Effect of Mineral Water from Trenčianske Teplice (Drinkable Source, Drill SB-3) on Lipid Peroxidation *in Vitro*

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Abstract: Influence of mineral water from Trenčianske Teplice (drinkable source) on lipid peroxidation processes was determined in model situations under *in vitro* conditions using the brain tissue. The central nervous system was selected because it is especially sensitive to the radical-induced damage. In addition, there is a high content of polyunsaturated fatty acids in the brain which has a low antioxidant capacity and is relatively rich in iron ions – enhancers of lipid peroxidation processes. We present the inhibitory effect of the mineral water on the intensity of lipid peroxidation in the presence of iron ions. We assume that some component or combination of more components of the mineral water may act as chelators of iron ions.

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Introduction

Ischemia induces tissue changes which cause cell damages and creates conditions for their further damage by following reperfusion. A mutual relation of the cell processes which originated as a consequence of the lack of oxygen (decrease in ATP, pH change, ionic imbalance, limited proteolysis, etc.) and of subsequent reoxygenation (oxygen radicals production, oxidation of polyunsaturated fatty acids, etc.) can be an important factor for triggering longer lasting and even irreversible changes. Measurement of parameters for oxidative damage of macromolecules and cell structures contributes significantly to the assessment of the total state of a tissue or organism. The model approach of monitoring of these changes enables simultaneously the assessment of mechanisms participating in their impact on cell functions at different levels.

Metal ions play an important role in the health and disease by influencing cellular biochemical pathways. Increased concentrations of some metal ions may have cytotoxic effects because of their ability to modify biomolecules oxidatively, what may cause oxidative stress – induced brain cell death leading to neurodegenerative disorders. A hypothesis that aluminium (Al) exposure is aetiologically related to Alzheimer's disease has led to many discussions. Several studies have suggested that concentration of aluminium in drinkable water of 0.1–0.2 mg/l may increase a risk of Alzheimer's disease (Gauthier et al., 2000; Rondeau et al., 2000). On the other hand, some epidemiological and experimental studies suggested that silicium can reduce aluminium oral absorption and protect against aluminium-induced unfavourable effects (Birchall and Chappell, 1989; Gillette-Guyonnet et al., 2005).

The aim of our study was to assess the influence of mineral water from Trenčianske Teplice (drinkable source) on the level of lipid peroxidation (LP) products in a model situation with *in vitro* conditions. In addition to the actual content of LP products, it is also important to evaluate the effect of the mineral water on the intensity of these products formation in conditions of intensive oxidation of polyunsaturated fatty acids – using LP activators addition, sample incubation at higher temperature, as well as monitoring of pro-oxidative or anti-oxidative influence of effectors.

Material and Methods

Adult, male Wistar rats (n=6), weighing 300–400 g, were provided by the Faculty of Medicine, CZ-62760158 (Brno, Czech Republic). All animals were fed on commercial rat diet and water *ad libitum*. After decapitation, the brain was rapidly removed and homogenized in 8.3 mmol/l Tris-HCl filled up with re-distil water (pH 7.25), using the Potter-Elvehjem's homogenizer at 0 °C, during 1 min.

Products of lipid peroxidation (LP) were determined according to the modified method of Ohkawa et al. (1979) in 2% (w/v) brain homogenates. The homogenates were used for analysis immediately (control) and after 30 min incubation at 37 °C

with or without the activator of lipid peroxidation. $FeSO_4$ (5 μmol/l) with/without ascorbate (125 μmol/l) was used as the activators of LP. These activators were combined without (A) (deionized water) and with mineral water SB-3 (B) 1:1 (v/v). TBA reaction was carried out by mixing of 0.3 ml homogenate in Tris-HCl and 0.1 ml of the LP activator. For control samples, 0.1 ml of deionized water was used instead of the LP activator. Subsequently, 0.75 ml of 0.2 mol/l phosphoric acid and 0.5 ml of 0.6% TBA (w/v) were added after 30 minute incubation at 37 °C. This mixture was heated at 100 °C for 30 minutes and than cooled in iced water. The produced coloured complexes were extracted into 1 ml of *n*-butanol and centrifuged at 1,000× *g* for 10 min.

Amounts of LP products were determined by the assay of thiobarbituric acid reacting substances (TBARS) by spectrophotometry at 532 nm.

Calibration curve was made using 1,1,3',3'-tetraethoxypropane (TEP), a precursor of MDA, in the range 7.5–30 μmol/l. These standards were treated in the same way as samples.

Results were expressed as nmol of MDA per mg of protein. Proteins were determined by the method of Lowry et al. (1951), using bovine albumin as a standard. Statistical analysis was performed using Man-Whitney U-test. Results were expressed as means ± SEM of 6 separate experiments in triplicates. The level of significance was set to $p<0.05$.

Results

Content of TBARS in control samples was low (0.79 nmol/mg proteins) in rat brain homogenates. The intensity of LP process, expressed by amounts of TBARS after 30-minute incubation at 37 °C, was not significantly different from control samples (0.96 nmol/mg proteins). We found out that iron ions after 30-minute incubation at 37 °C caused 5-times in increase of LP products in samples, compared to samples incubated without an effector. Moreover, iron in combination with ascorbate, after the same incubation, resulted in 10-times increase of LP products versus "no effector" samples.

In the second part of our study the influence of the mineral water on the level of LP products was monitored. The mineral water did not alter the content of TBARS products in conditions without any incubation of samples (control (B) versus (A) in Table 1). The production of TBARS was significantly increased by the mineral water in conditions with "no effector" compared to control samples (B).

The mineral water added to incubated samples (B) significantly decreased the formation of LP products in samples with iron ions, compared to samples without mineral water (A) (Table 1). Similarly, the mineral water added to samples with the LP activator (Fe^{2+} + ascorbate) had a tendency to decrease its effect. It has been shown that the LP activators (either Fe^{2+} or Fe^{2+} + ascorbate) were effective also after the addition of mineral water (B), however, their influences were lower than without the mineral water (A).

Discussion

Lipid peroxidation (LP) is a set of radical-mediated chemical chain reactions by which double bonds in polyunsaturated fatty acid (PUFA) side chains are rearranged. In the presence of a transition metal (such as Fe^{2+}, or Cu^{+}), lipid peroxidation reactions can expand geometrically. Ascorbic acid has the potential to protect both cytosolic and membrane components of cells from oxidative damage. In the cytosol, ascorbate acts as a primary antioxidant scavenging free radical species which are generated as by-products of cellular metabolism. For cellular membranes, ascorbate may play an indirect antioxidant role to reduce the α-tocopheroxyl radical to α-tocopherol. On the other hand, under certain conditions, ascorbate can promote generation of active oxygen species ($\cdot OH$, $O_2^{-}\cdot$) (Bast et al., 1991). The prooxidant activity of ascorbate is derived from its ability to reduce transition metals, Fe^{3+} or Cu^{2+}, by a one-electron transfer mechanism.

The central nervous system is especially sensitive to the damage caused by free radicals. In the brain, there is a high content of PUFA, which are the main substrate for lipid peroxidation. The brain has a low antioxidant capacity represented mainly by ascorbic acid and superoxiddismutase (SOD). This tissue is relatively rich in iron ions. The highest iron amount is in substantia nigra and globus pallidum (Gerlach et al., 2003). The brain is predominantly perfused by blood because it needs high amount of oxygen for it functional and metabolic processes. These are the reasons why the oxidative brain injury can be developed, negatively influencing cell integrity and functions by lipid peroxidation, as well as by damage of DNA and proteins, under pathological conditions.

Determination of an actual amount of LP products in a tissue can reflect mainly the ratio of anti-oxidative and pro-oxidative mechanisms in a specific state. The content of LP products in the rat brain tissue is small under physiological conditions. It seems to be important also to find out changes which need not be displayed by the increase in actual amount of LP products in tissue during physiological state, however, they can be manifested under pathological

Table 1 – Effect of mineral water SB-3 from Trenčianske Teplice on TBARS productions in rat brain homogenates expressed as nmol/mg proteins (means ± SEM)

		(A)	(B)
Control		0.79 ± 0.09	0.68 ± 0.11
Intensity of LP:	no effector	0.96 ± 0.08	1.42 ± 0.13
	Fe^{2+}	3.97 ± 0.28	2.40 ± 0.19*
	Fe^{2+} + ascorbate	10.00 ± 0.56	9.28 ± 0.70

(A) – no addition of mineral water SB-3; (B) – addition of 50 μl mineral water SB-3; intensity of LP: 30-minute incubation of samples at 37 °C; *significantly decreased amount of LP products after addition of SB-3 compared to samples without mineral water (A) ($p<0.001$)

conditions. Monitoring of this phenomenon is enabled by the incubation of samples with the possibility to change conditions in the incubation medium. In this way, the intensity of LP can be evaluated in different conditions, e.g. by incubation of the samples at higher temperature in the presence of substances taking part in the activation of peroxidation (Minotti and Aust, 1987).

In this paper, we present an inhibitory effect of the mineral water from Trenčianske Teplice (drill SB-3) on activated lipid peroxidation processes, by iron ions without/with ascorbate, in rat brain homogenates (Table 1). We found out that the intensity of LP after sample incubation with "no effector", without mineral water, versus control samples had a tendency for increasing (A). However, the intensity of LP after the sample incubation with "no effector", with mineral water addition, was increased significantly by 108% versus control samples (B). This finding can be explained by the presence of aluminium in mineral water. The activation effect of Al to lipid peroxidation was described in "*in vivo*" experiments (Nehru and Anand, 2005). On the other hand, we found out significant inhibitory effect of mineral water on LP activated by iron ions compared to samples without mineral water.

It seems that any component or combination of more components of the mineral water may act as effective chelators of iron ions. The used mineral water contains e.g. ions of aluminium (3.5 μmol/l), strontium (51 μmol/l), calcium (11.86 μmol/l), magnesium (4.7 μmol/l), silicium (0.68 mmol/l), as well as chlorides, carbonates and hydrogen sulphide. Only when iron is tightly bound to a chelator, its capacity for promoting the peroxidation of biomolecules is minimal. Some chelators of iron, e.g. desferrioxamine, o-phenathroline and others are able to bind Fe^{3+} and thus, it will be less Fe^{3+} for reduction to Fe^{2+} by ascorbate and other reducing compounds. However a question remains, if the required Fe^{3+}-chelator is contained in the used mineral water.

In addition, it would be interesting to examine, to which extent a long-time intake of mineral water, as well as its composition, might cause a disbalance between pro-oxidants and anti-oxidants in the organism, or might have an antioxidative effect. In our further experiments, we focuse on monitoring of individual components of the mineral water which might be supposed to have an inhibitory effect on LP. This part of our study will be the subject of our future article.

Conclusion

Inhibitory effect of the drinkable mineral water from Trenčianske Teplice (drill SB-3) on lipid peroxidation processes may be caused by the optimal combination of its ion component.

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