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Original Article

Staphylococcus epidermidis promotes the dissociation of selenium sulfide *in vitro*

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Abstract: Selenium sulfide is an agent used in treatment of dandruff and seborrheic dermatitis. We design this experiment to investigate selenium sulfide transformation *in vitro* and the impact of *Staphylococcus epidermidis (S. epidermidis)* on that process. The agar plate dilution method was used in this study for bacteriostasis test. A lead acetate detection method was used to monitor potential H_2S production by *S. epidermidis*. The soluble selenide was detected by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). We find that selenium sulfide can inhibit the *S. epidermidis* growth when it reaches concentration of 0.50‰ (*m/V*). And the average concentrations of 2.43, 3.91, 8.23, 18.08, 39.83 (μg/L) soluble selenide are detected in suspensions with selenium sulfide concentrations of 0.10‰, 0.25‰, 0.50‰, 1.00‰ and 2.50‰ (*m/V*) respectively. Our results also show that the average concentrations of 6.18, 24.04, 39.09 (μg/L) soluble selenide are detected in Broth Medium, Broth Medium + 0.10‰ (*m/V*) selenium sulfide, and Broth Medium + 0.10‰ (*m/V*) selenium sulfide + *S. epidermidis*, respectively. In conclusion, both the Broth Medium and the biological metabolism of *S. epidermidis* can significantly promote selenium sulfide dissociation level *in vitro*.

Keywords: Selenium sulfide, dissociation, *Staphylococcus epidermidis,* ICP-MS, selenide

Introduction

Selenium sulfide is an effective anti-dandruff agent often used in shampoos [1, 2] for treating patients suffered dandruff [3] or seborrheic dermatitis [4]. Topical selenium sulfide may act by cytostatic action, resulting in an inhibitory hyperproliferation of epidermal cells in dandruff and seborrheic dermatitis. Data also suggest that it has antibacterial and mild antifungal activity, which may contribute to its therapeutic action. But the exact mechanism of selenium sulfide therapeutic action on dandruff and seborrheic dermatitis is still poorly understood.

Selenium sulfide is generally regarded as incapable of absorption by intact skin. A clinical trial has demonstrated that application of selenium sulfide containing shampoo results in unmeasurable increase selenium levels in serum [5]. But the absorption has been reported in a patient with open lesions [6] on the scalp and in a patient using 1% cream on the back [7]. How can the insoluble selenium sulfide transform into soluble selenide and migrate into human body? Maybe this step would be critical for selenium sulfide acting its therapeutic action. A study in the French subjects has suggested that the dandruff show higher incidence of *Staphylococcus epidermidis* (*S. epidermidis*) [8]. Therefore, we design this experiment to investigate the potential transformation of selenium sulfide *in vitro* and the impact of *S. epidermidis* on that transformation.

Materials and methods

Chemicals and reagents

The chemicals used in this study included selenium sulfide (Sigma, St. Louis, MO, USA), lead acetate, sodium hydrosulfide (Aladdin Industrial Corporation, Shanghai, China) and carboxymethyl cellulose sodium (Shandong Liaocheng Ahua Pharmaceutical Co., Ltd). Nutrient agar and Broth Medium were obtained from Hangzhou Tianhe microorganism reagent co., LTD. Water was distilled and purified using a Milli-Q Water Purification System (Millipore, MA, USA). All other chemicals used were of analytical grade.

Table 1. Operating conditions of the Agilent 7500ce ICP-MS used for soluble selenide assay

ICP-MS conditions			
Plasma parameters			
RF power	1550 W	Carrier gas	0.88 L/min
RF matching	1.90 V	Option gas	0.0%
Smpl depth	8.0 mm	Nebulizer	0.10 rps
Lenses parameters			
Extract 1	-140.0 V	Omega lens	4.8 V
Extract 2	-200.0 V	Cell entrance	-30 V
Omega bias	-85 V	Cell exit	-70 V
Cell parameters			
OctP bias	-18.0 v	He flow	4.3 mL/L
OctP RF	200 V	H2 flow	0.0 mL/L
Use gas	True	3rd gas flow	0.0%

Bacterial strain and culture conditions

S. epidermidis strain originating from a patient who suffered seborrheic dermatitis was obtained by skin swab. This *S. epidermidis* strain was previously collected and kept by Clinical Laboratory of General Hospital of Jinan Military Command. This *S. epidermidis* strain had identified by VITEK 2 Compact (bioMerieux, France). The *S. epidermidis* strain used in this study was cultivated on 5% sheep blood agar medium one day before experiment. During experiment, *S. epidermidis* was cultured in 37°C, aerobic condition with supplement of 5% CO_2.

1% (m/V) selenium sulfide stock suspension preparation

Selenium sulfide (1.0 g) and carboxymethyl cellulose sodium (2.0 g) were weighed, mixed and grinded. And the mixture was spread in 100 mL water evenly for swelling 12 h. Then the suspension was grinded until it showed to be uniform and smooth. After being sterilized by pasteurization, the suspension was used in experiment immediately.

Bacteriostasis test

The agar plate dilution method used in this study was following the international guidelines suggested by National Committee for Clinical Laboratory Standards (NCCLS). The nutrient agar which contains 1.0% peptone, 0.3% beef extract, 2.0% agar, 0.5% sodium chloride and distilled water was used to support *S. epidermidis* growth. The 1% (*m/V*) selenium sulfide stock suspension was added into nutrient agar (50°C) with different volume, mixed and poured into plates (9 mm). Each plate represented different selenium sulfide concentration with 2 or 2.5 fold dilutions: 0.00‰, 0.10‰, 0.25‰, 0.50‰, 1.00‰, 2.50‰ (*m/V*). The *S. epidermidis* suspension was inoculated into plates with 0.5 McFarland standard. The cultural plates were incubated 24 h and then examined for *S. epidermidis* growth. The Minimal Inhibitory Concentration (MIC) of selenium sulfide for *S. epidermidis* was measured.

Soluble selenide assay and sample preparation

An Inductively Coupled Plasma-mass Spectrometry (ICP-MS) (Agilent 7500ce, Agilent Technologies, CA, USA) was used throughout the assay. The standard ICP-MS operating conditions used in this study are presented in **Table 1**. Samples were filtrated by filter paper first, then centrifuged (RCF=18000×g) to remove particles. The supernatant were collected and stored at -20 °C. Before being assayed, samples were melted under room temperature, filter-sterilized by using a membrane filter (0.22 μm pore size), and injected into ICP-MS.

H_2S detection

To monitor potential H_2S production by *S. epidermidis*, a lead acetate detection method was used [9]. The filter paper was saturated by 2% of lead acetate and affixed to the inner wall of cultural plates (being kept above the nutrient agar surface). After being incubated for 24 h, cultures were examined for H_2S production.

Statistics

The significance of the differences among groups was determined using one-way analysis of variance (ANOVA) and SNK-q test. A *p* value of 0.05 was considered to be statistically significant.

Results

Selenium sulfide inhibits the growth of S. epidermidis

The agar plate dilution method is used to test the bacteriostasis action of selenium sulfide. Our results show that selenium sulfide can inhibit the *S. epidermidis* growth when it reaches concentration of 0.50‰ (*m/V*) (**Figure 1A**). The *S. epidermidis* grow normally in plates without or with 0.10‰ (*m/V*) selenium sulfide-a

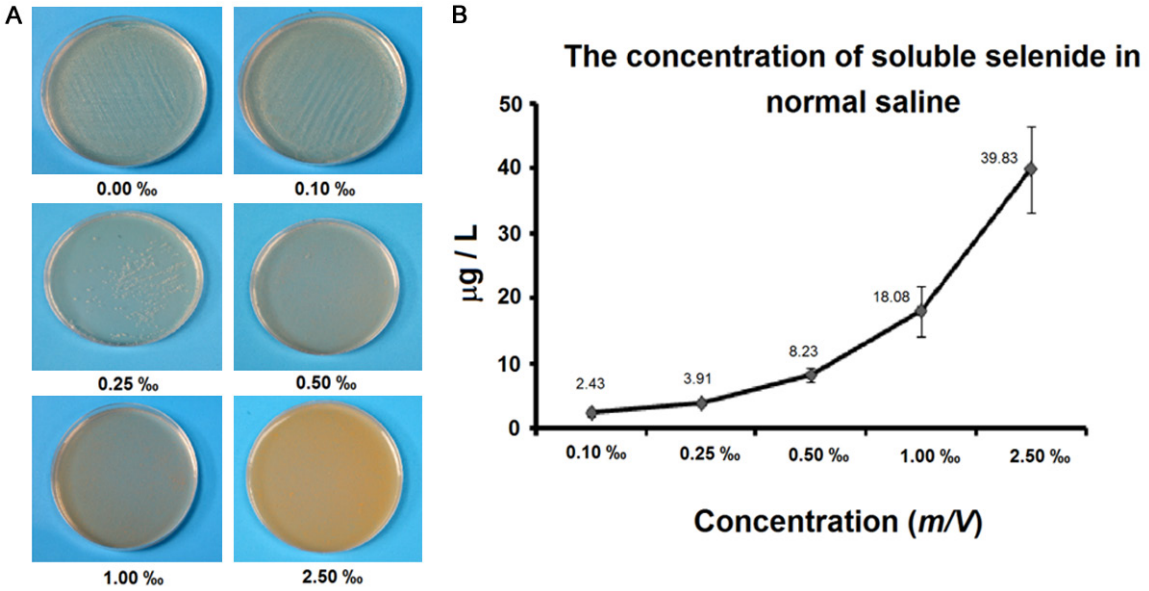

Figure 1. Bacteriostasis test and dissociation in normal saline of selenium sulfide. A. Bacteriostasis test. The agar plate dilution method was used in bacteriostasis test. Please refer to "Materials and Methods" section of this report to obtain further detailed information. This result shown is representative of 3 independent experiments. B. The dissociation in normal saline of selenium sulfide (N=5). This figure shows the soluble selenide concentrations by mean value ± SD. The different volume 1% (*m/V*) stock suspension of selenium sulfide was added into normal saline (50 mL) to reach different selenium sulfide concentrations: 0.10‰, 0.25‰, 0.50‰, 1.00‰, 2.50‰ (*m/V*). All the samples were collected and prepared for ICP-MS assay after stiring for 24 h under room temperature.

kind of mist-like growth. And the *S. epidermidis* only forms sporadic bacterial colony in plate which contains 0.25‰ (*m/V*) selenium sulfide. The MIC of selenium sulfide for *S. epidermidis* is 0.50‰ (*m/V*).

The selenium sulfide dissociates soluble selenide into normal saline

The concentrations of soluble selenide in selenium sulfide suspensions are detected by ICP-MS. Our results show that the average concentrations of 2.43, 3.91, 8.23, 18.08, 39.83 (μg/L) soluble selenide are detected in suspensions with selenium sulfide concentrations of 0.10‰, 0.25‰, 0.50‰, 1.00‰ and 2.50‰ (*m/V*) respectively (**Figure 1B**). This result confirms that selenium sulfide dissociates soluble selenide into normal saline medium at low level.

The selenium sulfide dissociates soluble selenide into Broth Medium at higher level and S. epidermidis accelerates that process without H_2S production

The concentrations of soluble selenide in Broth Medium are detected by ICP-MS. Our results show that the average concentrations of 6.18, 24.04, 39.09 (μg/L) soluble selenide are detected in Broth Medium, Broth Medium + 0.10‰ (*m/V*) selenium sulfide, and Broth Medium + 0.10‰ (*m/V*) selenium sulfide + *S. epidermidis,* respectively (**Figure 2B**). This result suggests that the Broth Medium contains a certain amount of soluble selenide which maybe exist in organic selenium form; selenium sulfide shows higher dissociation rate in Broth Medium than in normal saline; and the biological metabolism of *S. epidermidis* can promote that dissociation process significantly. At the same time, we fail to discover any H_2S production in this biological metabolism process (**Figure 2A**).

Discussion

Selenium sulfide used as shampoo has been shown to be an effective treatment in dandruff, seborrheic dermatitis and tinea capitis. Despite its established impression of bacteriostasis, selenium sulfide retarding bacterial growth still lacks ample supporting evidence. Our experiment *in vitro* has confirmed that selenium sulfide can inhibit the growth of *S. epidermidis* with concentration over 0.50‰ (*m/V*). Although the concentration of residue selenium sulfide on skin after topical application is still unclear, anyhow, it is almost impossible to reach concentration of 0.50‰ (*m/V*). Therefore, it is hard

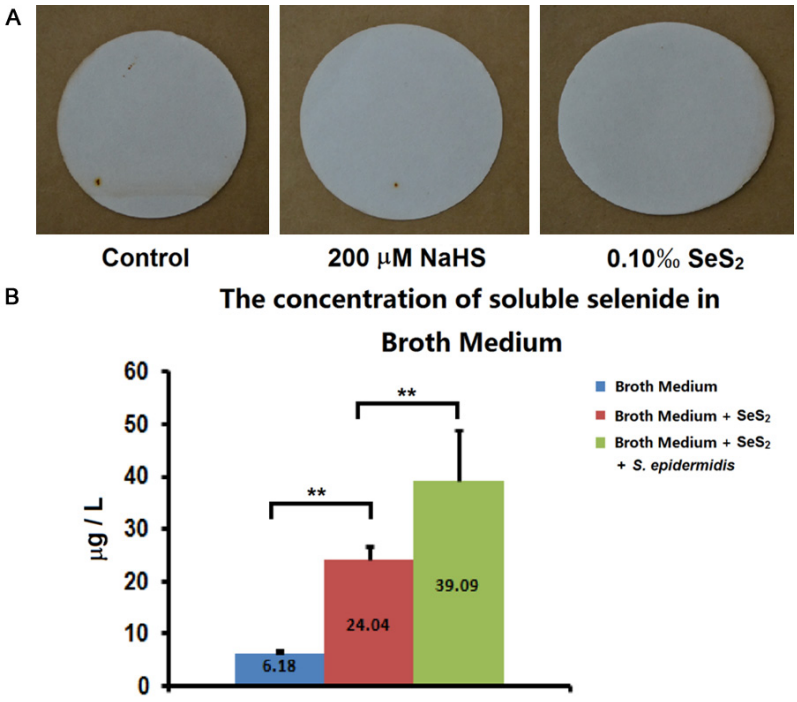

Figure 2. H_2S production test and selenium sulfide dissociation in Broth Medium. A. H_2S production test. A lead acetate detection method was used in H_2S production test. Please refer to "Materials and Methods" section of this report to obtain further detailed information. This result shown is representative of 3 independent experiments. B. The dissociation in Broth Medium of selenium sulfide (N=5). This figure shows the soluble selenide concentrations by mean value ± SD. The concentrations of soluble selenide in groups of "Broth medium", "Broth medium + 0.10‰ (*m/V*) SeS_2" and "Broth medium + 0.10‰ (*m/V*) SeS_2 + *S. epidermidis*" were detected by ICP-MS. Before assay, the *S. epidermidis* suspension was inoculated with 0.5 McFarland standard and incubated 24 h. All the samples were cultivated shakily in 37°C. Aerobic condition with supplement of 5% CO_2. SeS_2, selenium sulfide; **, $P<0.05$.

to establish the correlationship between bacteriostasis action and therapeutic action of selenium sulfide in anti-dandruff.

Clinical evidences have indicated that the topical use of selenium sulfide can increase the selenium level in serum. As we known, selenium sulfide is a sort of difficult-dissolved compound. The process of selenium sulfide dissociating into soluble selenide must be a key step which enable selenium to migrate into human body. A study in human keratinocyte model suggests that the oxyanion selenite (SeO_3^{2-}) and selenide (HSe^-) can be uptaken by keratinocyte [10]. In this study, we find that selenium sulfide shows low level dissociation in normal saline, but higher level in Broth Medium. And much more than this, the biological metabolism of *S. epidermidis* can significantly promote selenium sulfide dissociation level in Broth Medium. Analysis of bacterial genomes has revealed that most, if not all, have genomes of cystathionine beta-synthase (CBS), cystathionine gamma-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3MST), which are in charge of H_2S production using cysteine as substrate. And that bacterial synthetic process can be promoted by extra NaHS supply as H_2S donor. But our results indicate that *S. epidermidis* involved selenium sulfide metabolism should be a different process which is irrelevant to H_2S production even with NaHS supply.

The hypothesis of microorganisms inciting or contributing to the production of dandruff leads to long-time-lasted debates. Lots of data indicate *Malassezia* yeasts may contribute to dandruff formation. However, neither the number nor the morphology of microorganism is related to the skin lesions. Maybe we should take another look at this matter, for instance, the nutrient unbalance of skin. As we known, selenium acts important roles in keeping normal function of skin through selenoproteins [11, 12]. The most important involved selenoproteins are deiodinase family, which includes three subtypes of D1, D2, and D3. D3 is a crucial enzyme to ensure lower T3 level in keratinocytes, which is an extremely important condition for normal proliferation and differentiation of keratinocytes. Studies have confirmed that D3 is active in epidermis of goat [13], mouse [14], rat [15], and human [16]. Actually, selenium deficiency has shown potential correlation to seborrheic dermatitis in some disease, such as Acquired Immune Deficiency Syndrome [17].

Anyway, all of our results prompt the soluble selenide dissociated from selenium sulfide maybe associated with the pharmacological actions of selenium sulfide. Undoubtedly, our

findings will improve and perfect the evidences chain of selenium migration into human body when patients topical use selenium sulfide for anti-dandruff.

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Disclosure of conflict of interest

None.

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