Effect of topical minoxidil solution in promoting hair growth in male mice

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Abstract

The present study was designed to assess the efficacy of topical minoxidil solution in promoting hair growth in mouse model. This study aimed at finding the more efficacious drug for the treatment of androgenetic alopecia since the available regimens have many limitations. Alopecia or Hair loss is the absence or thinness of the hair. Androgenetic alopecia is commonly referred as male pattern hair loss or female pattern hair loss in genetically susceptible men and women. 45 albino-Webster male mice were included in this study, they were divided randomly into two groups, each one included 15 mice. One day before starting the experiment the dorsal skin of each mouse was shaved by an electric shaver, then stained by using commercial dye and then the animals were photographed. group No. 1 was considered as control group. It included mice which did not get any treatment. Group No. 2 received topical minoxidil solution 5%. At the end of treatment (21 day), the animals were photographed to assess the degree of hair growth, it was carried out by means of special computer program called Photoshop-visual basic-8 program, this program found the ratio of area showing hair regrowth to the ratio of area denuded of hair. Histological sections were obtained from each mouse to assess the effect of drugs on the number and diameter of hair follicles.
The results of this study showed that significant hair growth (P-Value < 0.05) occurred with topical minoxidil solution as compared to the control group, insignificant increase in the number of hair follicles as compared to the control group, significant increase in diameter of hair follicles as compared to the control group. From the results of this study, we concluded that topical minoxidil solution can promote hair growth efficiently and can increase the diameter of hair follicles. Also in the present study the concentration of the following hormones were measured after 21 day treatment with topical cyproterone acetate. These hormones are (17β-Estradiol, Free Testosterone and Progesterone). The results showed that there was no significant difference in concentration of these hormones among control and treatment groups after 21 day treatment, they are all within the normal range.

1. Introduction
Androgenetic alopecia is commonly referred to as male pattern hair loss or female pattern hair loss in genetically susceptible men and women (1). Thinning of hair diameter as well as miniaturizing of the terminal hair follicle is characteristic of androgenetic alopecia. With time, miniaturized hair follicles produce shorter and thinner hair (2,3) the onset of androgenetic alopecia in both sexes is between 12 and 40 years of age (1). Androgenetic alopecia is a result of the effects of androgens in genetically susceptible individuals (4). The mechanism of androgenetic alopecia development does not seem to be limited to dihydrotestosterone levels. The number of androgen receptor expressed by an individual may have an effect on androgenetic alopecia as well. It is well established that individuals with androgenetic alopecia have higher levels of androgen receptor (5,6,7) when compared, higher levels of androgen receptor have been found in the hair follicles in thinning regions of the scalp versus non-thinning regions (6). The present study was conducted to assess the efficacy of minoxidil solution in stimulating coat hair growth in mouse model. Minoxidil (trade names Rogaine and Regaine) is a vasodilator medication used primarily as antihypertensive drug. It was discovered to have the interesting side effect of hair growth and reversing baldness (8). In 1980 Upjohn corporation produced a topical solution that contained 2% minoxidil to treat baldness under the brand name Rogaine in the USA, and Regaine elsewhere. The treatment usually includes a 5% concentration solution that is designed for men, and 2% concentration solution that is designed for women. In 2007 a new foam based formulation of 5% Minoxidil was shown to be an effective treatment of male pattern baldness without the usual side effects of the topical solution (9). Topical minoxidil solution causes a significant increase in hair growth (10,11,12).

2. Materials and methods
In the present study 30 albino-Webster male mice were included, they were divided randomly into two groups, each one included 15 mice. One day before starting the experiment the dorsal skin of each mouse was shaved by an electric shaver, then stained by using commercial dye and then the animals were photographed as shown in the figure (1), (2). Group No. 1 was considered as control group. It included mice which did not get any treatment. Group No. 2 received topical minoxidil solution 5%. At the end of treatment (21 day), the animals were photographed to assess the degree of hair growth, it was carried out by means of special computer program called
Photoshop-visual basic-8 program, this program found the ratio of area showing hair regrowth to the ratio of area denuded of hair. Histological sections were obtained from each mouse to assess the effect of drugs on the number and diameter of hair follicles. Also, the concentration of the following hormones were measured, these hormones are (17β-Estradiol, Free Testosterone and Progesterone). The procedure for hormonal measurement based on ELISA kit which include approximately 0.1 ml of serum is required per duplicate determination. About 1 ml of blood from each mouse was collected and put into appropriately labeled tube and allowed to clot. The blood is taken from each mouse by direct cardiac puncture (using 1 ml syringe). It was immediately centrifuged and carefully removed the serum layer and stored in refrigerator at 4°C for up to 24 hours.

Figure-1: mice after clipping of the dorsal coat hair

Figure-2: The mice after staining of the clipped area.

3. Statistical analysis
Statistical analysis was done by using one way ANOVA test with post Hoc test at level of significance $\alpha = 0.05$ to compare between control and treated groups. Chi square test had been used to compare between the proportion of histological changes in various groups. Values of $P<0.05$ were considered statistically significant. The statistical analysis had been done by using computer program, SPSS version 15.

4. Results
4.1 Effect of minoxidil solution on hair growth
There was a significant hair growth ($P$-Value < 0.05) in group treated with minoxidil solution as compared to the control. The results are shown in table 1 (figure-3).
Table-1: Mean ratio of white color (area showing hair regrowth) to the ratio of black color (area denuded of hair) after 21 days of treatment with minoxidil solution 5%. (15 mice in each group).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Ratio of color difference</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>1.67 ± 0.35</td>
<td>N.S</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>48.52 ± 6.52</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

All data expressed as mean ±SEM.

4.2 Effect of minoxidil solution on the number of hair follicles

By examining the histological sections obtained from the control and treated groups under the light microscope, the number of hair follicles increased insignificantly (P-Value>0.05) in the group treated with minoxidil solution as compared to the control group as shown in (Figures 4, 5), (Table-2).

Table-2: The mean number of hair follicles in control and treated groups after 21 day of treatment with minoxidil solution 5%. This table shows that hair follicle number increased insignificantly (P-Value>0.05) in group treated with minoxidil solution. (15 mice in each group).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean number of hair follicle per 10mm</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>5.400 ± 1.98</td>
<td>N.S</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>15.800 ± 2.30</td>
<td>N.S</td>
</tr>
</tbody>
</table>

All data expressed as Mean ±SD.

4.3 Effect of minoxidil solution on hair follicles diameter

There was a significant increase in hair follicle diameter (P - Value<0.05) in group treated with minoxidil solution as compared to the control. The results are shown in table 3 (Figure- 4).

Table-3: The mean diameter (in micrometer) of hair follicles in control and treated groups after 21 days of treatment with minoxidil solution 5%. (15 mice in each group).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean diameter of hair follicles (in µm)</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>1.35 ± 0.0</td>
<td>N.S</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>3.70 ± 0.16</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

All data expressed as Mean ±SE
Figure-3: Mice treated with minoxidil showing significant hair growth after 3 weeks of treatment.

Figure-4: Histological section from the skin of mouse treated with minoxidil(x40) showing normal skin layers, increase in hair follicle diameter.
4.4 Hormonal Measurement

There was no significant difference in the concentration of hormones among control and treatment groups after 21 days treatment. All are within the normal range. The results are shown in the table (4).

Table-4: the mean concentration in (pg /ml) of 17β- Estradiol, Free Testosterone, and Progesterone in control and treated groups after 21 day treatment with 5% minoxidil solution.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean 17β-Estradiol concentration in (pg per ml)</th>
<th>Mean Free Testosterone concentration in (pg per ml)</th>
<th>Mean Progesterone concentration in (pg per ml)</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>24 ± 3.06</td>
<td>3.5 ± 0.18</td>
<td>0.5 ± 0.88</td>
<td>N.S</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>23 ± 3.02</td>
<td>3.2 ± 0.16</td>
<td>0.6 ± 0.98</td>
<td>N.S</td>
</tr>
</tbody>
</table>

All data expressed as Mean ± SEM

5- Discussion

Effect of Minoxidil

This study demonstrated a significant increase in hair growth in the group treated with topical Minoxidil solution. This result is in agreement with Olsen et al.\textsuperscript{(10,11,12)} The possible explanations for increasing hair growth are:
1- Vascular effects:

The idea that Minoxidil stimulates hair growth by increasing cutaneous blood flow has been the subject of two studies:

Wester 1984\textsuperscript{(13)} studied the effect of topical minoxidil (1%, 3%, 5%) on blood flow in balding scalp using Laser Doppler velocimetry (LDV) & photopulse plethysmography. Both showed an increase in skin blood flow after the application of Minoxidil solution 5%. Bunker and Dowd(1987)\textsuperscript{(14)} also using LDV failed to find any change in skin blood flow following the application of 3% topical minoxidil solution to the scalp in 10 balding men. The difference in results may be due to the higher concentration of minoxidil used in the first study.

2- Minoxidil sulphation:

Minoxidil induces rapid relaxation of vascular smooth muscle induced by its sulphated metabolite, Minoxidil sulphate\textsuperscript{(15)}. The conversion of Minoxidil to Minoxidil sulphate is catalysed by sulphotransferase enzyme, which was initially demonstrated in rat liver\textsuperscript{(15)} and has since been found in human Liver\textsuperscript{(16)} and platelets\textsuperscript{(17)}, as well as in mouse vibrissae follicles\textsuperscript{(18)}. In scalp skin of stump-tailed macaque, sulphotransferase activity is largely localized in the hair follicle\textsuperscript{(19)}. Biochemical evidence for Minoxidil sulphation by two phenol sulphotransferase has been found in human scalp skin\textsuperscript{(20)}. There are individual variations in scalp sulphotransferase activity and this correlates with the level in platelets\textsuperscript{(20)}. Also in the present study the concentration of the following hormones are measured to see if there is any change in the levels of these hormones after 21 day treatment with topical cyproterone acetate, topical finasteride and minoxidil. These hormones are (17β-Estradiol, Free Testosterone, and Progesterone). The results showed that there was no significant difference in concentration of these hormones among control and treatment groups after 21 days treatment. All are within the normal range. The explanation for these results is that very little amount of drugs are absorbed because these drugs given topically so very slight change in the levels of hormones will occur. it is statistically insignificant.

6- Conclusions

From the results of this study we can conclude that topical minoxidil solution promotes hair growth and increases hair follicle diameter in mouse model.

7- References

6- Pandall VA, Thornton MJ, Messenger AG. Cultured dermal papilla cells from androgen-dependent human hair follicles (e.g. beard) contain more androgen receptors than those from non-balding areas of scalp. J Endocrinol 133(1): 141-7. (1992).

7- Hibberts NA, Howell AE. Balding hair follicle dermal papilla cells contain higher levels of androgen receptors than those from non-balding scalp. J Endocrinol 156(91): 59-65. (1998).


