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ORIGINAL ARTICLE

Possible antioxidant role of SPA therapy with chlorine–sulphur–bicarbonate mineral water

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Abstract The aim of our research was to analyze the antioxidant role and efficacy of thermal or salus per aquam (spa) therapy with chlorine–sulphur–bicarbonate mineral water. The study has been performed on 30 rats. The animals were randomized in three groups, each of them composed by ten animals, denominated A, B and C. The A group was the control group and was not subjected to any specific treatment (placebo); the B group has been treated with a standard cycle of hydropinics treatment with mineral water of Therme of Stabia in Castellammare (Naples, Italy) denominated STABIA; the C group was treated with a standard cycle of hydropinic treatment with mineral water of Therme of Stabia in Castellammare (Naples, Italy) denominated SULFUREA. After two weeks of treatment all the rats were sacrificed and blood was collected for the plasmatic determination of reactive oxygen species (ROS). The results demonstrated a significant ($P < 0.05$) reduction of ROS in B (374 Carr. U. ±73) and C group (399 carr. U. ±62) treated with mineral waters if compared with control group (571 + 69 Carr. U.). In conclusion this study suggests a possible antioxidant effect of chlorine–sulphur–bicarbonate spa hydropinic treatment with a consequent suitable intestinal physiology, with reduction of the functional and organic modifications that can lead to pathological disorders of the gastroenteric diseases in whose pathogenesis the oxidative stress can develop an important role.

Keywords Spa therapy · Sulphur mineral water · Reactive radicals of the oxygen · Free radicals · Oxidative stress · Polyamines

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Introduction

In the last years it is becoming even more evident the demand of a scientific validation of thermal therapy according to the modern pharmacology and biology methods in order to give scientific dignity to the so-called therapy with mineral water (SPA therapy).

Until today the mechanisms of action of spa therapy in treatment of gastroenteric diseases are only partially known (Pignataro et al. 1998; Paolucci et al. 2001; Cuomo et al. 2002; Costantino et al. 2005).

The free radicals are atoms, ions or molecules that introduce one or more electrons separated in the orbital outside and therefore they are very unstable and reactive. This brings them to seek the equilibrium either by lacking an electron, or gaining it from the molecules that enter the reaction, consequently making them unstable (Cornelli et al. 2000; Alberti et al. 2000). This mechanism produces a chain of reactions that modifies in irreversible way the chemistry of some cellular compounds (Franzini et al. 1996).

Depending upon the nature of the atom to which the separated electron belongs, we can distinguish nitrogen, chlorine, carbon and oxygen radicals.

Among the radicals of the oxygen the most important and dangerous are the hydroxyl (HO^-) radicals and the radical (or anion) superoxide (O_2^-) that together with the hydrogen peroxide (H_2O_2) and with the oxygen singlet (O_2^+) constitute the reactive radicals of the oxygen (Dionisio et al. 1999).

The destructive action of the free radicals is directed toward lipids of cellular membranes, nucleic acids and proteins with consequent cellular structural alterations that are then at the basis of different diseases including cellular premature aging, cancer, cardiovascular diseases, diabetes, osteoarthritis, rheumatoid arthritis, psoriasis, gastroenteric diseases etc. (Tanganelli et al. 2000; Olguín-Matínez et al. 2006). Furthermore, an important anti-oxidant and medical scavenging role of polyamines has been reported. The model system in use to demonstrate protection by polyamines against lipid peroxidation and DNA cell damage was reported by Lovaas (1997).

On the basis of these considerations the aim of our study was to investigate on the possible antioxidant role of spa hydropinic therapy used in the treatment of various gastroenteric diseases.

Materials and methods

In vivo studies

The chlorine sulphur bicarbonate mineral waters of Stabia Spa in Castellammare (Naples, Italy), denominated STABIA and SULFUREA, were used for this study (Tables 1, 2).

The experiments have been performed on 30 rats of both sexes (not pregnant). In details, 15 males, have a mean weight of 286 ± 18 g and 15 females, have a mean weight of 282 ± 21 g. All the animals, maintained in fit conditions of nutrition and environment ($T = 21°C$; Humidity ratio = 55% ± 10 with cycles light–dark of 12 h) in agreement to the dispositions of the DL 116/1992, have been treated only after an adequate time of acclimatization.

Treatment schedule

At the beginning of the experimental work the animals were randomized in three experimental groups (A, B and C) each formed by ten animals, all including five males and five females.

The three groups of animals have been treated for 2 weeks with standard diets in pellets and drank to libitum with tap water (A group control-placebo), with chlorine sulphur bicarbonate mineral water denominated STABIA (B group) and with chlorine sulphur bicarbonate mineral water denominated SULFUREA (C group).

Table 1 Chemical composition of the chlorine sulphur bicarbonate mineral water from Therme of Stabia denominated STABIA

Total dissolved solids (mg/l)	15,670
Electrical conducibility (ms/cm)	19.96
pH	6.44
Free carbon dioxide (mg/l)	712
H_2S (mg/l)	2.74
Na^+ (mg/l)	5,236
K^+ (mg/l)	182
Ca^{++} (mg/l)	588
Mg^{++} (mg/l)	488
Li^+ (mg/l)	0.099
Cl^- (mg/l)	9,658
F^- (mg/l)	2.00
HCO_3^- (mg/l)	759
SO_4^- (mg/l)	759
SiO_2 (mg/l)	9.4

Table 2 Chemical composition of the chlorine sulphur bicarbonate mineral water from Therme of Stabia denominated SULFUREA

Total dissolved solids (mg/l)	15,482
Electrical conducibility (ms/cm)	20.29
pH	6.45
Free carbon dioxide (mg/l)	794
H_2S (mg/l)	2.40
Na^+ (mg/l)	5,028
K^+ (mg/l)	173
Ca^{++} (mg/l)	597
Mg^{++} (mg/l)	401
Li^+ (mg/l)	0.093
Cl^- (mg/l)	9,145
F^- (mg/l)	2.1
HCO_3^- (mg/l)	847
SO_4^- (mg/l)	554
SiO_2 (mg/l)	2.8

Reactive oxygen species (ROS) determination

ROS were evaluated in plasma samples by the use of "d-ROM test" (Diacron s.r.l., Grosseto, Italy) (Cesarone et al. 1999).

After 2 weeks of treatment, each rat, after anaesthesia due to the intraperitoneal injection of ketamine (80 mg/kg), was sacrificed and blood samples were collected in order to

determine the plasmatic ROS. In this test, plasmatic ROS (mainly hydroperoxides), in presence of iron (that is released from plasma proteins by an acidic buffer), are able to generate alkoxyl and peroxyl radicals, according to the Fenton's reaction. Such radicals, in turn, are able to oxidize an alkyl-substituted aromatic amine (that is dissolved in a chromogenic mixture) thus transforming them in a pink derivative photometrically quantified at 505 nm. The results of d-ROM test are expressed in arbitrary units called "Carratelli Units" (CARR U), 1 CARR U corresponds to 0.08 mg of H2O2/dl. The linearity range of d-ROM test is between 50 and 500 CARR U, intra-assay coefficient of variation is 2.1%, while inter-assay is 3.1%. Reference values of healthy subjects are between 250 and 300 CARR U; conditions of slight, medium and high oxidative stress are defined, respectively, by values of 320–360, 360–400 and >400 CARR U).

Statistical analysis

Results are expressed as mean ± standard deviation (SD); n represents the number of studied animals. The significance of differences was evaluated by Student's *t*-test for unpaired data. Probability values <0.05 were accepted (Lison 1989).

Results

Adverse effects

During the experiments no adverse reactions have been observed in the groups of animals subjected to thermal or spa treatments.

The analysis of the data has demonstrated that the two types of mineral waters used, respectively denominated STABIA and SULFUREA, did not induce some case of death, neither diarrhoea, neither ruffled hair, neither lethargy.

In comparison to the basal values, we have not observed, at end of the treatment, any variation of the body weight (Table 3).

Effects on ROS plasmatic concentration

The spa treatments with mineral water denominated STABIA (B group: 374 Carr.U. ± 73) and SULFUREA (C group: 399 Carr.U. ± 62) induced a significant ($P < 0.05$) reduction of ROM plasmatic levels if compared with the placebo group (A group: 571 Carr.U. ± 69) (Table 4). The reduction results slightly greater in the group treated with mineral water denominated STABIA.

Discussion

Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage (Cornelli et al. 2000). The production of peroxides and free radicals damages all components of the cell, including proteins, lipids, and DNA leading to cell death, mutations, and other toxicities (Alberti et al. 2000; Olguín-Matínez et al. 2006). Recent studies have shown a correlation between the oxidative stress and the development of diseases as diabetes, gastric carcinoma, Helicobacter Pylori, hepatic steatosis (Dionisio et al. 1999; Tanganelli et al. 2000; Oliveira et al. 2003; Zhang and Farthing 2005; Parkas et al. 2005; Bancel et al. 2006).

Clinical studies have suggested that mineral waters are valid tools in the treatment of a large number of disorders including liver, intestine and digestive apparatus (Pignataro et al. 1998; Paolucci et al. 2001; Cuomo et al. 2002; Costantino et al. 2005).

The SPA hydropinic therapy is particularly indicated for disease of the gastrointestinal apparatus and consists in drinking mineral waters.

As regard the disorders of digestive apparatus very little is known on the biochemical effects of mineral waters.

Mineral waters contain different types of dissolved substances, namely minerals and other biological compounds. Mineral water's "special" mineral composition might have the properties favourable to health.

For example: *carbonic waters*, prevalently characterised by the presence of the HCO_3^- ion, improve gastric and cholecystic emptying and the intestinal transit time in

Table 3 Variations of the body weight in rats watered through drank to libitum with chlorine–sulphur–bicarbonate mineral waters versus control group before and after spa hydropinic treatment for 2 weeks

Group considered (treatment)	weight (g ± DS) before treatment	weight (g ± DS) after treatment	Student's "*t*" test
A group (CONTROL) $n = 10$	270.2 ± 20	272.1 ± 21	$P > 0.05$
B group (mineral water denominated STABIA) $n = 10$	292.5 ± 16	293.6 ± 15	$P > 0.05$
C group (mineral water denominated SULFUREA) $n = 10$	290.1 ± 14	290.4 ± 13	$P > 0.05$

Table 4 Effects of the hydropinic treatments with chlorine–sulphur–bicarbonate mineral waters considered versus control on the plasmatic concentration of the ROS (expressed in Carr.U. ± SD) in rats watered through drank to libitum for two weeks

Group considered (treatment)	(ROM) in Carr.U. ± SD (% of reduction vs control group)
A group (control) $n = 10$	571 ± 69
B group (mineral water denominated STABIA) $n = 10$	374 ± 73** (−35%)
GRUPPO C (mineral water denominated SULFUREA) $n = 10$	399 ± 62** (−30%)

* $P < 0.05$ versus control group

** $P < 0.01$ versus control group

patients with functional dyspepsia and with hydiopathic constipation (2); improve the function of fibers on stool frequency in adult patients with functional hydiopathic constipation (Pignataro et al. 1998); carbonic water supplementation improves the symptoms of abnormal gastrointestinal motility (Paolucci et al. 2001).

The mechanisms of action on gastrointestinal activity of the *sodium-chlorine mineral water* are not completely clear. The biological effects of ions contained in mineral water may directly or indirectly (via neuroendocrine secretion of vasointestinal active peptides) stimulate smooth muscle to increase the gastrointestinal motility.

The spa therapy can improve gastric acid output, orocecal transit time in patients with functional dyspepsia or irritable bowel syndrome (Paolucci et al. 2001).

The effects exerted by mineral water include stimulation of chemoreceptors and baroceptors in the gastric walls. The well known effect of water on intestinal motility (due to osmotic properties that induce a water adsorption) increases intestinal transit time and induces an early duodenal transit of food with an earlier relaxation of pylorus and a faster transit of bolus from stomach to duodenum. These actions reduce the transit time of stool and increase the number of evacuations in a day.

The *sulphur mineral water*, containing sulphydric acid (H_2S), shows evident anti-inflammatory properties (Gupta and Nicol 2004; Rinaldi et al. 2006), and induces an increase in the levels of IgA, IgM and IgG.

In literature, a report demonstrated the role of sulphur in the biosynthesis of vital cofactors and in sulphating of glycoproteins in the gastrointestinal tract (Mulder and Jakoby 1990).

The sulfation is a key step in detoxication or in the process of excretion of toxic metabolites in excess.

On the basis of the considerations we have evaluated the possible antioxidant role of spa hydropinic therapy with chlorine–sulphur–bicarbonate mineral water.

The results seem to suggest a possible antioxidant action of the spa hydropinic treatment with chlorine–sulphur–bicarbonate water for the significant reduction of the (ROM) in comparison to the control in the treated animals.

During the processes of digestion and progression of the food, a series of cellular reactions, that induced the massive production of ROS, are involved. If the ROS are not under control they could inhibit antiproteases, causing an increase in proteases activity and consequent damages in the intestinal microvasis, and therefore an anomalous passage of substances (not digested proteins, fibres, etc.) that produce local inflammatory reactions.

These observations raise the importance of reducing the action of ROM to favour the maintenance of a suitable intestinal physiology and to prevent the appearance of gastroenteric pathological conditions.

The spa hydropinic treatment with chlorine–sulphur–bicarbonate mineral water seems to be able to guarantee the significant antioxidant effect shown.

These antioxidant effects can be correlated to the chemical characteristics of the mineral water used, and in particular to the presence of Mg^{++}, bicarbonate, sulphate ions and sulphydric acid.

In literature it is reported the protective role of the Mg^{++} salts against the oxidative injury through the enzymatic activity of the catalase, glutathione peroxidase and superoxide dismutase (Orrenius 1984; Tsan et al. 1985; Ketterer 1986; Bronzetti 2000) and the importance of the food integration with bicarbonate ions in the prevention of the aging (Bacci 2003); it is also known that sulphur containing mineral water represents an important source for human organism and enhances the levels of glutathione thus neutralizing the free radicals (Ciaccia et al. 1983).

In conclusion, the results of this study suggest a possible antioxidant effect of chlorine sulphur bicarbonate spa hydropinic treatment in the gastroenteric oxidative stress diseases with a consequent improvement in the intestinal physiology.

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