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Improving homocysteine levels through balneotherapy: effects of sulphur baths

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Abstract

Background: Plasma homocysteine (tHcy) is a risk factor for cardio-vascular diseases. Furthermore it has been associated with antioxidative status. Additionally balneotherapeutic sulphur baths have been shown to influence antioxidative status. *Methods*: 40 patients with degenerative osteoarthrosis were randomised into two equal groups, a treatment group, receiving stationary spa therapy plus daily sulphur baths (*sulphur group*) and a control group receiving spa therapy alone (*control group*). Blood tHcy levels and urinary 8-OHdG (an indicator for oxidative stress) were measured at the beginning and the end of spa therapy. *Results*: tHcy (μmol/l) was significantly reduced from 11.41 (± 2.91) to 10.55 (± 2.28) in the *sulphur group* ($p = 0.016$) and rose insignificantly from 12.93 (± 2.28) to 13.80 (± 3.87) in the *control group*. 8-OHdG (ng 8-OHdG/mg creatinine) declined from 18.00 (± 18.28) to 11.16 (± 5.33) in the *sulphur group* (n.s.) and from 17.91 (± 5.87) to 18.17 (± 5.70) in the *control group* (n.s.). Differences between the two groups showed significant effects of sulphur baths for tHcy ($p = 0.006$) but not for 8-OHdG ($p = 0.106$). *Conclusions*: Sulphur baths exert beneficial effects on plasma tHcyt whereas effects on 8-OHdG seem to be unlikely.

Keywords: Homocysteine; Antioxidative status; 8-OHdG; Sulphur; Sulphur baths

1. Introduction

Since 1969 plasma homocysteine (tHcy) has been associated with cardio-vascular diseases [1]. Meanwhile tHcy has not only been established as an independent predictor for coronary heart disease but it has also been associated with congestive heart failure, systolic hypertension, artherothrombotic event rates [2–14], complications in diabetes mellitus [15], cancer [16,17], and oxidative stress [18,19]. Free radicals, i.e. oxidative stress, are constantly formed in the human body within tissues and can damage DNA, lipids, proteins, and carbohydrates [20]. Elevated levels of radicals are associated with increased risks for various diseases, like atherosclerosis, cancer, and diabetes mellitus [21–23]. Biochemically oxidized DNA is continuously repaired whereby deoxy-

Abbreviations: tHcy, total plasma homocysteine; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; SAH, *S*-adenosyl-L-homocysteine.

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ribonucleoside is 8-hydroxylated and the product, 8-hydroxy-2′-deoxyguanosine (8-OHdG), is excreted in the urine. Therefore urinary 8-OHdG is a biomarker of the total systemic oxidative stress in vivo [24,25].

To minimize damages by free radicals it is necessary to maintain well functioning antioxidative defence systems. One of the factors contributing to the antioxidative potential and its deleterious effects apparently is sulphur. On the one hand it is an essential part of antioxidative enzymes, like glutathione peroxidase [26], on the other hand it is a key component of tHcy. Thus there might be a connection between the oxidative stress level and the cardiovascular risk factor tHcy by sulphur. Therefore sulphur and sulphur containing compounds are under intense investigation [27].

On the other hand sulphur has been applied for therapeutic purposes since ancient times. Sulphur baths are a well established balneotherapeutic method primarily used in the field of muscular and skeletal disorders [28]. Still, the mechanisms how balneotherapy can alleviate suffering from joint disease are not definitely clear.

Our study aimed to analyse whether balneotherapeutic sulphur baths can influence the level of tHcy and/or the status of oxidative stress. Therefore we assessed blood levels of tHcy and urine excretion of 8-OHdG at the beginning and the end of a three weeks period of spa therapy, whereby one of the two groups received additional daily balneotherapeutic sulphur baths.

2. Materials and methods

2.1. Study individuals

Forty patients with non-inflammatory osteoarthrosis participated in the study. Exclusion criteria were: cancer, rheumatoid arthritis, diseases of the gastrointestinum, nephropathies, alcoholism, and receiving an antioxidative therapy prior to the study. In addition diabetes mellitus and smoking were ruled out as both of these are associated with increased oxidative stress [23,29]. Individuals were randomly allocated to two groups. One of the group received spa therapy and daily baths in naturally mineralized water containing sulphur ($N=20$, *sulphur group*), while the other group served as controls, participating in spa therapy without receiving sulphur baths ($N=20$, *control group*). During their 3 weeks stay at the spa resort both the groups received identical balneotherapeutic applications, such as massages, electrotherapies, and underwater-exercise. Characteristics of all subjects are illustrated in Table 1. Tables 2a (all subjects) and 2b (discrimination between *sulphur group* and *control group*) show the values for three major characteristic laboratory parameters for chronic inflammatory processes (ESR, Hb, and CRP). As all these values were within the normal ranges it was clearly demonstrated that patients did not suffer from inflammatory diseases. Additionally Table 2a shows the values of serum creatinine of all the participants. Plasma creatinine was examined to rule out renal disorders as impaired renal function may effect any parameter excreted into

Table 1
Characteristics of subjects

	Sulphur group	Control group
N=(gender: m/f)	20 (12/8)	20 (11/9)
Age (years, mean±SD)	48 (±5)	50 (±7)
Body mass (kg, mean±SD)	72 (±9)	68 (±10)

Table 2a
Laboratory parameters, all subjects; mean (±SD)

	Beginning	End
ESR	9.3 (±3.0)/23.1 (±3.0)	9.0 (±3.4)/21.7 (±4.4)
Hb	14.8 (±1.4)	15.4 (±1.6)
CRP	0.4 (±0.2)	0.3 (±0.2)
Crea	1.1 (±0.2)	1.1 (±0.2)

ESR=erythrocyte sedimentation rate [mm/hour] 1st hour/2nd hour; Hb=hemoglobin [g/dl]; CRP=C-reactive protein [mg/dl]; Crea=serum creatinine [mg/dl].

Table 2b
Laboratory parameters, discrimination between sulphur group and control group; mean (±SD)

	Beginning	End
ESR sg	9.0 (±3.2)/22.6 (±6.3)	7.3 (±1.7)/19.1 (±4.3)
ESR cg	9.5 (±2.8)/23.6 (±3.6)	10.8 (±3.4)/24.4 (±4.1)
Hb sg	13.9 (±4.3)	15.7 (±1.4)
Hb cg	15.0 (±1.2)	15.0 (±1.6)
CRP sg	0.5 (±0.2)	0.2 (±0.2)
CRP cg	0.3 (±0.2)	0.4 (±0.2)

ESR=erythrocyte sedimentation rate [mm/h] 1st hour/2nd hour; Hb=hemoglobin [g/dl]; CRP=C-reactive protein [mg/dl].

the urine. As all individuals had normal levels there were no indications for any malfunction.

2.2. Sulphur baths

The S^{-2} concentration in the bathing water (pH 6.85) was 7.3 mg/l. Patients of the *sulphur group* received sulphur baths of 20 min duration every other day except Sundays. Thus they had a total of 6 h of sulphur baths. According to Austrian legislation [30] water has to contain more than 1 mg titratable sulphur in 1 kg water to be assignable as medical water.

2.3. Assessment of plasma homocysteine

Blood samples by venipuncture were obtained at the clinical and laboratory medical check up each spa patient had to undergo at the beginning and end of spa therapy. Complementarily we assessed plasma creatinine concentration to control the participants to be free from renal diseases. Immediately after blood taking blood samples were centrifuged and stored at − 70 °C until laboratory analysis. Microplate enzyme immunoassay homocysteine (*Axis® Homocystein*) by *Bio-Rad Laboratories®*, Bio-Rad Laboratories Diagnostic Group, Axis Shield AS, Oslo, Norway, (http://www.bio-rad.com) were applied. This assay is based on an initial enzymatic conversion of tHcy to *S*-adenosyl-L-homocysteine (SAH). Consequently SAH from the sample is under competition with immobilizied SAH bound to the walls of a microtiter plate for binding sites on a monoclonal anti-SAH antibody. After removal of not bound SAH, a secondary rabbit anti-mouse antibody labelled with the enzyme horseradish peroxidase is added. Finally the peroxidase activity is measured spectrophotochemically. The absorbance is inversely related to the concentration of tHcy in the sample. The assay precision is 5.7% (see manual of *Axis® Homocystein* by *Bio-Rad Laboratories®*).

2.4. Assessment of urinary 8-OHdG

8-OHdG was determined at the first and last day of the 3 weeks spa therapy. Urine samples were obtained by collecting urine from 10 p.m. until the next morning with a terminal emptying of the bladder at 7 a.m. Thus we received two urine samples of 9 h each. All the subjects had to leave their rooms latest at 7 a.m. to join the breakfast and the therapies. They were asked to urine into the bottles at this time no matter when they got up. This made us sure that none of the participants were confronted with additional stress by a predetermined time to get up. After registration of the sample volumes small portions of the urine were stored at − 70 °C immediately without any additives until laboratory analyses. Following thawing and centrifugation the supernatants were applied to competitive ELISA plates. An ELISA kit from the Japan Institute for Control of Aging, Fukuori City, Japan (http://www.jaica.com/biotech) was used for quantitative measurement of 8-OHdG. Thereby 8-OHdG monoclonal antibody reacts competitively with 8-OHdG bound on the plate and in the sample solution. After washing, an enzyme-linked secondary antibody binds to the monoclonal antibody which is bound to the 8-OHdG coated on the plate. Finally addition of chromatic substrate results in development of colour. The quantity of 8-OHdG is proportional to the measured absorbance. The assay precision is specified by the Japan Institute for Control of Aging with 5–8% at physiological urinary 8-OHdG levels. Urinary creatinine was determined by the HiCo Creatinine Jaffé-Method (rate-blanked with compensation) using equipment by Roche/Hitachi®. Data are shown as the urinary [8-OHdG (ng/ml)/creatinine (mg/ml)].

2.5. Statistical analysis

The laboratory analyses was performed blinded to the investigator. A *t*-test for equality of means was calculated to verify that there were no differences between the two groups in tHcy or 8-OHdG at the beginning of the spa therapy ($p>0.14$ and $p>0.98$, respectively). Paired *t*-tests were calculated to demonstrate changes of the measured variables within the two groups. Finally a multivariate analysis of variance (MANOVA) for repeated measures was used to analyse differences between the *sulphur group* and the *control group* from the beginning to the end of treatment. A *p*-value of <0.05 was defined as statistical significant.

3. Results

Mean values and standard deviations of tHcy and urinary 8-OHdG (8-OHdG relative to Creatinine-

Table 3
Values of tHcy and 8-HdG of subjects with and without complementary sulphur baths at beginning and end of spa therapy; means (±SD)

	Beginning	End	Change, p	Difference, p
tHcy sg	11.41 (±2.91)	10.55 (±2.28)	0.016	0.006
tHcy cg	12.93 (±2.28)	13.80 (±3.87)	n.s.	
8-OHdG sg	18.00 (±18.28)	11.16 (±5.33)	n.s.	n.s.
8-OHdG cg	17.91 (±5.87)	18.17 (±5.70)	n.s.	

tHcy=homocysteine [μmol/l blood]; 8-OHdG=urinary 8-hydroxy-2′-deoxyguanosine [ng]/creatinine [mg].

excretion) of both groups are illustrated in Table 3. As shown in Fig. 1 tHcy decreased in 13 subjects and increased in seven persons of the *sulphur group* whereas in the *control group* a decline was observable in nine individuals and an increase in 11. Fig. 2 shows that 8-OHdG dropped in 13 persons and increased in seven subjects of the *sulphur group* and declined in 8 but rose in 12 individuals of the *control group*. However, statistical analysis revealed that the decline of tHcy levels in the *sulphur group* was significant ($p = 0.016$) but did not change in the *control group* ($p =$ n.s.). Changes of 8-OHdG were insignificant in either group ($p =$ n.s.). Differences

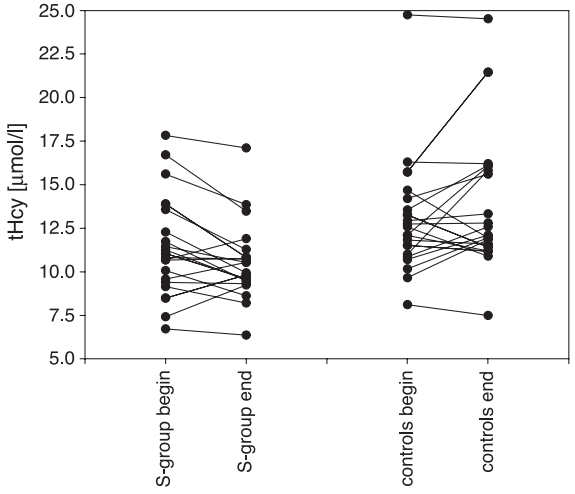

Fig. 1. tHcy at beginning and end of spa therapy in sulphur group and control group.

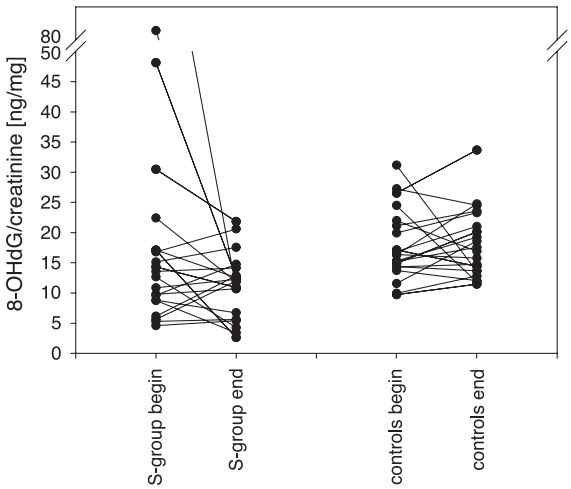

Fig. 2. 8-OHdG/creatinine at beginning and end of spa therapy in sulphur group and control group.

between the two groups showed significant effects of sulphur baths for tHcy ($p = 0.006$) while differences of 8-OHdG were insignificant ($p = 0.106$). The large SD of 8-OHdG at the beginning of the *sulphur group* is caused by one subject with an outstanding high value. However, dropping this value and calculating statistics with the remaining 19 subjects did not change the results.

4. Discussion

We determined the effect of a 3-week sulphur bath therapy on the risk factors tHcy and oxidative stress level. We found differences between the group receiving sulphur baths and the *control group* for tHcy but no clear effects on 8-OHdG. Values of 8-OHdG were very constant around 18 ng/mg (mean values) with the only exception of the *sulphur group* at the end of the spa therapy. However, as the p-value is around 0.100 this obvious (although insignificant) dynamic of 8-OHdG might be interpreted as a tendency. If this tendency can be affirmed might be definitely cleared up by further studies including an extended number of subjects.

In addition to the long practical experience recent studies have tried to analyse the mechanisms of the effects of sulphur baths scientifically. Karagülle et al. [31] demonstrated that a sulphur bath therapy has anti-

inflammatory effects on chronic experimental arthritis in rats. Ekmekcioglu et al. [32] demonstrated that sulphur baths can reduce the antioxidative defence system (Gluthathione-Peroxidase and Superoxide-Dismutase) in the blood and moderately improve the lipid status. They discussed that the decline of these enzyme-activities in their *sulphur group* may be caused by two reasons: either as consequence of reduced oxidative stress during sulphur therapy leading to a lower expression of the enzymes or as an enhanced generation of superoxide radicals exhausting the superoxide scavenging enzyme. Our findings would rather support the prior explanation as we could not find increased 8-OHdG levels. Therefore the changes of the enzyme levels Ekmekcioglu et al. found might rather be explained independently of actual oxidative stress.

In regard to tHcy our results clearly showed an effect of baths containing sulphide. However, the most likely possibility how administration of sulphide could influence the biosynthesis of tHcy in man is via cysteine and cystathionine. Alternatively tHcy is synthesized only by demethylation of methionine, which is an essential amino acid. Thus the amount of methionine ingestion can influence tHcy levels. In our study this factor was excluded as all the participants got similar meals prepared in the same kitchen with the same ingredients. Hildebrandt and Gutenbrunner [33] described relevant penetrations of sulphide through the skin by sulphide baths. However, they mentioned that the further metabolism of sulphide after skin penetration is not clear yet. A possible explanation for our finding might be based on the presumption that transdermal sulphide uptake can cause changes in tHcy via cysteine. Still, final conclusion in this regard might be found by further biochemical studies.

Some authors emphasize that screening and treatment recommendations for tHcy can or should not be provided yet [4]. Others reported that the screening of tHcy actually is useful for assessing individual risk profiles for cardiovascular or atherothrombotic vascular diseases [34–37]. Thus this matter is still intensively debated and trials are currently under way to evaluate benefits of tHcy lowering treatment on risk modification.

However, as mentioned we found a decline of tHcy. This may either be discussed as a consequence of a lower biosynthesis or an enhanced disintegration. Sulphur easily reacts with dilsuphide bonds of proteins and amino acids, such as cysteine, and therefore probably shortens the half life of this compound. Recently sulphur was found to participate in the disintegration of cysteine, as the Fe–S-Cluster is a key structure of the cysteine desulphurase [27]. Thus the metabolism of sulphur does not only influence the biosynthesis but also the disintegration of cysteine. However, at the moment we can not give a final clear explanation of how transdermal resorbed sulphide might influence tHcy metabolism.

Investigations considering sulphur are much rarer than those dealing with sulphur compounds, like e.g. sulphite, whose importance in antioxidative mechanisms and immunological functions have been described repetitively [27,38–40]. Only few authors reflected on effects of oral or percutaneous administered sulphur and restricted their studies on patients with chronic degenerative osteoarthritis [41]. On the contrary to Scheidleder et al. [41], who found advantageous effects on the antioxidative defence system and a reduction of the peroxide levels by sulphur drinking cures, we could not demonstrate analogous effects with our therapeutic setting. The reason for this difference might be that the actual resorption of sulphur through the skin is decisively lower than by drinking even if the total amount of sulphur the subjects are confronted with is much higher in a sulphur bath than by drinking. Scheidleder et al. administered 3×250 ml daily of a mineral water containing 11 mg/l titratable sulphur^{2+}, i.e. a daily ration of 8.25 mg.

The present results support the findings of previous investigations that therapeutic sulphur baths have clear effects on biochemical parameters. In regard of tHcy we cannot explain the effects found from sulphur baths on tHcy levels conclusively. However, we could demonstrate that sulphur baths positively influence plasma tHcy which is anyway a positive effect.

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