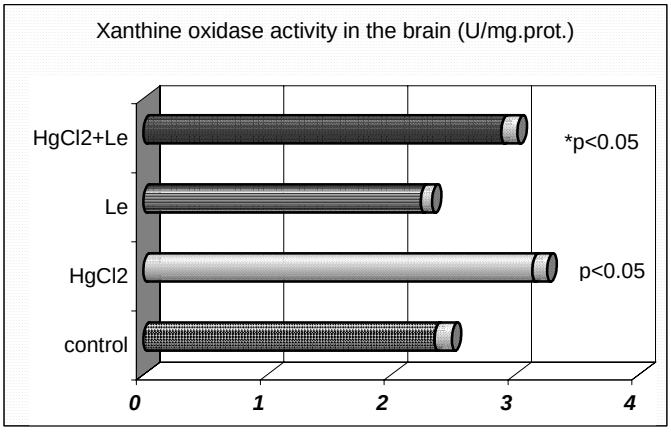

Figure 2. Effect of mercury chloride on xanthine oxidase activity
1-control, 2-$HgCl_2$, 3-Lespeflan, 4-$HgCl_2$+Lespeflan
$p<0.05$, vs. control
*$p<0.05$, vs. $HgCl_2$

Discussion

Heavy metals poisoning is frequently involved in neurodegeneration. Acute and chronic neurodegenerative diseases are illnesses associated with high morbidity and mortality. Neurodegenerative diseases of the human population like Alzheimer's, Parkinson's diseases and others are a result of the effect of various neurotoxins. Various forms of mercury can leads to neurodegeneration.

Free radicals. are molecules such as superoxide anion (O2-·), hydroxyl radical (HO·), nitric oxide (NO·) and lipid radicals. In reactions between free radicals and polyunsaturated fatty acids may result in a fatty acid peroxyl radical (R-COO·) that can attack fatty acid side chains and initiate production of other lipid radicals. End products of lipid peroxidation have cytotoxic and mutagenic properties. Cells under oxidative stress display various dysfunctions due to damages caused by reactive oxygen species to lipids, proteins and DNA. Oxidative stress in cells can be partially responsible for the toxic effects of heavy metals. Mercury chloride induces neurotoxicity in part by increasing of free radical production (12,13).

Xanthine dehydrogenase (XDH) can be converted reversibly to xanthine oxidase (XO) by oxidation of cysteine residues or irreversibly by limited proteolysis. XO has high

reactivity toward O_2 but negligible reactivity toward NAD^+. As XO can reduce molecular oxygen to superoxide and hydrogen peroxide, XO is thought to be one of the key enzymes producing reactive oxygen species. Increased activity of xanthine oxidase to dehydrogenase form increases production of free radicals ad lipid peroxidation can lead to gout and mutagenesis. Superoxide anion, hydrogene peroxide and hydroxyl radical is formed as by-products in reaction. Inhibitors of xanthine oxidase reaction have therapeutically application in treating of gout and hepatic injury (14,15).

Recent study indicates that bioflavonoids, bioactive components in medicinal and food plant extracts may be used in neuroprotection (16). The pharmacological properties of bioflavonoids occur through inhibition of enzymes involved in the production of free radicals and to their free-radical scavenging capacity. They also function indirectly as antioxidants through: inhibition of the redox-sensitive transcription factors, nuclear factor-kapaB (17); inhibition of "pro-oxidant" enzymes, such as inducible nitric oxide synthase, lipoxygenases, cyclooxygenases and xanthine oxidase (18); induction of phase II and antioxidant enzymes and have an antilipoperoxidative capacity (19) and they show a protective effect on DNA (20).

According to Mondal and Mitra (21) the inhibition of bovine xanthine oxidase activity by Hg2+ and other metal ions. in vitro was detected. . Results of our study show that mercury chloride administration to rats leads to increase of xanthine oxidase activity in rat brain and to elevation of free radical production.. Bioflavonoids are antioxidants that can reduce oxidative stress. Inhibition of xanthine oxidase by various bioflavonoids was detected. Therefore, catechins, some Chinese medicinal plants, purpurogallin and genistein were found as xanthine oxidase inhibitors (22,23,24). We have found that Lespeflan decreases XO activity. Literature data show that the structure-activity relationship of flavonoids as inhibitors of xanthine oxidase is necessary for their high inhibitory activity (25,26,27).

Therefore, recent results studies point the importance of thiol groups of aminoacids, peptides, proteins and specific signal mechanisms in mercury action, including interaction with nuclear kappa-B factor, specific stress proteins and expression of metallothioneins in mechanisms of mercury toxicity (28,29).

Conclusion

Acute mercury chloride intoxication increases lipid peroxidation level and xanthine oxidase activity in the brain. Pretreatment intoxicated rats with bioflavonoid Lespeflan decreases xanthine oxidase activity and MDA level. Obtained results indicate that bioflavonoid Lespeflan could be of importance in prevention of mercury chloride toxicity. These results confirm the hypothesis that oxidative stress is one of mechanisms of mercury chloride induced neurotoxicity.

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