THE PROTECTIVE PROPERTIES OF SELECTED NATURALLY OCCURRING ANTIOXIDANTS OF PLANT ORIGIN AGAINST FLUORIDE-INDUCED NEUROTOXICITY

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ABSTRACT: Fluoride is a substance with toxic properties and has been proven to adversely affect many soft tissues including nervous tissue. Chronic exposure to fluoride can result in neurodegenerative disorders. Results of many studies indicate that neurodegenerative processes induced by high concentrations of fluoride may occur through two main mechanisms: excitotoxicity and oxidative stress. One of the proposed strategies for the treatment of neurodegenerative diseases associated with excessive oxidation involves the use of medicinal substances with antioxidant activity. Natural antioxidants, such as those found in plants, may prove to be useful in the reducing the progression of various pathologies associated with oxidative stress including neurodegeneration. The present work describes a few of the chemical compounds, found in plants, which have been recorded in the scientific literature as acting as natural antioxidants in situations of oxidation due to the fluoride exposure. This paper focuses on the antioxidant and potentially anti-neurodegenerative properties of curcumin, silymarin, arjunolic acid, resveratrol, and gallic acid and their potential use in the treatment of nervous tissue degeneration caused by fluoride exposure by reducing the rate of disease progression.

Keywords: Arjunolic acid; Curcumin; Fluoride; Gallic acid; Natural antioxidants; Neurodegeneration; Oxidative stress; Resveratrol; Silymarin.

INTRODUCTION

Fluoride is a substance with toxic properties.[1,2] Chronic exposure and a significantly increased supply of fluoride can lead to degenerative disorders, for example those affecting the nervous system.[3,4,5]

NEUROTOXICITY OF FLUORIDE

Fluoride affects many soft tissues in the body, including nervous tissue.[6] It easily crosses the blood-brain barrier and accumulates in nervous cells.[7] The accumulation of fluoride in the hippocampus has been observed to contribute to a degeneration of neurons and altered metabolism of oxygen, promoting the formation of reactive oxygen species (ROS).[3,8] This indicates that neurodegenerative processes induced by high concentrations of fluoride may occur through two main mechanisms: excitotoxicity and oxidative stress.[9] The excitotoxicity consists of damage to nervous cells caused by a pathological overstimulation by neurotransmitters such as glutamate.[10] The oxidative stress is due to the growth of factors favoring oxidation, as well as a deficiency of antioxidants. Fluoride stimulates oxidative cascade reactions, at the same time as reducing the relatively small antioxidative potential of the brain. The imbalance between the synthesized ROS and defense mechanisms inside the cell, i.e.,

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oxidative stress, results in damage to neurons.[9] In addition, an increased content of polyunsaturated fatty acids and reduced iron binding capacity in the brain promote sensitization of this organ to elevated ROS levels.[11]

Damage to nervous tissue is also associated with gene suppression and the inhibition of enzymes involved in energy accumulation and transport.[12] Fluoride reduces transmembrane and synaptic transport in neurons by reducing the number of nicotinic cholinergic receptors. The impairment of the central nervous system (CNS) neurons is also reflected in reduced metabolic activity, especially the transformation of glucose, the main energy source for the brain (e.g., due to the suppression of glucose transporter-1 [GLUT-1]). Similar to patients with neurodegenerative diseases such as Alzheimer's disease, blood in fluoride-poisoned individuals has lower glucose levels.[13] Persons chronically exposed to fluoride, for example due to water fluoridation, exhibit disorders of the CNS, such as decreased intelligence quotient (IQ), lethargy, and insomnia.[14,15]

Given these highly adverse effects of fluoride on the CNS, we cannot rule out its role in the pathogenesis of neurodegenerative diseases. This impact can be significant, especially in areas heavily polluted with fluoride.

A proposed strategy for the treatment of neurodegenerative diseases associated with abnormal oxidation involves the use of medicinal substances with antioxidant activity. A particularly interesting method is the use of natural antioxidants, such as those found in plants, which may prove to be useful in the prevention of fluorosis or in reducing the progression of various diseases associated with oxidative stress. The advantage of plants is their high availability and often low labor intensity and costs in processing. The present work describes only a few of the chemical compounds, representing a very small fraction of substances with similar properties mentioned in numerous scientific publications.[16,17,18]

CURCUMIN

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, or diferuloylmethane) is a substance extracted from the rhizome of the turmeric (*Curcuma longa*).[19] Turmeric has been used for centuries in India, where it grows freely. Curcumin is a polyphenolic compound with antioxidant, anti-inflammatory, and anti-hyperglycemic properties, protecting against the harmful effects of ROS.[20,21,22] The beneficial effects of curcumin appear to be associated with the stimulation of endogenous antioxidants, which has been confirmed by the presence of a curcumin-induced increase in the concentration of glutathione in various tissues.[23] Particularly interesting are the potential therapeutic effects of curcumin in the treatment of neurodegenerative diseases (e.g., Alzheimer's disease and dementia).[24] The neuroprotective effects of curcumin have been studied in the context of neurogenesis of hippocampal neurons.[25,26] The observed biological activity may be beneficial for nervous tissue regeneration.[9]

Sharma et al. conducted a study on the effect of curcumin on the neurotoxicity of fluoride in mice *in vivo*. The control group consisted of mice on a non-fluoride diet. The experimental group was divided into 3 groups: (i) mice receiving fluoride

in drinking water, (ii) mice receiving fluoride and curcumin in the diet, and (iii) mice receiving curcumin. The levels of neuronal degradation were determined indirectly by measuring the concentration of malondialdehyde (MDA). The neurodegenerative process was most pronounced in the animals from the first group (especially in the hippocampus and cerebral cortex), while the addition of curcumin to the fluoride-containing diet resulted in a much less severe neurodegeneration in group 2 than in group 1. The third group did not show any significant differences when compared to the control group.[9] The co-administration of curcumin and fluoride reduced the risk of degeneration caused by this element.[9] The described properties of curcumin have been confirmed in other experiments showing its preventive action against oxidative stress induced by hydroxyl radicals generated via the Fenton reaction.[27]

SILYMARIN

Silymarin is a complex of flavonoid derivatives of plant origin, which includes, among others, silybin, silydianin, and silychristin. It is obtained from the fruits and seeds of the milk thistle (*Silybum marianum* L.).[28] It is known mainly for its hepatoprotective properties and beneficial effect on digestion.[29] It also shows antioxidant properties, whereby the compounds belonging to the silymarin complex work synergistically to protect against ROS.[30,31]

As fluoride is involved in ROS generation,[32] studies have been conducted to verify whether silymarin has a protective effect on nerve cells in the brains of rats exposed to sodium fluoride (NaF). In one such experiment, the animals were divided into five groups: (i) control group, (ii) group treated with NaF, (iii) group first subjected to the action of silymarin (10 mg/kg) and then NaF, (iv) group first subjected to the action of silymarin (20 mg/kg) and then NaF, and (v) group first subjected to the action of vitamin C and then NaF.[33] In rat brain homogenates the levels of thiobarbituric acid reactive substances (TBARS) were determined, accompanied by the determination of the activity of enzymes such as catalase and superoxide dismutase, as well as the level of reduced glutathione. The results showed that in the group treated with NaF the level of TBARS (reflecting the processes of oxidation) was significantly higher than in the control group. In the groups which had been additionally treated with antioxidants such as silymarin or vitamin C, the level of TBARS were very similar to the control group. The activity of catalase and superoxide dismutase, and the level of reduced glutathione were lowest in the group given only NaF, and were highest in the control group and in the group which had been given silymarin (20 mg/kg) before the administration of NaF.[33]

The aforementioned experiment confirmed (i) the neurotoxicity of fluoride via oxidative stress and (ii) the antioxidant and neuroprotective properties of silymarin. The likely molecular mechanism of action of this flavonoid ligand complex involves the inhibition of ROS and nitric oxide (NO) formation, reduction in lipid peroxidation, chelation of metal ions, and a reduction in the expression of genes involved in increased inflammatory responses by their effect on NFκB.[33,34]

ARJUNOLIC ACID

The arjun tree (*Terminalia arjuna*) has long been used in traditional medicine,[35] and laboratory tests indicate a broad spectrum of therapeutic properties.[36,37] Arjun tree bark preparations contain active substances such as arjunolic acid (AA) and triterpene saponins,[38] i.e., saponins in which aglycone is replaced by a triterpene.[39] These substances exhibit, among others, diuretic, expectorant, anti-inflammatory, and anti-microbial properties. They enhance intestinal absorption and stimulate the secretion of gastric juice and bile. They also influence lipid metabolism, intensifying the digestion of fats, and regulate cholesterol levels.[40] Some sources indicate that regular supplementation with arjun tree bark extract reduces low-density lipoproteins (LDL) and simultaneously increases high-density lipoproteins (HDL).[41] Some also indicate a beneficial effect on the prevention and treatment of cardiovascular diseases, especially coronary heart disease.[42]

Interesting results have been shown in research on the properties of AA in the context of protection against the effects of oxidative stress.[3] A study on isolated mouse hepatocytes incubated with AA and/or fluoride analyzed the functioning of cells in terms of the continuity of cell membranes and intact structure of hepatocytes, measured by the efflux of alanine aminotransferase (ALAT) and alkaline phosphatase (ALP) from cells. The level of damage to the hepatocytes was also estimated by measuring the levels of ROS and membrane lipid peroxidation, as indicated by the concentrations of MDA and protein carbonylation products. The study also evaluated the intracellular activity of antioxidants (superoxide dismutase, catalase, glutathione, glutathione reductase, and glutathione peroxidase).[43]

Hepatocytes exposed to the toxic effect of fluoride showed decreased cell viability due to an increase in ROS levels and reduced activity of antioxidant enzymes, as well as an increase in ALAT and ALP activity in plasma due to their efflux from the cell. At the same time, the developing oxidative stress caused by the intensified synthesis of ROS resulted in increased lipid peroxidation. Importantly, the simultaneous addition of AA to the culture resulted in a reduction of adverse changes in hepatocytes through increased antioxidant enzyme activity up to the level found in control cells, and a significant reduction in ROS levels and membrane lipid peroxidation compared to the group receiving fluoride.[43]

The aforementioned study confirmed the ability of AA to protect hepatocytes from cytotoxicity and cell death resulting from damage due to the accumulation of ROS and increased membrane lipid peroxidation. In addition, AA contributed to a reduced efflux of ALAT and ALP enzymes from the cell, indirectly indicating the integrity of the cell and continuity of the membrane. The antioxidant properties of AA were shown to be similar to those of vitamin C. For example, AA can be oxidized in reactions with free radicals, thanks to the presence of one primary and two secondary hydroxyl groups.[43] These groups may be readily oxidized to corresponding carbonyl groups, analogously to reactions involving vitamin C. Besides, the hydrogen atom in the carboxyl group in AA can be transferred to a nucleophilic molecule of a radical, neutralizing it.[43] The antioxidant properties of

AA are also indicated by other tests, e.g., one using arsenic as an oxidative stressor within the mouse brain.[44]

RESVERATROL

Resveratrol (3,5,4'-trihydroxystilbene) (polyphenol stilbene derivative) is obtained from the roots of the Japanese knotweed (*Polygonum cuspidatum*), a plant commonly used in traditional Eastern medicine to treat fungal infections, cardiovascular diseases, and skin and liver problems.[45,46] Resveratrol occurs naturally in many plant species such as grapes (highest concentration in the skin), plums, peanuts, cranberry, mulberry, and black currant.[47] It is believed to be responsible for the so-called "French paradox."[48] It shows antifungal activity and is therefore added to foods as a natural preservative.[49] On the one hand, resveratrol has antioxidant properties on its own,[50] and, on the other, it increases the activity of endogenous antioxidants (such as superoxide dismutase). Furthermore, it contributes to the reduction of LDL, promoting the normal function of the vascular endothelium.[51]

The protective effects of resveratrol against neurotoxicity have been shown in a number of studies.[11,52] In one such study, the protective properties of resveratrol against the effects of ROS were shown in the liver and nervous tissue of rats intoxicated with fluoride.[11] The results in the group exposed to NaF had significantly elevated levels of total oxidants (ROS and other oxidants) and 8-hydroxy-2'-deoxyguanosine (8-OHdG). In addition, the overall level of antioxidants in plasma was reduced, which might have resulted from an increase in consumption of enzymes or fluoride-induced inhibition of these enzymes. In addition, there was an increase in MDA and TBARS, i.e., markers of lipid peroxidation. The elevated levels of 8-OHdG indicated that the mechanisms of DNA repair were not able to effectively counteract the damage caused by fluoride. Quite different results were achieved in the group receiving NaF together with resveratrol. The values of the determined parameters did not differ significantly from those measured in the control group. In comparison with the group receiving fluoride, it had a significantly reduced concentration of oxidants and increased levels of antioxidants.[11]

Resveratrol is a polyphenolic compound and the most active among the stilbene phytoalexins. The antioxidant properties of resveratrol may be related to its chemical structure.[53,54,55,56] Its polyphenolic structure provides reduction properties due to the presence of hydroxyl groups and a delocalized electron potential.[57,58] The resveratrol molecule comprises highly lipophilic and hydrophobic regions, which increase its effectiveness as an antioxidant compared to vitamins C and E. Three mechanisms of the antioxidant action of resveratrol include (i) interruption of the free radical chain reaction in conjunction with CoQ; (ii) neutralization of superoxide radical formed in the mitochondria; and (iii) protection of lipids against peroxidation induced by Fenton reaction products.[57,58]

GALLIC ACID

Gallic acid (3,4,5-trihydroxybenzoic acid) is a compound with a broad spectrum of medical properties. It is found in brambles, raspberries, mango fruit, seeds and leaves of the catechu palm, and certain varieties of tea.[59,60] A monomer in which a polymerization reaction produces a number of tannins,[61] it is used in the food, cosmetic and pharmaceutical industries.[62,63]

Gallic acid has antiseptic, antifungal and antiviral effects.[64] It is a powerful antioxidant that enhances the activity of other antioxidants, including curcumin, in terms of the protection and regeneration of hepatocytes damaged by free radicals.[60] Extracted from natural sources, gallic acid has been used in the treatment of chronic diseases (diabetes, tuberculosis, and pulmonary and gastrointestinal hemorrhages), and skin lesions caused by venereal diseases (gonorrhea). Similar properties have also been exhibited by modified gallic acid compounds (e.g., ester of gallic acid).[65]

The antioxidant activity of gallic acid has been tested in experiments on rats in which oxidative stress in the nervous tissue of the brain was induced with NaF.[31,66] In one study, neuronal damage was measured by the level of TBARS in the brain. Groups that had been administered gallic acid (group 1) and vitamin C (group 2) prior to fluoride poisoning had lower concentrations of TBARS when compared with the control group. The level of oxidative stress was also estimated by establishing changes in the activity of antioxidant enzymes, which decreased significantly as a result of fluoride poisoning. Importantly, in the group receiving antioxidants prior to poisoning, the activity of glutathione reductase and superoxide dismutase did not differ from normal levels, and gallic acid caused a return to the optimum activity of antioxidant enzymes more effectively than vitamin C.[31]

In nature, gallic acid appears in two forms: as a methylated derivative of the acid, and as galotanins. Its activity as the scavenger of free radicals is associated with the presence of three hydroxyl groups on the benzene ring in the para position relative to the carboxyl group. Their chemical nature, favoring activation of a delocalized bond in the aromatic ring, facilitates the binding of free radicals.[31,67]

CONCLUSIONS

Fluoride occurs commonly in the environment. Exposure of the body to excessive amounts of this element may damage many tissues and organs, including the nervous system, as evidenced by many of the aforementioned papers.[3,9,11,33,52,68] That is why researchers focus on understanding the mechanisms of the destructive action of fluoride in organs, including nervous tissue. The involvement of fluoride in the processes of oxidation and reduction is unquestionable. Significantly however, the results of studies on the effects of fluoride on the activity of antioxidant enzymes are contradictory, depending on the research model, with various experiments having repeatedly confirmed the prooxidative properties of fluoride, leading to increased ROS production.[69,70,71] Therefore, it is suggested to use antioxidants to prevent and treat the effects of

overexposure to fluorine compounds.[72] The literature shows that a wide range of compounds are capable of inhibiting the fluoride-induced generation of ROS.[3,9,11,33,72] Particularly interesting are those that occur naturally in the environment. This paper has presented selected substances of plant origin with antioxidant properties. As shown by the cited papers and in the various test models, curcumin, silymarin, arjunolic acid, resveratrol, and gallic acid all have a protective role against the effects of oxidation caused by excessive exposure to fluoride, and thereby prevent damage at the tissue or cell level.[3,9,11,33,52]

The generally applied protection against the effects of excessive exposure to fluoride, involving decontamination of the hydrosphere and surface layers of the lithosphere is expensive and inadequate. Therefore, the use of naturally occurring antioxidant substances, such as those discussed above, could strengthen protection against the negative effects of fluoride on the body, including neurodegenerative diseases.[73]

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