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Preventive effects of cedrol against alopecia in cyclophosphamide-treated mice

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ABSTRACT

Although numerous hypotheses have been proposed to prevent chemotherapy-induced alopecia (CIA), effective pharmaceuticals have yet to be developed. In our study, the back hairs of C57BL/6 mice were factitiously removed. These mice were then treated with cedrol or minoxidil daily. Mice with early-stage anagen VI hair follicles were treated with cyclophosphamide (CYP, 125 mg/kg) to induce alopecia. The CYP-damaged hair follicles were observed and quantified by using a digital photomicrograph. The results demonstrated that the minoxidil-treated mice suffered from complete alopecia similar to the model 6 days after CYP administration. Simultaneously, the cedrol-treated (200 mg/kg) mice manifested mild alopecia with 40% suppression. Histological observation revealed that anagen hair follicles of the cedrol-pretreated mice (82.5%) likely provided from damage compared with the sparse and dystrophic hair follicles of the model mice (37.0%). Therefore, the use of topical cedrol can prevent hair follicle dystrophy and provide local protection against CIA.

1. Introduction

Although various cancer treatments have been reported, anti-cancer agents, such as doxorubicin, daunorubicin, cyclophosphamide (CYP) and etoposide, are preferred for cancer prevention or treatment (Susan et al., 2012). However, these agents elicit negative effects. For instance, chemotherapy-induced alopecia (CIA) is a serious and distressing adverse effect of cancer therapy. In general, human scalp hair comprises anagen, catagen and telogen hair follicles. Anagen hair follicles actively accumulate cytochrome and rapidly produce hair shaft. Telogen hair follicles are incapable of producing neonatal hair shafts until these follicles mature into anagen hair follicles (Hayumi and Tatsuya, 2001). During cancer chemotherapy, hair follicles shift to the subsequent telogen phase in advance as a consequence of anti-cancer drug-induced apoptosis. Thus, cancer chemotherapy causes serious hair loss and inhibits hair follicle proliferation because of damaged hair matrix keratinocytes that divide cellular subsets or because of disrupted follicular structure and function (Lindner et al., 2012; Roh et al., 2005).

Multiple agents isolated from Chinese medicinal herbs or biologics have elicited modulatory effects on CIA in various models (Satish et al., 2014; Ranjitha et al., 2014; Cheryl and William, 2015; D'Agostini et al., 2013), although not all have anti-apoptotic activity. Several trial approaches, such as scalp cooling during chemotherapy, prevent hair loss in substantial patients (Grevelman and Breed, 2005; van den Hurk et al., 2012). However, cancer scalp metastasis may occur because of tumor drug uptake reduced by scalp cooling (Lemieux et al., 2009; Lemieux et al., 2011). Furthermore, minoxidil can induce hair regeneration and shorten the telogen phase of hair follicles after CIA and alopecia areata are treated; however, this treatment ineffectively prevents or alleviates CIA (Duvic et al., 1996). Therefore, effective CIA prevention agents should be further developed.

Cedrol has been extensively investigated as an anti-inflammatory constituent, anti-microbial ingredient and antibiosis agent (Jantan et al., 2005; Oh et al., 2011; Umeno et al., 2008). This substance is a crystal-type compound found in the volatile oil of *Thuja orientalis*. Cedrol is also the major ingredient of *Platycladus orientalis* (L.) Franco essential oil. With cedrol, the extracts and volatile oil of *P. orientalis* can promote hair growth and alleviate hair loss by protecting hair follicles from chemotherapy-induced damage (Shan et al., 2013). The extract of *T. orientalis* as a traditional herb has also been used to inhibit hair loss and stimulate hair growth (Zhang and Park, 2013). Therefore, we supported the

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main component cedrol may prevent or alleviate hair loss in CIA. To investigate the effects of cedrol on CIA, mature C57BL/6 mice with anagen hair follicle were treated with CYP. Then the effects of topically applied cedrol on alopecia prevention and hair follicle protection were examined. Our study elucidated cedrol may have preventive or alleviative effects on CIA by mitigating dystrophic follicle atrophy.

2. Materials and methods

2.1. Preparation of herbal extracts

The specimens of *P. orientalis* leaves (150228) used in our experiment were purchased from the Traditional Chinese Medicine Market of Bozhou City in Anhui Province (Anhui China), identified and authenticated by Prof. Jincai Lu in the school Shenyang Pharmaceutical. First, the leaves were crushed to powder and soaked in water at 40 °C for 60 min. Volatile oils were acquired from plant materials via a steam distillation method under the following conditions: distillation temperature of 150 °C, total distillation time of 3 h, and material-to-solvent ratio of 1 g: 12 mL. Cedrol was separated from the volatile oils via a freezing method at 2 °C for 120 h and recrystallised from ethanol (Yang et al., 2009). Cedrol was dissolved in 85% ethanol before the trial was performed.

2.2. Experimental animals

Sixty adult female C57BL/6 mice (certification: No. 211002300005208) aging 6 weeks and having an average body weight of approximately 20 g were housed in standard cages and fed with basal feed. Mice were maintained on 12-h light/dark cycle with *ad lib* water and lab chow. They were acclimatized for at least one week prior to experimentation. The temperature of the animal room was 23 ± 2 °C with the suitable humidity. All experiments and procedures were conducted in accordance with the protocol of the Shenyang Pharmaceutical University.

2.3. Grouping of animals

After acclimatization, the healthy mice were randomly divided into the following six groups (Table 1). Each group was consisted of 10 mice.

2.4. Induction of anagen

Catagen or telogen hair follicles in the treated skin patches of the mice should mature into the subsequent anagen phase to assess the efficacy of protecting mouse neonatal hair (Paus et al., 1990). The mice were anesthetised by intraperitoneally injecting 4% chloral hydrate (1 mL/100 g). A 6 cm^2 area (longitudinal length, 3 cm; horizontal length, 2 cm) of telogen-phase hair was removed carefully by using an animal clipper from the dorsal portion of 6-week-old C57BL/6 N mice before topical drugs were applied to their back. The skin of the mice appeared pink, which suggested that the hair follicles were in anagen I to anagen III.

2.5. Induction of alopecia

Freshly dissolved CYP in saline (125 mg/kg body weight) was administered by single intraperitoneal injection to the mice except the normal mouse group on the 9th day of depilation when the hair follicles reached the early stage of anagen VI (Paus et al., 1994a). The normal mouse group received equal volumes of saline.

2.6. Experimental studies with cedrol

Acute toxicity, skin irritation and skin sensitization tests of cedrol were conducted before the experiment, which suggested that cedrol had hardly toxic and side effects. The dosage of cedrol was determined according to the tests described above. Three different doses of cedrol prepared in 85% ethanol were spread on the dorsal skin of mice 2 days after depilation for several consecutive days until the mice were all sacrificed under an ether atmosphere to evaluate whether topical treatment with cedrol may prevent or alleviate CYP-induced alopecia. The treatment for each group is summarized in Table 1.

2.7. Mice alopecia and hair regrowth assays

After CYP was injected to the mice, their back skin was examined daily to determine alopecia symptoms. Hair loss was visually quantified using the photographs obtained daily. Alopecia scores were assessed for 5 consecutive days when the mice experienced hair loss. Hair loss scores were graded as follows: Grade 0, without hair loss; Grade 1, with 0–20% hair loss; Grade 2, with 20–40% hair loss; Grade 3, with 40–60% hair loss; Grade 4, with 60–80% hair loss; Grade 5, with 80–100% hair loss; and Grade 6, with complete hair loss. The animals were not sacrificed until they reproduce hair shaft. Taking the thickness, shade and length of the hair grow back as indexes, the hair regrowth score was assigned as reported previously (Zhang et al., 2016).

2.8. Histological analysis of hair follicles

After the mice were treated with different solutions for 15 consecutive days when the hair follicle in anagen VI or in catagen was damaged by CYP, four mice from each group were sacrificed in an ether atmosphere. Representative skin samples were excised and maintained in 10% formalin fixative to prepare permanent paraffin sections. Longitudinal sections were observed and photographed by using a digital photomicrograph to examine follicle morphology. Anatomical location in the transverse sections was quantitatively examined to assess the percentages of hair follicles in anagen or catagen (Paus et al., 1994b).

3. Results

3.1. Effects of cedrol on alopecia prevention

Blackened skin areas, especially in Groups E and F were observed 9 days after shaving. Hair production continued within the first 3 days. All of the mice presented an almost full coat hair 3 days after CYP administration (data not shown). Fig. 1, time scheme, illustrates the models of the C57BL/6 N female mice with alopecia and subjected to topical treatments.

Macroscopic assessment and alopecia score (Fig. 2) showed that complete alopecia could be significantly observed 6 days after CYP administration in minoxidil and model groups ($p > 0.05$). By contrast, the cedrol-treated mice manifested incomplete hair loss with approximately 10%, 20% or 40% coat hair. This finding indicated that high dose cedrol was associated with protective response against CYP alopecia. The efficacy of topical cedrol in inhibiting CIA was dose dependent when the cedrol solution was initially titrated from 50 mg/kg to 200 mg/kg (C, D and E in Fig. 2).

3.2. Effects of cedrol on protecting hair follicle from damage

The numbers of relatively dystrophic hair follicles with disrupted melanin granules were observed in the longitudinal section of the treated skin patches (empty circles shown as solid lines or