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Original article

Sulphurous medicinal waters increase somatostatin release: It is a possible mechanism of anti-inflammatory effect of balneotherapy in psoriasis

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Abstract

Aim: Balneotherapy has been used in the treatment of immune-mediated skin diseases, but its molecular mechanism has yet to be elucidated. The aim of the present study was to observe the effect of sulphurous medicinal water in a murine dermatitis model and on psoriatic patients; moreover to investigate the role of hydrogen sulphide in the release of somatostatin during bathing treatment.

Materials and methods: Inflammation was induced by oxazolone in the paw skin of mice. Oedema, TNF-α concentration, histological changes and myeloperoxidase level were investigated. Mice were bathed in medicinal water or distilled water for 20 min/day. To define the effect of hydrogen sulphide on somatostatin release mice were bathed in sodium hydrosulphide solution for 2 weeks. Somatostatin plasma concentration was detected by nanoHPLC-ESI-Q-TOF-MS.

In the clinical study nineteen patients (PASI: 2.2–21.6) received 2 × 25-min bath treatment for 21 days. Somatostatin-like immunoreactivity of the plasma was determined by radioimmunoassay. Before and after the balneotherapy skin biopsies were performed.

Results: Oxazolone caused 29.43–33.73% paw swelling which was significantly reduced by the medicinal water. Myeloperoxidase, TNF-α levels and histological changes of the skin were unaltered. Somatostatin plasma concentration significantly increased in response to the bathing treatment.

In the clinical study PASI markedly declined (0–13.4) and the plasma level of somatostatin increased significantly. Langerhans-cells migrated from the dermal pool to the epidermis.

Conclusion: We conclude that balneotherapy is an effective treatment in psoriasis. Our results provided evidence that somatostatin released by H_2S plays role in the mechanism of action of sulphurous medicinal water.

Keywords: Balneotherapy; Hydrogen sulphide; Somatostatin; Psoriasis; Allergic contact dermatitis

Introduction

Several reports suggest the anti-inflammatory role of sulphurous medicinal waters in psoriasis by its inhibitory effect on proliferation and cytokine production of T and dendritic cells [1,2]. There is little information in the literature on the molecular mechanism of action of thermal mineral waters. The present study gives a possible explanation of mechanism of sulphurous thermal mineral water investigated in experimental model of dermatitis and on psoriatic patients.

Psoriasis is a Th1/Th17 mediated [3] chronic inflammatory incurable skin disease with remarkable impact on quality of life [4–7]. Genetic, environmental and psychological factors play

Abbreviations: ACD, allergic contact dermatitis; CGRP, calcitonin gene-related peptide; COS, carbonyl sulphide; H_2S, hydrogen sulphide; LCs, Langerhans cells; MPO, myeloperoxidase; NaHS, sodium hydrosulphide; nanoHPLC-ESI-Q-TOF-MS, nano-high-performance liquid chromatography in on-line conjunction to electrospray ionization quadrupole time-of-flight mass spectrometry; OD, optical density; PASI, Psoriasis Area and Severity Index; RIA, radioimmunoassay; SOM, somatostatin; SOM-LI, somatostatin-like immunoreactivity; SPE, solid phase extraction; TFA, trifluoroacetic acid; TRPA1, transient receptor potential ankyrin 1 ion channel.

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role in the development of this disease [8–11]. Complex neuroimmune interactions influence the progression of the disorder. The role of the sensory nerve endings, sensory neuropeptides (calcitonin gene-related peptide (CGRP), substance-P, vasoactive intestinal peptide, somatostatin (SOM)) and nerve growth factor have been suggested to be involved in the pathomechanism of the disease. These neuropeptides are increased in the psoriatic skin and modulate the function of keratinocytes, the antigen presenting Langerhans cells (LCs) and microvasculature [12–14]. Symptoms can be controlled with topical and systemic drugs, or biological treatments [15,16]. Besides these drugs and procedures balneotherapy has been used effectively as a safe, natural alternative therapy [17]. It is believed that the physical and chemical characteristics of medicinal waters influence the success of the therapy, however some researchers have pointed out that the spa environment also has beneficial effect [18–21].

A unique sulphurous thermal water spring was found near Harkány (Hungary) in 1823. Total mineral content of Harkány medicinal water is 1045.5 mg/l (Fig. 1A) [22] and contains carbonyl sulphide (COS), which is converted to hydrogen sulphide (H_2S). H_2S is a small, diffusible gas, it can penetrate through the skin or it is absorbed by airways during the bath treatment.

H_2S is known as an endogenous inter- and intracellular signaling molecule with multiple effects [23]. Its most investigated roles are related to cardiovascular, nervous system and inflammatory diseases. It has pro- and anti-inflammatory effects depending on the investigated model, the type of inflammation and the applied concentration. H_2S acts on different thiol group-containing molecular targets [24]; inhibits mitochondrial cytochrome *c* oxidase and ATP production; modifies proteins (S-sulphurhydration) [25]; affects on kinases and transcription factors; inhibits L-type Ca^{2+} channels in cardiomyocytes [26], chloride channels in rat heart lysosomal vesicles [27] or activates K_{ATP} channel [28] or transient receptor potential ankyrin 1 (TRPA1) ion channel [29]. TRPA1 receptors are expressed on the capsaicin sensitive sensory neurons which make more than 50% of the cutaneous nerves [30]. Beside the nociception these nerve endings have a local efferent function by releasing pro-inflammatory (CGRP, tachykinins) neuropeptides and producing neurogenic inflammation [30,31]. An additional function of these nerves is the systemic anti-inflammatory effect due to the release of anti-inflammatory and analgesic neuropeptides such as somatostatin [32,33]. SOM has immunoregulatory effect on the T and B cells and other inflammatory cell types [34]. Moreover, in lymphoid organs numerous accessory cell-types exist such as epithelial, dendritic cells, fibroblasts, endothelial and smooth muscle cells of blood vessels and they are innervated by sensory and autonomic nerve fibres which express somatostatin receptors [35]. Besides the neural pool somatostatin is produced by neuroendocrine cells and the gastrointestinal tract. Immune cells themselves have also been shown to contain somatostatin which may act as an autocrine or paracrine regulatory agent in the local environment [36].

SOM has been used as treatment of psoriasis [37–40]. Its presence has been demonstrated in dendritic cells located in the dermis and epidermis of normal skin and psoriatic lesions [41,42]. It has been established that the number of SOM-positive dendritic cells are reduced during the healing of the disease, and a non-significant negative correlation has been found between Psoriasis Area and Severity Index (PASI) and SOM plasma concentration suggesting a role for the neuropeptide [14].

We suggested that the absorbed H_2S stimulates capsaicin-sensitive afferents in the skin and releases SOM which has inhibitory effects acting on inflammatory and vascular endothelial cells. Therefore we investigated the effects of sulphurous medicinal water in the treatment of psoriasis and the possible role of SOM in the improvement of the disease. Furthermore we examined the effect of the balneotherapy in an oxazolone-induced mouse model of allergic contact dermatitis (ACD). There is no suitable animal model which shows the complexity of the disease [43], but a delayed type hypersensitive reaction which is in the background of ACD presumably plays role in the pathomechanism of psoriasis [44].

Materials and methods

Animal model

Animals

BALB/c (20–25 g) mouse strain were obtained from Charles River, Hungary and bred in the Animal Centre of the University of Pécs under standard pathogen-free conditions. Mice were maintained on standard diet and water ad libitum in climatically controlled environment. Nine–eleven mice were used per group and each protocol was executed twice. Experiments were performed according to the 1998/XXVIII Act of the Hungarian Parliament on Animal Protection and Consideration Decree of Scientific Procedures of Animal Experiments (243/1988). The studies were approved by the Ethics Committee on Animal Research of Pécs University.

The examiner taking the measurements was blinded from the treatment applied on the animals.

Induction of ACD

ACD was induced by oxazolone on the basis of our previous study [45] modified to mice paw. Sensitisation was induced by smearing the abdominal skin of mice with 50 μl of 2% oxazolone (Sigma–Aldrich) on two consecutive days under ketamine (100 mg/kg i.p., Richter, Hungary) and xylazine (5 mg/kg i.p., Lavet, Hungary) anaesthesia. Six days later elicitation phase was induced by 30 μl of 4% oxazolone on the skin of the right paw. On the left leg vehicle (96% ethanol) was used as control. 24 and 48 h after the induction of inflammation anaesthetised animals were killed by cervical dislocation. The skin of the paw was dissected and stored at −80 °C for further processing or fixed in paraformaldehyde for histology.

Bath treatment

Mice were bathed in 37 °C medicinal water (freshly obtained from the spring of Harkány), tap water or distilled water (control groups) for 20 min/day. Three different bathing protocols were applied: (A) bathing started one day before the sensitisation and