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A novel rat model for chemotherapy-induced alopecia

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Summary

Background—More than half of all people diagnosed with cancer receive chemotherapy, and approximately 65% of these develop chemotherapy-induced alopecia (CIA), a side-effect that can have considerable negative psychological repercussions. Currently, there are very few animal models available to study the mechanism and prevention of CIA.

Aim—To develop a clinically relevant adult rat model for CIA.

Methods—We first tested whether neonatal pigmented Long–Evans (LE) rats developed alopecia in response to the chemotherapeutic agents etoposide and cyclophosphamide. We then determined whether the rats developed CIA as adults. In the latter experiment, rat dorsal hair was clipped during the early telogen stage to synchronize the hair cycle. and starting 15 days later, the rats were treated with etoposide for 3 days.

Results—Neonatal LE pups developed CIA in response to etoposide and cyclophosphamide, similar to other murine models for CIA. Clipping of the hair shaft during early telogen resulted in synchronized anagen induction and subsequent alopecia after etoposide treatment in the clipped areas only. Hair follicles in the clipped areas had the typical chemotherapy-induced follicular dystrophy (dystrophic catagen). When the hair in the pigmented alopecic areas regrew, it had normal pigmentation.

Conclusions—A novel, pigmented adult rat model has been established for CIA. By hair shaft clipping during early telogen, synchronized anagen entry was induced that resulted in alopecia in response to chemotherapy. This is the first clinically relevant adult rat model for CIA and will be a useful tool to test agents for the prevention and treatment of CIA.

Introduction

Hair follicles (HFs) undergo cycles of growth (anagen), regression (catagen) and quiescence (telogen). The rapid cell proliferation in anagen HFs makes them particularly susceptible to the cytotoxicity of antineoplastic chemotherapeutic agents. Because the vast majority (~86%) of human scalp HFs are in the anagen phase at any given time, patients develop clinically evident anagen effluvium, termed chemotherapy-induced alopecia (CIA), within days or weeks after chemotherapy.^{1,2}

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More than half of all people diagnosed with cancer receive some form of chemotherapy. With an incidence estimated at 65%, CIA is one of the commonest side-effects occurring during chemotherapy.^{1,2} Although the hair loss is almost always reversible, CIA can have considerable negative psychological repercussions for patients, even leading to refusal of treatment.¹⁻⁴ Consequently, pharmacological and physical (nonpharmacological) measures have been developed to reduce the severity and duration of CIA, aiming to either prevent hair loss or promote hair regrowth.^{1,2,5-9} Some of these treatments have been shown to reduce the severity or shorten the duration of alopecia in animal models or clinical trials, but have not been able to prevent CIA or consistently promote hair regrowth.

Good animal models are invaluable tools in developing prevention and treatments for CIA. There have been two neonatal rat models developed, a Sprague-Dawley (SD) model⁵ and a Wistar model,¹⁰ in which alopecia can be induced by chemotherapeutic agents during perinatal follicular morphogenesis. Similarly, a young (12 days old) mouse model has also been developed,¹¹ as well as an adult (6–8 weeks old) mouse model using the C57BL/6 strain, in which synchronized anagen is induced by depilation during telogen before administration of the chemotherapeutic agent.⁷ To date, however, there has been no pigmented, adult rat model developed for CIA. We therefore set out to develop a model of CIA using the pigmented Long–Evans (LE) rat strain.

In this study, we tested whether LE rats developed alopecia in response to chemotherapeutic agents, as seen in young albino rats^{5,10,11} and adult mice.⁷ We chose etoposide (a topoisomerase inhibitor) and cyclophosphamide (an alkylating compound) for our experiments because they are common chemotherapeutic agents that are known to cause severe alopecia in patients and have been shown to induce complete loss of body hair in young albino rats.¹¹ We then investigated whether clipping (not plucking) of the hair shaft affected the hair cycle, and whether clipping influenced the HF response to subsequent chemotherapy.

Methods

All animal care and use procedures were approved by the University of Miami Institutional Animal Care and Use Committee.

Animals and experimental procedures

Nursing LE rat pups (Charles River Laboratories, Wilmington, MA, USA) were kept under standard conditions. In our first two experiments, we investigated the effects of etoposide and cyclophosphamide during different phases of the HF cycle.

In the first experiment, pups were injected intraperitoneally (IP) with 1.5 mg/kg etoposide (Sigma-Aldrich, St. Louis, MO, USA) or vehicle alone (sham group) once daily for 3 consecutive days: days 7–9, 14–16 or 21–23. In the second experiment, pups were injected IP with 35 mg/kg cyclophosphamide (Sigma-Aldrich) or vehicle alone (sham) once on days 9, 16 or 23.

In a third experiment we investigated whether clipping affected the HF cycle and HF response to subsequent chemotherapy. Pups were weaned on day 21, and randomly divided into three groups: group I (sham/control) received vehicle-only injections on days 36–38; group II received IP injections of 25 mg/kg etoposide once daily on days 36–38; and group III rats had the hair shaft on their dorsal skin clipped with an electric clipper on day 21 (under anaesthesia), then received IP injections of 25 mg/kg etoposide once daily on days 36–38. Rats were monitored for hair loss and killed at different time points for histological analysis of the skin.

Sample collection and histological analysis

For the first two experiments, pups were killed by CO₂ asphyxiation 7 days after the last etoposide or cyclophosphamide injection, i.e. on days 16, 23 or 30. For the third experiment, rats from different treatment groups were killed on days 25, 30, 36 and 47 for skin sample collection. Two hours before being killed, rats were injected IP with 5-bromo-2'-deoxyuridine (BrdU) Labelling Reagent (10 µL/g) (Invitrogen Corp., Carlsbad, CA, USA).

Dorsal skin biopsies were fixed in 10% formalin, dehydrated and embedded in paraffin wax, before being cut into sections (5 µm) and stained with haematoxylin and eosin. BrdU-positive cells were detected using an immunohistochemistry kit (Invitrogen Corp., Carlsbad, CA, USA), and counterstained with haematoxylin. Images were captured using a microscope (Observer D1; Carl Zeiss Microimaging Inc., Thornwood, NY, USA).

Results

Effects of etoposide and cyclophosphamide on hair follicles

HF's on the rat trunk are generally in anagen before day 14, in catagen on days 14–18 and in telogen on days 19–27, depending on the location.¹² Because antineoplastic agents target proliferating cells, pups treated with etoposide on days 7–9 or cyclophosphamide on day 9 developed alopecia on the entire trunk (Fig. 1b,c) whereas sham-treated pups had no apparent hair loss (Fig. 1a). Pups treated with etoposide on days 14–16 or cyclophosphamide on day 16 had complete alopecia posterior to the nape of the neck on the trunk, whereas there was no apparent hair loss anterior to the nape of the neck (Fig. 1e,f). This is because HF's anterior to the nape of the neck had already entered catagen at this age, whereas HF's posterior to the nape of the neck remained in anagen. More hair was preserved in cyclophosphamide-treated (Fig. 1f) than in etoposide-treated (Fig. 1e) rats, probably because treatment started 2 days later for the former, thus more HF's had entered catagen. The hair regrowth on alopecic areas had normal pigmentation (data not shown). Pups treated with etoposide on days 21–23 or cyclophosphamide on day 23 were completely resistant to CIA (Fig. 1h,i) because the HF's were already in telogen. These results are consistent with observations made in albino SD rat pups,¹¹ and show that the pigmented LE pups can be used as a model for etoposide- or cyclophosphamide-induced alopecia.

In the third experiment, we clipped the dorsal hair of the group III rats on day 21 after birth, during early telogen. We took care to avoid any abrasion of the skin by the electric clipper. Four days later (day 25), there was already a slight difference in the phase of the HF's between the clipped areas of the group III rats (anagen IIIa) and the normal dorsal skin of matched locations in the group I (sham/control) rats (anagen II) (Fig. 2a', b').¹² This difference was confirmed by BrdU labelling of S-phase cells, which detected abundant BrdU-positive keratinocytes in the hair bulbs in the clipped areas of the group III rats (Fig. 2b''), but not in the group I rats (Fig. 2a''). The HF's of clipped areas were at more advanced anagenstages at all the time points examined. On day 30, HF's in the clipped areas of group III rats had penetrated deeper into the subcutaneous adipose tissue, and the new hair shaft had reached the hair canal (anagen IV) (Fig. 2d'), whereas the HF's in group I rats were still in anagen IIIc (Fig. 2c'). By day 36, the hair shafts in the clipped area of group III rats had penetrated the epidermis (anagen VI) (Fig. 2f'), whereas those from group I rats had entered the hair canal but had not penetrated the epidermis (anagen V) (Fig. 2e'). BrdU staining confirmed keratinocyte proliferation in the anagen hair bulbs (Fig. 2c''–f''). The more advanced anagen stages in the clipped areas from group III rats (Fig. 2b', 2d', 2f') suggest that clipping on day 21 during early telogen accelerated anagen entry, and the effects were detectable as early as 4 days after clipping.

On day 36–38, group III (clipped on day 21) and group II (unclipped) rats received IP injections of etoposide once daily. During the next few days, group III rats developed very marked alopecia, whereas group II rats did not (Fig. 2h, 2i, i"). Alopecia in group III rats occurred only in the areas where the hair was clipped (Fig. 2i, i"). On day 47, histological analysis of the clipped areas from group III rats detected typical dystrophic catagen HF, with distorted HF and accumulation of perifollicular ectopic melanin granules (Fig. 2i'). Skin from group II rats contained dystrophic anagen HF in response to chemotherapy (Fig. 2h'), whereas HF show prolonged anagen with little melanin.¹³ As expected for day 47 normal skin, HF in group I rat (unclipped, no chemotherapy) skin were already in catagen (Fig. 2g'). These results indicate that clipping on day 21 had significant effect on hair cycling and consequently the HF response to chemotherapy.

Discussion

Human scalp HF stay in anagen for a very long time (2–6 years), and the vast majority of scalp HF are in anagen at any given time. Therefore, a clinically relevant animal model for CIA needs to show synchronized anagen, hair loss and HF histopathology similar to CIA in patients. Two young rat models and a young mouse model for CIA take advantage of the fact that rat and mouse HF are synchronized during the perinatal HF morphogenesis.^{5,10,11} However, because human scalp hair is pigmented, it is preferable to have animal models with mature, pigmented terminal HF. To this end, an adult mouse model in the pigmented C57BL/6 strain has been established, in which synchronized anagen was induced by physical depilation (plucking).⁷ These models have been widely used in the study of CIA.

In the current study, we established an additional pigmented model for CIA, using the pigmented adult LE rat. We report that hair clipping of the telogen hair shafts, without removal of the entire hair shafts, was sufficient to induce synchronized anagen and subsequent CIA in young rats, a finding that, to our knowledge, has not been previously reported, although previous studies have shown that clipping can induce anagen in rabbits,¹⁴ mice^{15,16} and guinea pigs.¹⁷ The effects of synchronized anagen were detectable as early as 4 days after clipping, and the HF in the clipped areas had the typical HF dystrophy in response to chemotherapy.

It has been shown in previous studies on other animals, using dye on the existing hair shafts, that new hair growth was the result of new hair-shaft formation, not elongation of clipped hair shafts.¹⁵ Moreover, clipping causes no clinical and/or histological irritation of the dorsal skin immediately after the procedure, thus ruling out inflammatory response as a possible cause. Several possible mechanisms of this phenomenon have been proposed, including increased exposure of the clipped area to cooling and light; sensitivity of the exposed skin to grooming; alteration in the oxygen tension due to the increased amount of air in contact with the hair bulb; and changes in the direction of the remaining club hair due to the increased stiffness after clipping, causing friction and stimulating inflammatory responses at the level of the matrix and papilla.^{15,17} It has also been suggested that the transient pulling of the hair shaft by the clipper may mimic plucking to some extent. Future studies are required to investigate the precise timing of clipping-induced anagen entry and the underlying mechanisms. Manipulation of the hair cycle, especially through noninvasive methods without removal of the entire hair shaft, is an attractive approach to develop prophylactic measures for CIA.

Finally, because clipping is fast, simple and does not cause abrasion to the skin, it has been a preferred method for hair removal in experimental animals and clinical and product testing. It is also widely used in combination with other methods (e.g. shaving, chemical depilation) to remove the hair shaft. However, the effects of clipping on hair cycling and keratinocyte

activation have been largely overlooked. A better understanding of the effects of clipping on anagen entry and keratinocyte proliferation and migration will lead to more consistent experimental design and more accurate result interpretation.

Conclusion

We have established the first pigmented, adult rat model for CIA. Synchronized anagen could be induced by clipping during early telogen, without removal of the entire hair shaft.

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What's already known about this topic?

- There have been 2 neonatal albino rat models and a neonatal and an adult mouse model for chemotherapy-induced alopecia (CIA).
- Synchronized anagen was induced by plucking in the adult mouse model.

What does this study add?

- This study established the first pigmented, adult rat model for CIA. It may be useful for testing prophylactic approaches for CIA.
- Synchronized anagen was induced by clipping, without removal of the entire hair shaft.

Figure 1.

Gross phenotype of Long-Evans rat pups treated with(b,e,h) etoposide or (c,f,i) cyclophosphamide compared with (a,d,g) their sham-treated littermates. Pictures were taken 7 days after the last treatment.

Figure 2.

(a–i,i'') Gross phenotype and (a'–i') histology of dorsal skin sections from (group I) control rats and rats treated with etoposide with (group III) or without (group II) prior clipping. (a–i,i'') Hair on the dorsal skin of 21-day-old rats was either (b,d f,i,i'') removed with an electric clipper (group III) or (a,c,e,g,h) not clipped (groups I and II). (f) Small block arrows point to the boundary of partially regrown hair; the highlighted area in (i) is enlarged in (i'') to show that hair loss after etoposide treatment only occurred where hair was clipped on day 21. (a'–i') Dorsal skin sections from rats in (a–i), shown at the same magnification (haematoxylin and eosin, original magnification $\times 50$). (b',d',f') The thicker skin in group III rats reflected more advanced anagen than (a',c',e') control rats. Arrows point to the hair follicle (HF) features used to determine the stages of the HF cycle. (a''–f''). S-phase cells in the anagen hair bulb (arrowheads) (BrdU with haematoxylin counterstain, original magnification $\times 200$).