Stem cell conditioned media aids the successful treatment of androgenetic alopecia (AGA)

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Abstract

Androgenetic alopecia (AGA) is a common form of hair loss that affects up to 50% of males between 18 and 40 years old. 80% of Caucasian men will experience some form of AGA before they reach 70 years of age. Testosterone is necessary for AGA to develop, and a genetic susceptibility locus in the androgen receptor is present in a minority of men with AGA. However, additional factors contributing to this disorder remain unknown. Many previous studies indicate that there are a multitude of molecules involved in the control of the hair cycle. Although not normally used to treat this condition, stem cell conditioned media (CM) from adipose derived mesenchymal stem cells (ADMSCs) contains prostaglandins, cytokines, and anti-inflammatory proteins that are missing in AGA patients. For this reason, we believe that the CM will have a positive effect on hair growth.

To perform this study, CM, the prostaglandin PGE2 (found in CM and linked directly to hair growth), minoxidil (which has an unknown method of action, but likely signals through PGE2), redensyl (has an unknown method of action), and empty vehicle was used to see an effect on murine hair growth. A double-blind clinical trial was conducted with 10 mice (5 males and 5 females) per condition. Mouse bellies were depilated and 25uL of various treatments were applied daily to the ventral left side of the mice. Preliminary results indicate that all treatments somewhat accelerated hair growth, but the application of a combination of the factors worked the best to restore hair growth. While more studies need to be completed, it appears that stem cell conditioned media and supplements caused significant hair growth in mice.

Introduction

In AGA, terminal hair follicles forming thick hair shafts miniaturize over time to small follicles. This miniaturization is accompanied by a decrease in the duration of the growing phase of the follicle (anagen), which normally lasts several years to produce hair more than 1 m long, but which decreases to only days or weeks in AGA. This results in an increase in the percentage of resting (telogen) hair follicles containing microscopic hairs. In addition to these intrinsic changes to the hair follicle, infiltrating lymphocytes and mast cells have been identified around the miniaturizing follicle, especially in the area of the stem cell–rich bulge area. In balding scalp, the number of hair follicle stem cells remains intact, whereas the number of more actively proliferating progenitor cells markedly decreases, suggesting that balding scalp either lacks an activator or has an inhibitor of hair follicle growth.

The **purpose of this study** was to investigate the effects of:

1. prostaglandin PGE2 (prostaglandin that is downregulated in patients with AGA)
2. redensyl (an alternative treatment for hair loss that aims to reactivate hair division by stimulating the initial hair growth phase)
3. minoxidil (which has an unknown method of action, but likely signals through PGE2)
4. stem cell conditioned medium from human mesenchymal stem cells (which possesses cytokines, growth factors, angiogenic factors, and anti-inflammatory proteins)

The **other purpose of this experiment** was to expose Chico State students to real-life, impactful science, planning and executing a clinical trial for eventual FDA approval.

Our hypothesis was that PGE2, redensyl, and minoxidil should cause hair growth, but this will be amplified when stem cell conditioned media is added.

Methods

A double-blind clinical trial was conducted with 10 mice (5 males and 5 females) per condition. Groups are shown below:

Treatment 1: vehicle only

Treatment 2: vehicle + stem cell conditioned media

Treatment 3: vehicle + .5% redensyl

Treatment 4: vehicle + 2% minoxidil

Treatment 5: vehicle + 10uM PGE2

Treatment 6: vehicle + stem cell conditioned media, .5% redensyl, 2% minoxidil, and 10uM PGE2



10 black mice (5 female/5 male) per treatment condition were shaved, depilated, and cleaned.

24 hours later 25 uL of treatment was applied to the belly region and rubbed in with a gloved finger. Treatment was reapplied every 24 hours for 2.5 weeks. Images were obtained every 7 days and at completion of the trial.

Hair growth was scored and belly skin was removed for pathology.

Autopsies were performed to inspect kidneys, liver, spleen, and gonads.

Results

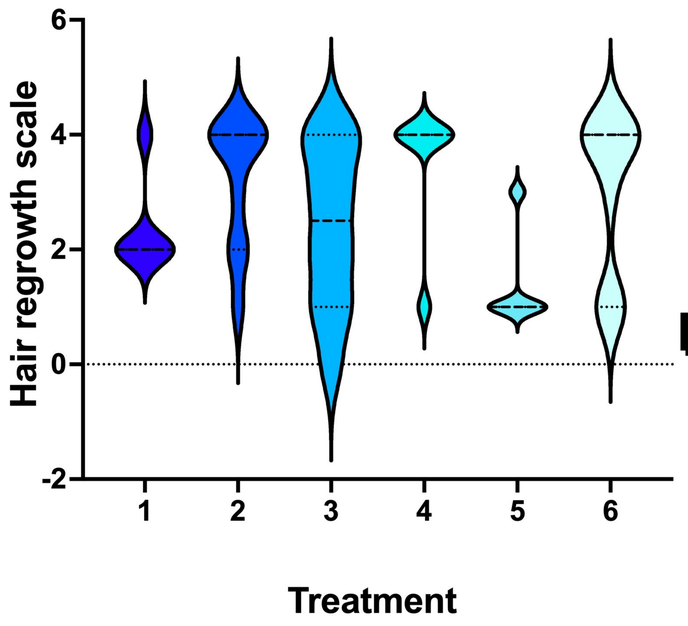
Table 1: Scoring system for hair regrowth

Growth	Numerical score	Notes
No growth	0	No hair seen- completely depilated
Patchy hair growth	1	Hair growing back unevenly and in random locations
Normal hair growth	2	Short, fine hair covering area is seen, but there is a distinct border around the area and skin can be seen behind the hair
More than normal hair growth	3	In-between "normal" and "full."
Full hair growth	4	Hair has completely regrown. No borders are seen, and hair is long and thick enough to completely cover over skin



Figure 1: Mouse hair regrowth after 2.5 weeks. Representative mice shown. Mice were grouped and scored as described in **Table 1**.

Stem cell medium, redensyl, and minoxidil, increase hair growth



Results

Treatment	Sex	Hair regrowth status	Autopsy result
1	♂	1	Unremarkable
1	♂	4	Unremarkable
1	♀	4	Unremarkable (pregnant)
1	♀	4	Unremarkable (pregnant)
2	♂	1	R kidney white lesions.
2	♂	4	Unremarkable
2	♀	4	Unremarkable
2	♀	4	Unremarkable
3	♂	1	Unremarkable
3	♂	4	Unremarkable
3	♀	4	Unremarkable
3	♀	4	Unremarkable
4	♂	1	Unremarkable
4	♂	4	Unremarkable
4	♀	1	1/3 spleen dark and necrotized
4	♀	4	Unremarkable
5	♂	1	Unremarkable
5	♂	4	Unremarkable
5	♀	1	Unremarkable
5	♀	4	Unremarkable
6	♂	1	Unremarkable
6	♂	4	Unremarkable
6	♀	4	Unremarkable
6	♀	4	Unremarkable

Table 2: Most mice had unremarkable autopsies after 2.5 weeks of treatment. Kidneys, liver, spleen, and gonads were examined. Skin was collected and sent for pathology. Pregnant mice: embryos were also examined and were normal

Conclusions

All treatments fared better than vehicle control except for PGE2 alone.

Stem cell media, redensyl, and minoxidil all encouraged hair growth on the mice over 2.5 weeks. PGE2 encouraged hair growth as well, but it was patchy and not full.

Further studies should be performed to analyze the method of action of these supplements on their own and in conjunction with other compounds.

Future studies

Belly tissue where hair growth occurred is being processed for pathology. We plan to count hair follicles present in each of the samples to quantitate hair regrowth better.

RNA samples of belly tissue was also collected from all autopsied mice so that we can examine gene changes occurring after treatment.