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Communication

Effect of Iodide on Total Antioxidant Status of Human Serum

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Free radicals and subsequent lipid peroxidation have been implicated in the pathogenesis of several degenerative and chronic diseases which are also treated frequently in spas. There are some data arising from previous studies which support an antioxidant or scavenging effect of iodide, being the essential ingredient of a therapeutically used local brine. The aim of the study was to test the antioxidant capacity of iodide in human serum. For this reason we measured the so-called Total Antioxidant Status determined by a colorimetric method, which reflects the protection against the attack of reactive oxygen species, including enzymic and non-enzymic antioxidants. Exogenous iodide applied as NaI, shows a significantly increased antioxidant capacity in comparison with NaCl at a concentration of 15 μM, which is quite comparable to the upper range of serum iodide levels achieved through balneo-therapeutical intervention. This result is in accordance with previous results from *in vitro* depolymerization experiments with hyaluronic acid. The antioxidant effect of 15 μM NaI has been found to be approaching the physiologically relevant concentration of ascorbic acid (50 μM). Copyright © 2000 John Wiley & Sons, Ltd.

KEY WORDS — antioxidant; iodide; free radicals; crenotherapy (balneotherapy); cures; degenerative diseases

INTRODUCTION

Oxidative damage is considered to play an important role in the pathogenesis of several disorders. For example patients with degenerative and chronic diseases e.g. atherosclerosis, diabetes mellitus, joint diseases, cataract or diseases of the respiratory system suffer from increased oxidative stress. In healthy subjects, oxidative damage is largely prevented by a very complex antioxidant system, consisting of several enzymic and non-enzymic components, which act cooperatively to provide better protection against free radical attack. Several methods to determine the degenerative effects of oxidative stress have been published. The determination of the Total Antioxidant Status (TAS) has been proven to be a relatively simple and useful assay to estimate the antioxidative capacity in serum or plasma without consideration of individual pro- or antioxidative components.

The aim of this study was to test the influence of iodide, added to human serum in concentrations comparable to those which could also be achieved with a local iodide brine by therapeutic treatments like drinking cures or iontophoretic treatments. There is some evidence for the antioxidant effects of iodine or iodide (I^-) from studies *in vitro*[1–3] as well as from studies on experimental animals and patients. One of these studies[1] revealed that iodide, thiocyanate and formate are scavengers of $OH^{\bullet}$, preventing the inactivation of phages by ascorbic acid at concentrations in the mM range. In addition, 1 mM potassium iodide has been shown to inhibit $DMPO^{\bullet}$ radical adduct formation by both human polymorphonuclear leukocytes and purified myeloperoxidase.[3] Concerning *in vivo* studies, an increase of plasma catalase and glutathione peroxidase activity was found in patients after drinking cures with an iodine brine.[4,5] The data of the present study were compared to ascorbic acid, an established water-soluble antioxidant.

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MATERIALS AND METHODS

Serum samples of 14 subjects, consisting of six males and eight females with a median age of 36·6 years were used for this study. To 1 ml of serum 10 µl of an appropriately diluted NaCl or NaI solution were added to reach 5, 10 and 15 µM final concentration. The 50 µM ascorbic acid sample was prepared in the same way. Furthermore, we measured the effects of NaI and NaCl in pooled serum, which consisted of three single sera, as external standards. The serum samples were analysed either freshly withdrawn or after storage at −20°C.

The TAS was measured using a colorimetric assay (Randox Laboratories Ltd., Diamond Road, Crumlin, Co. Antrim, UK). The chromogen ABTS® (2,2′-Azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase and hydrogen peroxide to produce the ABTS radical cation. The ABTS radical is detectable due to its blue–green colour, which is measured at 600 nm at 37°C. Antioxidants in the sample suppress the formation of the radical cation to a degree which is proportional to their concentration. All samples were measured in duplicate. Serum samples without any additions served as controls.

The values are expressed as means ± standard deviation. To determine significant differences the paired *t*-test was used. A value of $p < 0{\cdot}05$ was considered to be significantly different.

Table 1. Total antioxidant status (TAS mmol l^{-1}) of human serum after addition of NaCl/NaI and after addition of ascorbic acid.

Additive	*N*	TAS (mmol l^{-1})
—	19	1·180 + 0·286
5 µM NaI	8	1·006 + 0·092
5 µM NaCl	8	0·977 + 0·306
10 µM NaI	10	1·077 + 0·253
10 µM NaCl	10	1·058 + 0·261
15 µM NaI	13	1·428 + 0·106*,†
15 µM NaCl	13	1·221 + 0·228
50 µM ascorbic acid	6	1·573 + 0·020‡

*$p = 0{\cdot}004$ versus 15 µM NaCl, $p = 0{\cdot}022$ versus serum without addition.
†$p = 0{\cdot}004$ versus 50 µM ascorbic acid.
‡$p = 0{\cdot}017$ versus serum without addition.

Table 2. TAS of pooled human serum without and with additions of isomolar NaCl/NaI.

Additive	TAS (mmol l^{-1})
—	1·220
5 µM NaI	1·189
5 µM NaCl	1·165
10 µM NaI	1·159
10 µM NaCl	1·146
15 µM NaI	1·348
15 µM NaCl	1·232

RESULTS AND DISCUSSION

As recommended for the TAS assay, we determined the mean value of a healthy control group, because of regional differences with respect to the incorporation of antioxidants. The mean value obtained for 19 apparently healthy subjects was 1·18 ± 0·29 mmol L^{-1} (range: 1·01–1·67). This is somewhat lower in comparison with a Viennese population which was reported to reach 1·54 ± 0·116 mmol L^{-1} ($n = 156$).[6] The effects of 5, 10 and 15 µM NaI and NaCl and of 50 µM ascorbic acid, respectively, on the TAS of serum samples are summarized in Table 1. The addition of 5 and 10 µM NaI and NaCl was ineffective with respect to enhancing the antioxidative properties of the serum; if anything, decreased protection was shown in comparison to the control samples (without any addition). A significant increase in antioxidant capacity in comparison to the control ($p = 0{\cdot}022$) and to isomolar NaCl ($p = 0{\cdot}004$) was achieved with 15 µM NaI.

In the presence of 50 µM ascorbic acid our samples became most resistant to radical attack ($p < 0{\cdot}05$ compared to 15 µM NaI).

An overview of the antioxidative capacities of the serum samples in the presence of 15 µM NaCl, 15 µM NaI and 50 µM ascorbic acid is given in Figure 1.

As we were not provided with enough serum from one person for a whole test series (0–15 µM NaI/NaCl), we tested, in addition, a whole series produced with pooled serum from three individuals consisting of the same amount of each single serum, thus obtaining sufficient homogenous material. As can be seen from Table 2, there is principally the same result: 15 µM iodide showed an absolute antioxidant effect in comparison to the control, and a tendency to a relative antioxidant effect (I^- more efficient than isomolar CI^{-1}) could be seen even at lower concentrations (5–10 µM).

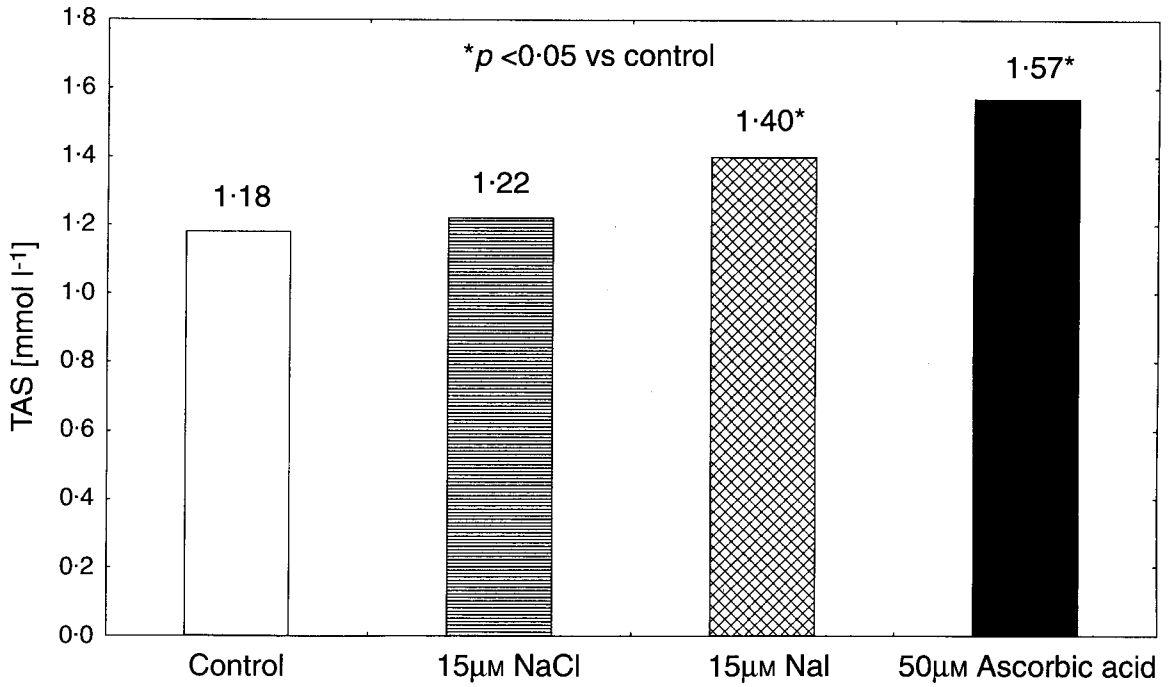

Figure 1. TAS of human serum after addition of 15 μM NaCl, 15 μM NaI and 50 μM ascorbic acid.

Our data support the results from some previous studies that iodide can act as an antioxidant *in vitro* (iodide inhibited the free radical-induced depolymerization of hyaluronic acid)[2] as well as *in vivo* (plasma catalase and glutathione peroxidase were raised in patients after drinking iodine bring).[4] The present effect can be explained either by a direct effect of I^- as an electron donor that quenches free radicals such as $OH^{\bullet}$ [1,3] or more likely, by an indirect mechanism (e.g. iodine as a cofactor of peroxidases or as an activator of other antioxidant or peroxide-eliminating enzymes),[7] leading eventually to an increase of the TAS. Moreover, an adequate iodine supply for the organism is necessary for optimal thyroid function which is also a requirement for a well-functioning antioxidant defence system.[8,9]

The question arises, of whether the effective iodide concentration in our model system can be achieved *in vivo* through the application of an iodine brine as used in the Austrian spa Bad Hall. The total iodine concentration of the local iodine brine is in the range of about 300 μM. According to Krizek *et al.*[10] serum levels can be increased by drinking an iodine brine up to a mean concentration of 3 μM in the 24 h following intake. Presumably there are short-term peaks, where the iodide level is comparable to the most effective concentration in our experiments, of 15 μM. Lieb and Spitzy[11] have found serum levels of total iodine up to ~8 μM after a 20-min inhalation of iodine vapour. Some body structures such as the stomach and connective tissue were found to concentrate iodine in comparison to serum. Above all, it should be emphasized that all concentrations tested indicated an increased antioxidative capacity of NaI in comparison to NaCl. During the course of the depolymerization of hyaluronic acid by UV-light-induced free radicals, a significant radical scavenging effect of iodide was found *in vitro* even down to 3 μM compared with chloride.[2] Ascorbic acid, at a physiologically relevant concentration (50 μM), was the most effective antioxidant used in our series. Vitamin C leads to a significant increase of antioxidant status in our external standard. It shows an enhanced protection of the serum to an extent which exceeded the control (without any addition) of about 30 per cent against attack by free radicals. It is interesting that the antioxidative capacity of 15 μM NaI is only a little below that of 50 μM ascorbic acid.

There are some critical arguments against the use of the TAS assay, namely the lack of specifity, there can be an overwhelming contribution of uric acid

as an antioxidant factor in this system,[12] and the possible occurrence of direct interactions of some antioxidants with the ABTS-radical.[13] Nevertheless, the application of the TAS is meaningful in providing fundamental information of the pro- and antioxidative effect(s) of certain substances. To improve the impact of the TAS, the measurement of the peroxide level and/or vitamin E concentrations in serum or plasma will be very valuable. In this context we recommend the application of the TAS especially in radical-induced or radical-implicated diseases like atherosclerosis, diabetes mellitus or some diseases of the eye.

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