Recovery of Oral Candidosis by Oral Ointment Containing Antifungal Naphthoquinone Derivatives Isolated from Shikon

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Abstract

In this report, we describe the cases recovering from oral candidosis by treatment with oral ointment containing antifungal naphthoquinone derivatives. The patients bearing persistent colonization with Candida genera were treated with our domestic oral ointment, three times a day for two weeks or for one month. During the observation period, the oral candidosis was gradually vanished at 3 days after the treatment and disappeared completely at the end of the period. This ointment contains naphthoquinone derivatives, which are constituents of Shikon (root of Lithospermum erythrorhizon), having been investigated against several fungal pathogens. When the biological activity of these compounds was tested against fungi, a wide range of sensitivity was recorded. With the determination of these naphthoquinones by HPLC, this ointment contains about 10 to 100-folds of each effective concentration.

This observation demonstrates that the oral ointment containing some antifungal naphthoquinone derivatives would useful for the patients bearing serious oral candidosis.

Key words: oral candidosis, antifungal activity, shikonin, deoxyshikonin, acetylshikonin, $\beta$-hydroxyisovaleryl shikonin

Introduction

Previously we reported\textsuperscript{1} shikonin and deoxyshikonin are notable compounds and/or important candidates of lead compounds for new anti-fungal agents. The present study was carried out to describe the clinical cases of recovering from serious oral candidosis by oral ointment containing antifungal naphthoquinone derivatives, which we had prepared domestically with Lithospermum erythrorhizon.
Materials and Methods

Preparation of the Extracts and Tested Compounds

*Lithospermum erythrorhizon* marketed as Shikon was purchased from Tochimoto-Tenkai-do (Osaka). Deoxyshikonin, acetylshikonin, 3-hydroxyisovaleryl shikonin and shikonin were isolated from the chloroform extracts of *L. erythrorhizon* as described previously\(^2\).

Preparation of oral ointment *Lithospermum erythrorhizon* (10 g) was extracted with olive oil (100 ml) heated to 140°C and filtered with sterilized gauze. This extraction oil (30 ml) was added into white petrolatum (70 g) giving oral ointment.

**Determination of naphthoquinone derivatives** Olive oil extract of Shikon was applied to Wakogel C-200 (Wako Chemical, Inc., Ltd., Kyoto, Japan) column to separate the naphthoquinones from fats and fatty oils. The CHCl\(_3\) elute was concentrated in vacuo. The solution was put into a 5 ml measuring flask and then made to 5 ml with CHCl\(_3\). The solution was applied to a Wakogel C-200 (Wako Chemical, Inc., Ltd., Kyoto, Japan) column to separate the naphthoquinones from fats and fatty oils. The CHCl\(_3\) elute was concentrated in vacuo. The solution was put into a 5 ml measuring flask and then made to 5 ml with CHCl\(_3\). The HPLC conditions were as follows: pump, W-515 (Waters, USA); UV-detector, W-2484 (λ = 274 nm, Waters, USA); column, Lichrosphere RP-18 (Cica-MERCK, USA); mobile phase, MeOH-H\(_2\)O-AcOH-CHCl\(_3\) (80:20:1:1); flow rate, 1.0 ml/min; column temperature, 30°C.

### Table 1. Antifungal Naphthoquinone-Derivatives in Oral Ointment

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (μg/g)</th>
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<tbody>
<tr>
<td>shikonin</td>
<td>64.47 ± 0.52</td>
</tr>
<tr>
<td>β-hydroxyisovaleryl shikonin</td>
<td>146.71 ± 0.43</td>
</tr>
<tr>
<td>acetylshikonin</td>
<td>367.54 ± 20.11</td>
</tr>
<tr>
<td>deoxyshikonin</td>
<td>211.00 ± 9.34</td>
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**Results**

**Concentration of Naphthoquinone derivatives**

HPLC profile of CHCl\(_3\) extract is shown in Fig.1. The major four peaks were determined and each peak were identified as acetylshikonin, shikonin, β-hydroxyisovaleryl shikonin and deoxyshikonin compared with the standards isolated by the previous report\(^2\). The concentrations were as follows: Acetylshikonin; 367.54 ± 20.11 μg/g, shikonin; 64.47 ± 0.52 μg/g, β-hydroxyisovaleryl shikonin; 146.71 ± 0.43 μg/g and deoxyshikonin; 211.00 ± 9.34 μg/g (Table 1).

**Clinical cases**

**Case 1.** A 65-year-old woman with subarachnoid hemorrhage swooning. After she had been hospitalized for 1 month, oral ulcer had diagnosed and Candida genera had been detected from an oral mucous membrane and/or ulcer tissue by cultivation test. Therapy with the oral ointment times three a day, about 15 g/day for two weeks was clinically successful. During the observation period, the oral candidosis was gradually vanished after 3 days treatment disappearing completely at the end of the period. The pictures of this progress are shown in Fig.2.

**Case 2.** A 74-year-old man with cerebral hemorrhage. After he had been hospitalized for 2 weeks, oral
sore had diagnosed and Candida genera had been detected from an oral mucous membrane and/or ulcer tissue by cultivation test. The oral candidosis was also disappeared after this same treatment. The picture of this progress is shown in Fig.3. At the end of this treatment, there was no detection of Candida genera from these oral tissues by cultivation test.

Discussion

Previously we reported shikonin and deoxyshikonin are notable compounds and/or important candidates of lead compounds for new anti-fungal agents. Shikonin and its derivatives had been reported to have various pharmacological effects: an anti-inflammatory effect, enhancement of the proliferation of granular tissue, anti-tumor promotion and anti-bacterial activity. Its anti-inflammatory effects were examined in detail and found to have useful medical applications. Because this activity seems potentially useful for clinical applications on inflammatory agents with antibacterial and antifungal activity: it could be applicable for canker sores and oral candidosis following the use of cytotoxic drugs, immunosuppressive therapy, and human immunodeficiency virus infection, or fungal infection in a compromised host with an opportunistic infection which does not allow use of steroidal anti-inflammatory drugs, we demonstrated the treatment to two cases bearing oral-persistent colonization with Candida genera.

We prepared the domestic oral ointment containing about 10-100 holds of each effective concentration of naphthoquinone derivatives. This ointment had prepared by only olive oil and white petrolatum. Its color is almost same as the gum and the applicatory feeling in not uncomfortable. Then we could see the complete recover-
ing from oral candidosis after two or four week’s treatment. We certainly defined that this anti-inflammatory and/or anti-fungal activities of naphthoquinone derivatives had remedied a serious oral candidosis. We are not aware of any report that described alleviation of oral candidosis after any oral ointment therapy. In conclusion, we presented cases of refractory oral candidosis satisfactorily with oral ointment containing naphthoquinone derivatives. This ointment may be effective not only in reducing the inflammation but also in preventing progression to oral-persistent colonization with Candida genera for the treatment of oral candidosis.

References
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