NORRETICULINE AND RETICULINE AS POSSIBLE NEW AGENTS FOR HAIR GROWTH ACCELERATION

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(S)-Norreticuline and (S)-reticuline have been shown to stimulate the proliferation of cultured cells from the murine hair apparatus significantly. Furthermore, these activities were found on cultured hair cells, but not on cultured keratinocytes or fibroblasts from murine skin. In addition, (S)-norreticuline significantly stimulated mouse hair regrowth. These results suggest that (S)-norreticuline and (S)-reticuline could have specific activities on hair apparatus cells and might be useful as active compounds for accelerating hair growth.

KEY WORDS hair cell; proliferation; benzyloquinoline alkaloid; norreticuline; reticuline

Cepharanthine, a bisbenzyloquinoline alkaloid isolated from Stephania cepharantha Hayata (Menispermaceae), has been used in the treatment of alopecia areata and male pattern alopecia.1) We previously reported that some bisbenzyloquinoline alkaloids from S. cepharantha, such as cepharanthine and isotetrandrine, exerted proliferative effects on cultured cells from the murine hair apparatus, but had no significant effects on cultured keratinocytes or fibroblasts from murine skin.2) These results suggested that some bisbenzyloquinoline alkaloids including cepharanthine might have specific effects on hair apparatus cells and accelerate hair growth. However, little is known about the effects on hair growth of monomeric benzyloquinoline alkaloids, such as reticuline which is structurally similar to half of cepharanthine. In the course of our search for active compounds that could accelerate hair growth, we studied the effects of monomeric benzyloquinoline alkaloids on the proliferation of cultured hair apparatus cells. Through this screening, it was found that norreticuline and reticuline had significant activity on cultured hair cells. In this communication, we describe the proliferative effects of norreticuline and reticuline on cultured hair apparatus cells in vitro and their activity on hair regrowth in mice in vivo.

METHODS

Hair apparatus cells were isolated from 4-day-old C3H mice and cultured according to the method of Tanigaki et al.3) Keratinocytes were isolated from 4-day-old C3H mice and cultured using the method proposed by Boyce et al.4) Fibroblasts were isolated during the preparation of keratinocytes. For the assay of mitogenic activities, hair cells, keratinocytes, and fibroblasts were treated with norreticuline or reticuline (0.0001-1 μ g/ml)

![Cepharanthine](image1)

(S)-Reticuline: R=Me
(S)-Norreticuline: R=H
(R)-Reticuline: R=Me
(R)-Norreticuline: R=H

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on day 1. After culturing for 6 days, the cells were harvested and counted in a hemocytometer chamber. The assay of hair regrowth activity in mice was performed by the method of Ogawa et al. One percent norreticuline (50% ethanol solution) was applied topically once a day to the dorsal area of the mice beginning the day after shaving. Fifty percent ethanol was used as a control, and 1% minoxidil (50% ethanol solution) as a positive control. Hair regrowth was observed daily and photographed under uniform conditions. The percentage of new hair growth in the shaved area was calculated from photographs.

RESULTS AND DISCUSSION
To investigate the activities of norreticuline and reticuline on hair growth in vitro, we tested the effects of both benzylisoquinoline alkaloids on the proliferation of cultured cells from the murine hair apparatus. (S)-Norreticuline and (S)-reticuline stimulated the proliferation of cultured hair cells (Figs. 1 and 2). The dose responses of these alkaloids showed bell-shaped curves, and optimum cell proliferation was obtained with 0.01 μ g/ml alkaloid (175-180%). The decrease in stimulative activities at higher concentrations (0.1-10 μ g/ml) may have been due to cytotoxicity. On the other hand, (R)-norreticuline and (R)-reticuline were not as effective on cultured hair cells (Figs. 1 and 2). Thus stereo-specific activity was clearly observed between the S-form and R-form. Additionally, in order to characterize the stimulative activities of (S)-norreticuline and (S)-reticuline on cell growth, we tested their effects on the proliferation of other cultured cells from murine skin, i.e., keratinocytes and fibroblasts. (S)-Norreticuline and (S)-reticuline had no significant effects on the proliferation of cultured keratinocytes or fibroblasts (Fig. 3). This suggested that the activities of the two alkaloids may be specific for hair apparatus cells.

Since it was confirmed that (S)-norreticuline and (S)-reticuline were active on hair growth in vitro, we tested the effect of (S)-norreticuline on hair growth in vivo. In the group treated with 1% (S)-norreticuline, complete hair regrowth in the shaved area of all mice was observed 17 days after shaving, while in the control group regrowth was completed at 20 days (Fig. 4). This observation suggested that 1% (S)-norreticuline strongly accelerated mouse hair regrowth in vivo. Moreover, the activity of 1% (S)-norreticuline was about the same as 1% minoxidil, which is known to have a significant effect on hair growth.6,7

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Fig. 1. Effects of (S)- and (R)-Norreticuline on Proliferation of Cultured Cells from Murine Hair Apparatus
Cells were grown for 6 days with or without (S)- or (R)-norreticuline (0.0001-1 μ g/ml). Each value represents the mean ±SD (n=6). * p<0.05, ** p<0.01 as compared with the respective control.

Fig. 2. Effects of (S)- and (R)-Reticuline on Proliferation of Cultured Cells from Murine Hair Apparatus
Cells were grown for 6 days with or without (S)- or (R)-reticuline (0.0001-1 μ g/ml). Each value represents the mean ±SD (n=6). * p<0.05, ** p<0.01 as compared with the respective control.
In conclusion, the present data indicated that (S)-norreticuline and (S)-reticuline have specific activities on the proliferation of hair cells and may stimulate hair growth. It is well known that reticuline is located on an important key intermediate in the biosynthesis of certain pharmaceutically useful alkaloids such as morphine, berberine, etc., but little is known about its pharmacological effects. Our results suggest that (S)-norreticuline and (S)-reticuline may be useful as active compounds for accelerating hair growth.

Fig. 3. Effects of (S)-Norreticuline and (S)-Reticuline on Proliferation of Cultured Keratinocytes and Fibroblasts from Murine Skin

Keratinocytes were grown for 6 days with or without 0.01 μg/ml alkaloids. Fibroblasts were grown for 6 days with or without 0.01 μg/ml alkaloids. Each value represents the mean±SD (n=6). * p<0.01 as compared with the respective control.

Fig. 4. Effects of (S)-Norreticuline on Mouse Hair Growth

1% (S)-norreticuline, 1% minoxidil, and 50% ethanol (as a control) were topically applied to 8 mice once a day. Each value represents the mean±SD (n=8). * p<0.01 as compared with the control.

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