Mineral Water Vincentka and its Influence on Mucosal Ulcers

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Summary

Gastropathy is one of the most common diseases of the human gastrointestinal tract. Apart from its consequences in the stomach, it is also manifested in other parts of the digestive tract, particularly in the duodenum. The aim of this pilot study was to verify on animal model the empirically observed alleviation of gastropathy symptoms in patients who underwent a drinking treatment of Vincentka natural mineral water during their spa treatment. Sixteen male Wistar rats were included in the study. The animals were randomly divided into two groups: experimental group (E; n=8) and control group (C; n=8). The experimental protocol consisted of three phases: (1) handling phase (7 days); (2) mineral water (E)/tap water (C) administration (7 days); (3) acute gastritis induction (1 day). Twenty-four hours after the induction of acute gastritis, the animals were sacrificed. The collected tissues (stomach and duodenum) and blood were examined by standard histological microscopy, and by immunohistochemical and biochemical methods. Histopathological analysis revealed significantly reduced damage to the gastric mucosa in the experimental group. Significantly different values of blood plasma antioxidant capacity, oxidative stress parameters and blood plasma biochemical parameters were also found. Based on these results, we conclude that the mineral water Vincentka has a positive impact on development and symptoms of acute gastric ulcers.

Key words

Mineral water Vincentka • Gastric ulcer • Oxidative stress

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Introduction

Dyspepsia, epigastric pain, nausea, vomiting, and heartburn are common gastrointestinal symptoms, occasionally experienced by the majority of people at some point in their lifetime. The main cause of these symptoms is inflammation of the upper gastrointestinal tract, which begins as a response to different agents and can be manifested by various morphological and clinical symptoms [1-3]. Its extent and distribution are related to the etiology of the disease, its progression and recurrence. The most frequent diagnosis in the patients with these symptoms are acute or chronic gastritis and related gastropathy, where *Helicobacter pylori* (*H. pylor*i) infection and nonsteroidal anti-inflammatory drugs (NSAID) use are the major causative factors in the pathogenesis [4]. Therapy of these diseases is focused on elimination of the etiological factors and pharmacologically relies on the use of proton pump inhibitors [5]. In case of peptic ulcers, surgical therapy is used only in patients at risk of ulcer malignization [6].

Except for classical treatment, balneotherapy or herbal medicine are commonly used as a supporting therapy or as a preventive measure in the remission stage [7,8]. Balneotherapy is one of the most commonly used

non-pharmacological complementary therapies in gastrointestinal, metabolic, respiratory, cardiovascular, and excretory system diseases, as well as in inflammatory and degenerative processes [9-11]. Balneotherapy includes both local and whole-body baths, various types of showers, inhalation therapy, irrigation and bowel lavage, and last but not least drinking of mineral water. External therapy (baths, showers and wrap therapy) is mainly used in dermatology and musculoskeletal system disorders, whereas drinking therapy, inhalation and irrigation are widely used in respiratory or gastrointestinal diseases [8,11,12].

Vincentka is mineral water from a natural healing source. It is a residual sea water from the Tertiary period. Vincentka is highly mineralized water, with increased content of lithium, barium, fluoride and boric acid, and with pH 6.45. The water includes, among others: bicarbonates 4820 mg/l, sodium 2280 mg/l, chlorides 1480 mg/l, calcium 261 mg/l, potassium 126 mg/l, magnesium 16 mg/l, barium 11 mg/l, lithium 9 mg/l, iodides 6 mg/l, bromides 5 mg/l, iron 4 mg/l, fluorides 3 mg/l, strontium 3 mg/l. As of micronutrients, Vincentka includes manganese, aluminum, beryllium, vanadium, copper, nickel, zinc, cesium, rubidium, sulphate, and hydrogen phosphates. Boric acid (310 mg/l) and silicic acid (24 mg/l) are also present in this mineral water [9,13]. Due to its chemical composition, osmolality and pH, Vincentka is commonly used for inhalation, gargling and rinsing in almost all respiratory disorders (inflammations of the upper and lower respiratory tract, convalescence after respiratory tract surgeries, after trauma and occupational respiratory diseases, occupational injuries of the vocal cords, lung transplantations) [8,9]. Other indications for the drinking therapy are related to diabetes mellitus and other metabolic diseases, such as gastric or duodenal ulcers, postcholecystectomic syndromes and other postoperative GIT conditions, in chronic inflammation of the liver, chronic inflammation of the pancreas etc. [8,9].

The aim of this pilot study was to verify on animal model the empirically observed alleviation or disappearance of gastric ulceration symptoms in patients after drinking of the mineral water Vincentka during the spa treatment.

Methods

All experiments were carried out according to the recommendations of the European Community Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Committee for Ensuring the Welfare of Laboratory Animals of Masaryk University and licensed by the Ministry of Education, Youth and Sports of the Czech Republic.

Sixteen male Wistar rats (body mass of 240-280 g) were included in the study. The animals were housed at the Animal Breeding and Experimental Facility, Faculty of Medicine, Masaryk University, in a room accredited for housing of laboratory animals, in standard cages (40×26×20 cm) in groups in standard pressure, temperature (21.00±1.20 °C), and relative humidity (78-87 %), and with light cycle 12/12 (light/dark). Standard diet (pellet feed, Altromin Spezialfutter GmbH & Co., D) and water were accessible *ad libitum*.

The animals were randomly divided into two groups: experimental group (E; n=8) and control group (C; n=8). Experimental protocol consists of three phases: (1) handling phase (7 days); (2) mineral/drinking water administration (7 days); (3) acute gastritis (1 day). All animals were handled for seven days (the handling phase) to adapt to daily manipulation and use of orogastric tube. All the animals were also regularly weighted. For following seven days, mineral water Vincentka (VINCENTKA a.s., Luhačovice, Czech Republic) was administered to the animals from the group E in the volume of 9 ml/kg of body mass by orogastric tube twice a day (at 9 am and 4 pm). To the animals from the group C, tap water was administered in the corresponding volume. Both mineral water and tap water were administered after approx. one hour of fasting. The next day, acute gastritis was induced by indomethacin in both groups as described previously [14]. Indomethacin was administered by orogastric tube in the dose of 100 mg/kg diluted in distilled water (administered volume of 10 ml/kg). Twenty-four hours after indomethacin administration, the animals were deeply anesthetized by mixture of ketamine and xylazine (100 mg/kg i.p. and 10 mg/kg i.p. respectively). A sample of EDTA anticoagulated blood was collected by intracardiac puncture. The animals were then sacrificed and dissected. Stomach and duodenum were excised *en bloc* for histopathological analysis.

Excised stomach and duodenum were fixed in 10 % neutral buffered formalin for 24 h. Then, all lesions were examined, sampled, and embedded in paraffin. Specimens were sectioned at a thickness of 3 μm and applied on positively charged slides. Tissue sections were

stained with hematoxylin-eosin and reviewed by pathologist (MH). Peptic erosions or ulcers were assessed according to the ulcer score introduced by Kim *et al.* [15]. Each lesion was evaluated independently in length and depth and ulcer score was calculated according to the Table 1. Final score was assessed in both duodenum and stomach independently and represents sum of scores of all detected lesions per stomach/duodenum.

Table 1. Determination of ulcer score in stomach and duodenum.

Ulcer score = depth × length		
Depth	Erosion affecting 1/3 of mucosa	1
	Erosion affecting 2/3 of mucosa	2
	Ulcer affecting the whole mucosa	3
Length	Erosion or ulcer up to 1 mm	2
	Erosion or ulcer larger than 1 mm	3

Adopted from Kim *et al.* [15].

The blood plasma was separated by centrifugation immediately after blood collection and divided into aliquots. Plasma samples were stored at -80 °C until further analyses. Plasma was used for the estimation of the antioxidant capacity (SH – sulfhydryl groups; ABTS – 2, 2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid; FRAP – ferric reducing antioxidant power) and of the oxidative stress (MDA – malondialdehyde; ROS – reactive oxygen species; hydrogen peroxide; carbonylated proteins) parameters.

We used dichlorofluorescein assay for the ROS production estimation. The method is based on the formation of highly fluorescent 2',7'-dichlorofluorescein, intensity of the fluorescence is measured with spectrofluorometer [16]. Spectrophotometry was used for the SH, FRAP, MDA, hydrogen peroxide and carbonylated proteins estimation. MDA is an end product of lipid peroxidation, for MDA estimation we used a protocol which was described by Pederson, Reznick protocol was used for carbonylated proteins estimation, plasma SH content was determined by using the method described by Sedlak, and ABTS, FRAP, and hydrogen peroxide were estimated according to protocol which was represented by Chaturvedi [17-20]. Statistical analyses of obtained data were performed in GraphPad Prism 5 (GraphPad Software, CA, USA). Unpaired *t*-test was employed for comparison of measured parameters between the groups. p-value below 0.05 was considered as significant. Results are expressed as mean ± SD or as median (lower quartile – upper quartile).

Results

Ulcer score

Histopathological analysis revealed significantly reduced damage of gastric mucosa in the group E (ulcer score 3.38±4.93) as compared to animals in group C (ulcer score 14.38±7.39; p=0.0035). Five out of 8 animals from the group E showed no detectable erosion of gastric mucosa. For comparison, at least one gastric erosion was detected in every control animal. Duodenal erosions were modest and less frequent in both groups with no significant difference between them. Table 2 summarizes mean ulcer score in stomach and duodenum in both groups. Figures 1 and 2 represent histological specimen of the stomach and duodenum in group E and C.

Table 2. Ulcer score in stomach and duodenum in group E and C.

Ulcer score	**Group E**	**Group C**	***t*-test (E vs. C)**
Stomach	3.38±4.93	14.38±7.39	p<0.01
Duodenum	2.25±4.06	1.88±3.56	NS

Parameters are expressed as mean ± SD. Mineral water using group (group E); control group (group C); NS (not significant).

Blood plasma parameters

We found a significant difference in the following plasma antioxidant capacity parameters in groups E and C: SH (225.5 vs. 184.6 μmol/l; p<0.05), ABTS (0.104 vs. 0.103 mg GA/ml; p<0.05). FRAP did not show any significant difference between E and C groups.

In blood plasma markers of oxidative stress, we found significant difference only in MDA in groups E and C (4.9 vs. 5.5 nmol/mg of proteins; p<0.05). Carbonylated proteins, ROS, and hydrogen peroxide are not significantly different in a comparison between groups E and C. All blood plasma parameters are represented in Table 3.

Discussion

The main mechanism of action of the majority of NSAIDs including indomethacin is nonselective inhibition of cyclooxygenase 1 and 2 (COX), which

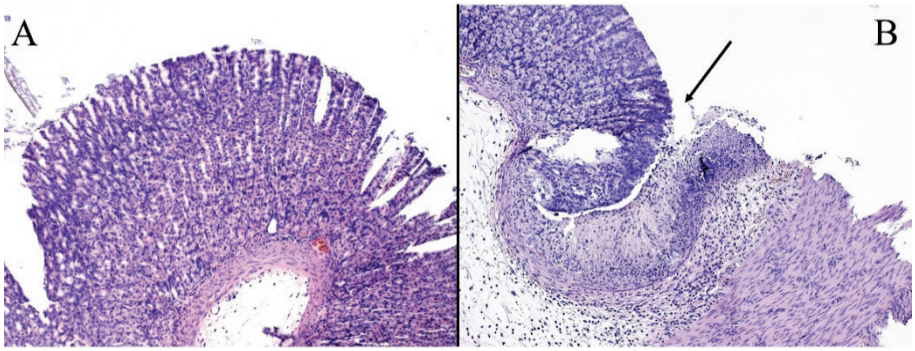

Fig. 1. Histological specimen of the stomach in group E and C. (**A**) – stomach without ulcer; (**B**) – stomach ulcer; group E – mineral water using group; group C – control group; the arrow is pointing on the mucosal ulcer.

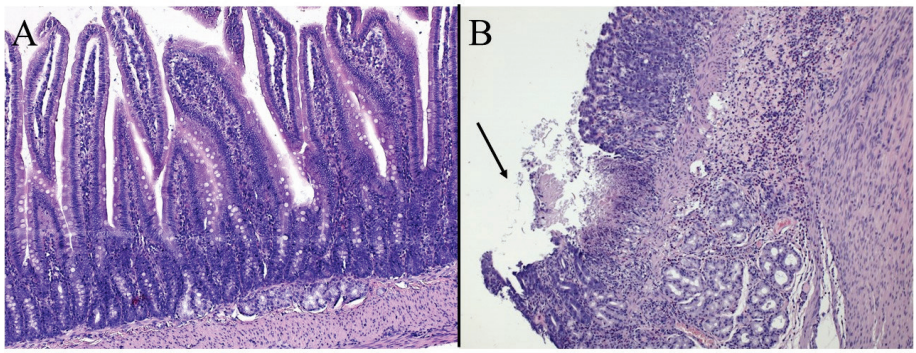

Fig. 2. Histological specimen of the duodenum in group E and C. (**A**) – duodenum without ulcer; (**B**) – duodenum ulcer; group E – mineral water using group; group C – control group; the arrow is pointing on the mucosal ulcer.

leads to reduced synthesis of prostaglandins (PGs) [21]. PGs are crucial in the regulation of homeostasis and the restriction of their synthesis leads to multiple negative consequences. Among the most important systemic consequences of COX inhibition are the vascular effects, which include stronger vasoconstriction and increased platelet aggregation. These changes disrupt the microcirculation of gastric mucosa and cause hypoxia/anoxia of its cells. NSAIDs also cause disinhibition of the synthesis of hydrochloric acid in the parietal cells of stomach mucosa and reduced secretion of bicarbonates and mucus. Previously mentioned effects of NSAIDs on the gastric mucosa explain the most common side effects of NSAIDs use – gastric ulcers. In this study, this side effect was observed in both groups, with higher ulcer scores in group C. The significant difference of ulcer scores between the groups E and C can be explained by the effects of mineral water Vincentka. The mineral water contents, mainly the bicarbonates, cause hyperemia [8,9]. Moreover, chloride and calcium ions, both abundantly present in the mineral water, have anti-inflammatory effect on gastric mucosa [9]. Based on these mechanisms, we assume that the mineral water Vincentka could be used as a preventive/prophylactic measure against gastric ulcers, possibly even as a part of pharmacotherapy in combination with conventionally prescribed drugs.

Another effect of the mineral water Vincentka is the stimulation of smooth muscle cells in the gastrointestinal walls, which leads to increased motility and more frequent emptying of the stomach [9]. Such effect of mineral water is well known and widely used in the treatment of almost all gastrointestinal diseases (common dyspepsia and functional gastric dyspepsia, gastric ulcers, convalescence after gastric resection, etc.) [8,9]. Surprisingly, we observed this effect in this study as a difference in the duodenal ulcer scores. In spite of the difference being statistically insignificant, we observed higher duodenal ulcer score in the group E, which allows us to suggest that the increased motility of the proximal GIT induced the side effects of indomethacin in the duodenum instead of the stomach.

Naito and Yoshikawa reported the role of reactive oxygen species (ROS) in indomethacin-induced gastropathy [22]. ROS are generated as a result of a variety of extracellular and intracellular processes and

increased ROS production could cause remodeling of multiple cell-signaling proteins [23]. The remodeling leads to further functional consequences, which manifest as atherosclerosis, diabetes, dysregulated growth, neurodegeneration, inflammation, and aging [24]. It has been shown that in addition to impaired microcirculation and disinhibition of the gastric glands function, indomethacin-induced gastropathy is caused by ROS-mediated oxidation of important biomolecules (including lipid, protein and DNA structures) which as a result causes increased gastric epithelial cell apoptosis [4,22].

Our results show lower parameters of oxidative stress in group E in comparison to group C. Interestingly, not all of the parameters are significantly lower in group E. A detailed analysis of indomethacin-induced gastropathy shows that the superoxide radicals or superoxide radical-derived toxic substances play a crucial role in gastric cells impairment, whereas hydrogen peroxide by itself does not participate in this process at all [22]. Similar results were obtained in the present study: insignificant changes regarding the hydrogen peroxide and significant changes in MDA, with lower values observed in group E.

An indirect marker of the oxidative stress is plasma antioxidant capacity. This parameter could reflect the altered oxidation-reduction balance in several health conditions such as lung cancer, inflammation, diabetes mellitus, aging, and cardiovascular system diseases [25,26]. Moreover, the plasma antioxidant capacity is crucial for the understanding of the relationship between type of diet, oxidative stress and human diseases [25]. It has been shown that the composition and quality of food affect the plasma antioxidant capacity in respondents with different medical condition (healthy young respondents, postmenopausal women) [27]. Herken *et al.* reported the positive effects of sulfites on total plasma antioxidant capacity [28]. Sulfites have various uses in the food industry as they prevent oxidation and color changes of light-colored fruits and vegetables, help to control microbial growth in fermented beverages, preserve flavor and prevent spoilage of food and drinks [28,29]. The sulfite anion has many possible targets in the human body and could participate in the stabilization of high blood pressure, management of ischemia, or conditions affecting the kidneys or the nervous system [30]. Moreover, the possible negative toxic effect of sulfite anion is eliminated via sulfite oxidase, which transforms the sulfite anion into the sulfate anion, which is easier to be excreted from the human body [28]. We observed the positive effects of sulfate and sulfite ions and their derivates on plasma antioxidant capacity where group E had significantly higher SH and ABTS values in comparison with group C.

Based on our results, it can be concluded that the mineral water Vincentka has a positive impact on development and symptoms of acute gastric ulcers.

Conflict of Interest

There is no conflict of interest.

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