A Heterocyclic Pyrrolopyrimidine Compound

as a Possible Candidate for Topical Application

to Induce Hair Restoration

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Abstract

Established from previous studies, a heterocyclic pyrrolopyrimidine (PyP) compound increases endogenous stem cell proliferation. Here, we utilized a topical PyP to increase cell proliferation of cells responsible for hair production. Varying doses (0.75, 1.5, 3.0 mg/ml) were applied to shaved, dorsal regions of mice for 5 consecutive days. Bromodeoxyuridine (100 mg/kg/day, i.p.) was injected on days 4 and 5 to identify proliferating cells. The PyP dose-dependently increased hair growth and cell proliferation within the subcutaneous region of the epidermis. This data suggests the PyP compound has the potential to be a novel compound for hair restoration.

Keywords: cell proliferation, alopecia, hair growth

1 Introduction

Alopecia, or hair loss, affects millions of men and women annually. Androgenic alopecia, a genetically inherited hair loss condition, is responsible for 80% of hair loss in men and 40% in women over the age of twenty [5]. Here, testosterone is converted in the body to Dihydrotestosterone (DHT) by the enzyme 5 Alpha-deductase (5AR). DHT binds to specific points in the hair follicle called androgen receptor sites and causes a mineralization, which shrinks the diameter of the hair and reduces the time spent in the growth cycle, known as the anagen phase [2; 17]. Cell proliferation has been investigated with androgenic alopecia and its role in hair loss. One study demonstrated cell proliferation is reduced within balding regions in men with androgenic alopecia when compared to controls [6].

The remaining percentage of hair loss can be associated with a variety of health conditions, stress and trauma, diet and nutrition, environmental toxins, and medications. Telogen effluvium results in a premature shedding of hair that is in telogen phase. Causes of telogen effluvium can be contributed to illness, shock, and medication, and can usually be reversed upon the removal of conditions [12; 16]. Traction or traumatic alopecia is demonstrated by patchy, scattered hair loss and is induced by heating elements used to dress hair or binding hair with bands; this is also reversible [3; 4]. Alopecia areata produces round irregular patchy spots of hair loss and is an autoimmune disease [11]. The commonality of all types of hair loss is the negative psychological effect it has on the men and women who experience these conditions [7; 8].

Recently, we became intrigued by several reports suggesting that a family of heterocyclic pyrrolopyrimidine compounds (PyPs) have a variety of growth promoting biological activities including increasing neurite outgrowth and repair.
of injured peripheral nerves and muscle [1; 9; 13-15]. These compounds were initially reported by Akira Awaya and his group for its ability to promote neurite outgrowth in isolated neurons maintained in vitro [1]. The mechanism of action of PyPs is not known, although some evidence suggests the activation of the mitogen activated protein kinase (MAPK) pathway, a cascade that is also activated by peptide growth factors [1]. Additionally, it suggested PyPs promoted the survival of rodent cortical neurons by reducing the rate of apoptosis. Early evidence from rodent studies suggest PyP is absorbed well after intraperitoneal administration as well as oral administration, and it has a half-life of 4 hours in rat circulation as shown by HPLC analysis [1]. The dosing method for PyPs has not been optimized and rigorous genotoxicity, absorption, distribution, metabolism, or excretion studies have yet to been done. Upon learning about the availability of PyPs, we hypothesized that these compounds may be involved in the proliferation process of stem cells and decided to examine the possible implication for cell proliferation within hair follicles. Based on the above discoveries, we tested a PyP in a basic, preliminary topical application study to increase hair growth.

2 Methods and Materials

C57 BL/6 mice were selected and all procedures were performed in accordance with institutional guidelines. The mice were prepared by shaving four specific locations lateral/dorsal in a circular pattern. The PyP was delivered to the specified areas (right, counterclockwise from lowest to highest dose, ending with the control in the left lower quadrant) in concentrations of 0.75, 1.5, 3.0 mg/ml, and control (50% ETOH), respectively. The solutions were applied to the center of these locations in a 5 µl dose for 5 consecutive days, and on days 4 and 5, bromodeoxyuridine (BrdU; 100 mg/kg/day, i.p.) was injected. On day 7, mice were euthanized using a high dose of sodium pentobarbital (70 mg/kg, i.p.) and the treated dermal layer was dissected and placed into 4% paraformaldehyde fixative for 48 hours.

Epidermal sections were mounted into square embedding molds with freezing medium (O.C.T. compound, Tissue Tek), sliced in 20 µm sections and mounted onto adhesive coated slides (Instrumedics, Inc) via tape transfer method. Slides were washed with PBS and placed in 2N HCl for 30 minutes to induce histone release. Slides were washed again with PBS and then blocked using 3% donkey serum in PBST for 1 hour. Primary anti-body suspended in blocking solution used was anti-BrdU (Sigma) (1:1000) overnight at 4°C. Slides were washed 3 times and placed in secondary anti-body, FITC (Jackson ImmunoResearch), suspended in blocking solution (1:500) for 2 hours at room temperature then washed with PBS. Slides were coverslipped using Vectashield with Dapi. Photographs were taken using an inverted fluorescent microscope (Leica, DMI 6000 B with Q-imaging Retiga exi camera). Statistical analysis was conducted using Image J software downloaded from NIH.gov.
3 Results

The PyP induced visible hair re-growth in treated areas, near to its original length within 7 days, while the control or untreated areas showed no visible re-growth within the same time frame (Fig. 1). By the seventh day, there was near complete hair re-growth within the treated sections on the mouse and the control areas remained predominately free of hair. The mice used had a hair phenotype of black with a mix of grey, however, the sections of hair that grew back were completely black.

Immunohistochemistry showed that the PyP dose-dependently increased proliferating cells in the skin. After five days of PyP treatments and following two days of BrdU injections, all of the mice demonstrated increased levels of BrdU positive cells in comparison with controls (Fig. 2). Statistical analysis revealed the PyP significantly increases the number of BrdU positive cells within the dermis of treated areas when compared with controls (Fig. 3). The highest concentration (3mg/ml) showed approximately a 7-fold increase in cell number over the control.

4 Discussion

The hair growth cycle consists of three phases: anagen (growth), catagen (reduction), and telogen (rest) [10; 11; 18]. The hair is subsequently shed at the end of telogen and the hair cycle repeats [11]. Our results show that topical application of a PyP increases hair growth and the number of BrdU positive cells in the dermis of mice. The increase in cell proliferation due to PyP application still requires further investigation, but we hypothesize that our compound is stimulating the endogenous stem cell population within the hair follicle to increase cell proliferation. If true, our compound should not require continual application to observe positive results. Our next feasible step will be to test this compound’s efficacy against similar proven compounds, such as minoxidil, continue mechanistic studies, and identify the stimulated cell population.

Originally introduced as a drug for hypertension, minoxidil has evolved into a treatment for androgenetic alopecia and alopecia areata [11]. However, its mechanism of action for hair growth is still unknown [11]. One draw back to minoxidil is the need for twice daily applications and perpetual use for optimal and sustained results [11]. On the other hand, since PyP is hypothesized to increases the proliferation of stem cells existing in the hair follicle, it may not require continual use.

Previous studies on alopecia have revealed that hair holds an important psychological and symbolic importance in society [8]. PyPs are rare and valuable with the potential to become possible therapeutic candidates for human conditions of alopecia by cell proliferation within the hair follicle. The reduction of cell proliferation within androgenic alopecia [6] leaves an opportunity for our
compound to overcome this and increase hair growth. PyPs represent a unique class of synthetic heterocyclic compounds that are stable, orally bioavailability, and easily manufactured. Although we still need detailed safety studies, including the effect of PyPs on the growth of other types of cells, the current preliminary study is a stepping-stone for further studies to investigate the effect of increasing cell proliferation within the hair follicle on the pathogenesis of various alopecia conditions, which may lead to new treatments.

References


Figure Legends

Fig 1. Mice treated with topical PyP. Green circles represent the area which were treated with a PyP and white circles represent control area. (A) and (D) are of the mice prior to beginning of experiment and (B) and (E) are after the mice were shaved. (C) and (F) show the animals on day seven after five days of topical application of the PyP. A majority of hair re-growth was observed within the PyP treated area with relatively little re-growth in the control areas.

Fig 2. Immunohistochemistry in the cross section of the dermis on day seven. The animals were topically treated with 5 µl of PyP (A and E: control, B and F: 0.75 mg/ml, C and G: 1.5 mg/ml, D and H: 3.0 mg/ml) solution dissolved in 50% ethanol for five days. Top row is a combination of BrdU (green) staining with counter staining of nuclei by DAPI (blue). Bottom row is the same picture without DAPI counter staining. The PyP dose dependently increased BrdU positive cells.

Fig 3. The effect of PyP on the number of BrdU positive cells in the dermis. The cell number was counted using NIH Image J software. The data represents mean +/- standard deviation. The PyP significantly (*P<0.05, **P<0.01, by ANOVA and post hoc) increased number of BrdU positive cells within the dermis of treated mice in comparison to the dermis of control mice. The highest concentration (3 mg/ml) showed approximately a 7-fold increase in cell number over the control.
Figures

**Figure 1**

![Figure 1](image1)

**Figure 2**

![Figure 2](image2)
Figure 3

![Graph showing the number of BrdU positive cells versus concentration of PyP (mg/ml)]

- Control
- 0.75
- 1.5
- 3

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