The Effect of Carbonated Natural Mineral Water on Oxidative Stress in Experimental Myocardial Ischemia

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Natural therapeutic factors are widely used as an important adjuvant therapy in various cardiovascular and cerebrovascular disorders. The aim of this study was to assess the role of balneal therapy on oxidative stress parameters in experimental myocardial ischemia induced in rats. 5 groups of 8 rats were used as follow: group 1- control group; group 2 - group swimming in distilled water (DW); group 3- group with myocardial ischemia (MI); group 4 - group with MI swimming in DW; group 5 - group with MI and swimming in carbonated mineral water (CMW). Myocardial ischemia was induced with Isoproterenol. The following oxidative stress/antioxidant blood parameters were assessed for each animal: nitric oxide (NOx), malondialdechyde (MDA), total oxidative stress (TOS), catalase (CAT) and total ant oxidative capacity of plasma (TAC). In group 5 all parameters assessed were significantly improved compared with group 3 and 4. Carbonated mineral water can be used as an adjuvant therapy for improving oxidative stress/antioxidant status in patients with cardiac ischemia, in order to reduce the amplitude of ischemic lesions and to contribute as a prophylactic therapy to a better quality of life for these patients. Continuing this research in humans through clinical studies would be warranted.

Keywords: carbonated mineral water, oxidative stress, antioxidant plasma capacity, myocardial ischemia

Baile Tusnad spa resort, through the presence of natural therapeutic factors including natural carbonated mineral waters and mofettes with a vasodilator effect, is recommended for the prophylaxis and rehabilitation treatment of cardiovascular diseases such as hypertension stage 1 and 2, stable chronic ischemic heart disease, chronic peripheral circulatory arterial and venous disorders [1]. Some authors believe that carbon dioxide (CO_2) baths can be an effective therapeutic method for the rehabilitation of coronary diseases, myocardial infarction, stroke [2], for the treatment of chronic venous insufficiency, inflammatory diseases [3]. The therapeutic effects of carbonated mineral baths are based on the action of carbon dioxide and less on the pharmacodynamics action of mineral salts in the composition of mineral water. A study on the molecular mechanism showed that CO2-induced vasodilatation in peripheral blood vessels is linked to the activation of the nitric oxide-cyclic guanosine monophosphate signalling cascade and angiogenesis by induction of synthesis of vascular endothelial growth factor (VEGF) [4]. Another mechanism of CO_2 is the Bohr effect, which seems to be responsible for changes following its application, a decrease in oxygen-haemoglobin concentrations, an increase in partial oxygen pressure, and a reduction of peripheral tissue *p*H [5]. A recent study (Italy) investigated the specific effects of successive carbon dioxide (CO_2) baths (carbon dioxide-enriched water, 1553 mg CO_2 per kg water) on the release of plasma free radicals and total antioxidant capacity in patients with stage 2 chronic obliterate arteriopathy, demonstrating a reduction of oxidative stress after 2 weeks of balneal therapy with carbonated mineral water [4].

Although numerous uses of carbonated mineral baths have been reported, their effect in ischemic heart disease is not yet sufficiently known. Ischemic heart disease is caused by coronary atherosclerosis, its pathogenesis being explained through the hypothesis of endothelial dysfunction; the role of oxidative stress and antioxidant systems in cardiovascular diseases is in the process of being researched [6]. The beneficial effects of CO_2 on ischemic vascularization have been documented in many animal models [7].

The aim of this study was to observe the effect of carbonated mineral water on oxidative stress/antioxidant

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parameters in plasma, associated with experimental myocardial ischemia in rats, in order to evaluate if there is a beneficial effect of this non-pharmacological treatment. The effect was evaluated by assessing plasma oxidative stress parameters and antioxidant status, and by histopathology examination of myocardial tissue.

Experimental part

All the procedures of this experimental study were approved by the Ethics Committee of Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca (protocol approval No.391/16.10.2018) and Veterinary Sanitary Committee (Approval No. 146/29.11.2018) and were in accord with the rules of European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. We used Wistar-Bratislava albino rats weighing 250-300 g, procured from de Department from the Animal Department of the Faculty of Medicine and Pharmacy Cluj-Napoca, Cluj-Napoca, Romania and were kept in polypropylene cages at constant temperature (24 ± 2 °C), 50 ± 15% humidity, and light-dark regime. All the measures were taken to reduce the animal suffering.

Experimental Design

Five groups with eight animals/group were used by random allocation of rats in the groups. Appropriate protocols were applied to each group (table 1). Myocardial ischemia was induced by an unique dose of Isoproterenol (45 mg/BW, s.c. route) [8]. The animals were introduced in water for swimming for 20 min, each day, for 2 weeks, water temperature 32ºC. Group 2 and 4 swam in distilled water, and the group 5 in carbonated mineral water (CMW) (spring no. 7-Tusnad, Romania). The composition of carbonated mineral water was as following: chlorine 49.999 mmoli/L, bromine 0.020 mmoli/L, sulphates 0.0666 mmoli/L, $HCO3^-$ 13.000mmol/L, sodium 44.161 mmol/L, potassium 2.307 mmoli/L, calcium 4.698 mmol/L, magnesium 3.088 mmol/L, iron 0.376 mmol/L, carbon dioxide 27.000 mmol/L, hydrogen sulphide (H_2S) 122.036/ L. *p*H 5.8 Total mineralization is of the water is 122.036 mmol/L.general characteristics of this water refers as an carbonated, ferruginous, chlorinated, bicarbonate, sodium, hypotonic natural mineral water. Oxidative stress parameters and antioxidant plasma status were assessed following the same method as Bulboaca *et al.* [9].

Histopathology examination

At the end of the experiment myocardial tissue samples were taken from each animals and prepared for optic microscopic examination. Myocardium tissue samples were about 4 mm thickness and were treated with Stieve solution for 48 h. The sample were further dehydrated with ethylic alcohol, treated with butyric alcohol and included in paraffin. The paraffin block was used for sample cutting (5 µm thickness each sample) and tricrom Goldner stained was made. The sample observation was made by optic microscope (Olympus BX 41).

Analysis of the data

Statistical analysis was made by Statistix 10 software, and presented as mean ± SD. Mann-Whitney test was used for statistical comparisons, and $p<0.05$ was considered statistically significant.

Results and discussions

The values of oxidative stress /antioxidant status parameters are presented in table 2.

The comparison between groups is shown in table 3.

Comparing group 2 with group 1 a statistical significant difference was noted ($p<0.0001$), regarding the plasma level of NOx and TOS without significant changes for antioxidative status. These results showed that physical activity as is swimming itself is associated with increased oxidative stress. Similar results were noted by other studies [10]. At comparison of group 3 with group 2 and 1, all the parameter changes were found to have a high significant statistic differences ($p<0.00001$). Similar results were found comparing group 4 with group 2 ($p<0.00001$) for all studied parameters. Comparison of oxidative parameters and antioxidant plasma status for group 4 and 3, was found

Group No	Group description
1	Control group
2	Swimming in distilled water
3	Isoproterenol induced myocardial ischemia
4	Isoproterenol induced myocardial ischemia + swimming in DW*
5	Isoproterenol induced myocardial ischemia + swimming in CMW**

*DW = distillate water, ** CMW = carbonated mineral water

Table 1
EXPERIMENTAL GROUPS, PROTOCOL DESCRIPTION

Group number	NOx (µmol/L)	MDA (pmol/L)	TOS (µmol/L)	CAT U/ml)	TAC (Eq/L)
1.	25.82 ± 1.03	2.275 ± 0.21	16.25 ± 1.39	18.78 ± 1.12	1.32 ± 0.14
2.	30.71 ± 1.19	2.30 ± 0.20	21.90 ± 2.06	18.96 ± 1.03	1.23 ± 0.11
3.	43.13 ± 1.27	3.355 ± 0.22	47.34 ± 1.07	14.8 ± 1.12	0.69 ± 0.064
4.	43.675 ± 1.04	3.34 ± 0.19	47.25 ± 0.96	14.68 ± 1.06	0.67 ± 0.06
5.	35.8625 ± 1.52	3.35 ± 0.22	39.57 ± 1.53	16.625 ± 1.60	0.931 ± 0.08

Table 2
OXIDATIVE STRESS PARAMETERS (MEAN ± STANDARD DEVIATION)

1 vs. 2	P< 0.00001	P=0.370923	P< 0.00001	P =0.375402	P=0.107321
3 vs. 1	P< 0.00001	P< 0.00001	P< 0.00001	P< 0.00001	P< 0.00001
3 vs. 2	P< 0.00001	P< 0.00001	P< 0.00001	P< 0.00001	P< 0.00001
4 vs. 2	P< 0.00001	P< 0.00001	P< 0.00001	P< 0.00001	P< 0.00001
4 vs. 3	P=0.186	P=0.472	P=0.436	P=0.419	P=0.279
5 vs. 2	P< 0.00001	P< 0.00001	P< 0.00001	P< 0.0018	P<0.000016
5 vs. 3	P< 0.00001	P=0.48	P<0.00001	P= 0.00973	P< 0.00001
5 vs.4	P<0.00001	P=0.49	P<0.00001	P=0.095	P=0.096

Table 3
GROUPS COMPARISONS (P VALUES)

to have no significant changes. Comparing group 5 with group 3 and 2, all the parameters had statistical significant changes except MDA levels. For group 5 vs. group 4, significant differences were found regarding NOx values and TOS. Improving NOx level can result in improvement of endothelial function, NOx being a valuable biomarker for endothelial function, and also improve endothelium dependent vasodilatation [11](table 3).

Histopathology assessment results

Histopathology assessment for animals in group 3 showed cells with homogenous cytoplasm, inflammatory cells, mostly in perivascular area, and perivascular edema. (fig. 1) The fibrotic tissue was also present in same territories (fig 2). These histopathology changes are associated with ischemia. The histopathology assessment for group 4 showed no remarkable differences compared with group 3, inflammatory cells, perivascular edema and extensive fibrotic tissue were still present and were similar to those in group 3 (fig. 3 and 4). Assessing the samples from group 5 significantly differences were observed in histopathology examination. The inflammatory cells were reduced as number but with perivascular edema persistency, and fibrotic tissue was almost absent (fig5). The conjunctive tissue proliferation was also observed in perivascular areas (fig 6). Histopathology results showed a visible improvement of tissue structure according with associated changes with myocardial ischemia regarding the inflammatory cells abundance, peri inflammatory edema and fibrotic tissue.

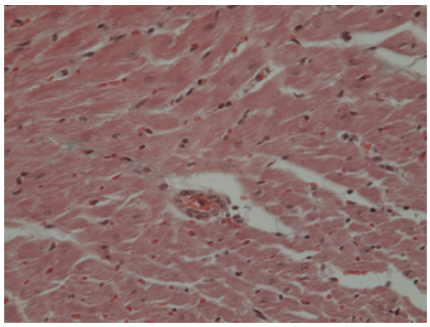

Fig. 1. Histopathological aspect of myocardial tissue - group 3

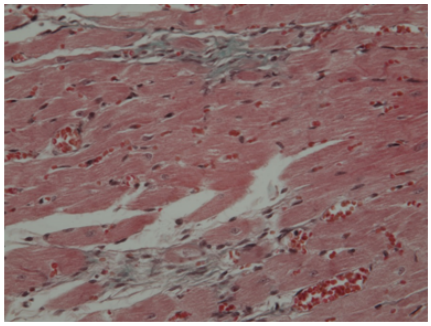

Fig. 2. Histopathology aspect of myocardial tissue - group 3

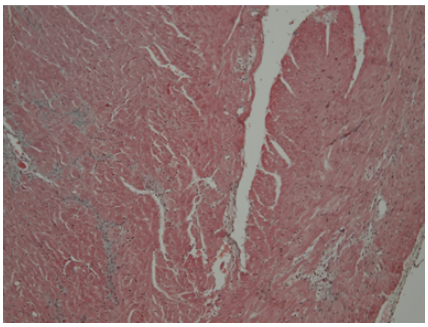

Fig. 3. Histopathology aspect of myocardial tissue -group 4

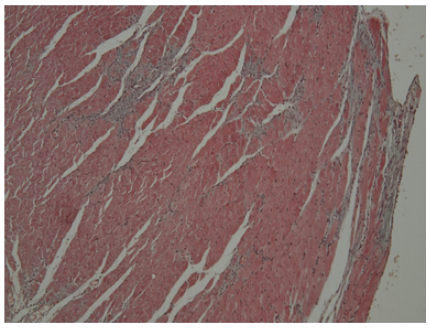

Fig. 4. Histopathology aspect of myocardial tissue -group 4

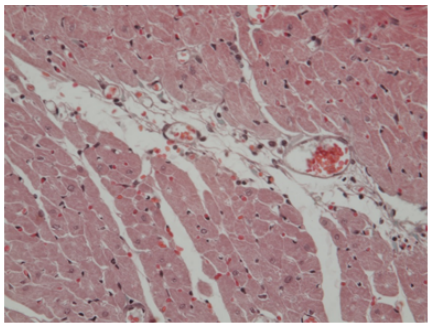

Fig. 5.Histopathology aspect of myocardial tissue -group 5

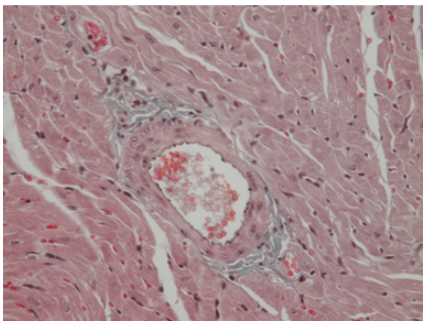

Fig. 6.Histopathology aspect of myocardial tissue -group 5

The effect of CMW on oxidative stress/antioxidant parameters

As a valuable therapy for cardiovascular diseases wide used in European countries, carbonated mineral water effects was studied in various papers. The beneficial effect of CO2, was demonstrated, its vasodilatation effect contributing to improving of vascular blood flow [12]. The composition of CMW used in this experimental study is rich in carbon dioxide, this property being important in reducing ischemic effects occurred after Isoproterenol administration, proved by significantly improving of all the oxidative stress parameter, and also improved characteristic of histopathology changes consequently to myocardial ischemia. The improving of myocardial blood flow under the carbon dioxide action was also found to occur at the similar effect as the adenosine action [13]. The effect of carbon dioxide seems to be similar with those exert by carbon monoxide and nitric oxide, inducing vasodilatation associated with smooth cells proliferation reduction, and having an inhibitor effect on platelets aggregation [14,15]. In vivo animal studies with coronary vessels vasoconstriction follow by stenosis (induced by caffeine), vasodilatation effect activity of carbon dioxide was demonstrated with significantly improved coronary blood flow (PET studies of the heart) [16]. The oxidative stress contribution to ischemia has been intensively studied, with a special attention given to quantitative assessment of oxidative stress parameters and their relationship with the ischemia progression and consequences. There are other experimental studies that proved that immersion in a bath with carbon dioxide content is floored by an efficient absorption of carbon dioxide trough the skin with an important improving of tissues perfusions. The absorption of carbon dioxide due

to immersion in CMW was sustained by elevated levels of carbon dioxide concentration in subcutaneous tissues [17]. Moreover, was also demonstrated, an improving of oxygen delivery to the tissues, by haemoglobin, due to shifts of the haemoglobin dissociation curve to the right [17]. By improving the NO levels CMW treatment can contribute to reducing endothelial dysfunction in patients with arterial stenosis due to atherosclerotic process [18]. Other cardiovascular effects of carbon dioxide rich baths could be bradycardia, reducing blood pressure [4]. Our study also proved a significant reduction of MDA and total oxidative stress that are important contributors in ischemia progression and lesions amplification. Antioxidant status improvement can also constitute an essential factor that is beneficial in ischemic process limitation and rehabilitation. Detection and quantification of periodontal pathogens is of great importance in the clinical management of myocardial ischemia who is often associated with periodontal disease [19].

Conclusions

From our knowledge, this is the first study which assessed the efficiency of CMW treatment effect in experimental myocardial ischemia with mineral waters with presented chemical and physical characteristics as above described. Baths with natural carbonated mineral water can be an effective therapeutic method in the treatment of cardiovascular diseases, but the periodic analysis of their physical and chemical properties is important. Further studies regarding their therapeutic efficacy and mechanism of action are required.

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