Oestrogen receptor antagonist and hair regrowth in dogs with hair cycle arrest (alopecia X)

Linda A. Frank

Department of Small Animal Clinical Sciences, University of Tennessee, Knoxville, Tennessee, USA
This project was funded by the American Kennel Club Canine Health Foundation. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the views of the Foundation.
Correspondence: Linda A. Frank, Department of Small Animal Clinical Sciences, University of Tennessee, Knoxville, TN, USA.
Tel.: 8659748387; E-mail: lfrank@utk.edu

What is known about the topic of this paper
- Alopecia X is a common disease in Pomeranian dogs.
- Oestrogen receptor pathway may be important for hair cycle initiation.
- Fulvestrant, a pure oestrogen receptor antagonist, failed to cause hair regrowth in dogs with alopecia X.

What this paper adds to the field of veterinary dermatology
- Oestrogen receptor pathway may be important for hair cycle initiation.
- Fulvestrant, a pure oestrogen receptor antagonist, was used to try to grow hair in dogs with alopecia X.
- Fulvestrant was unsuccessful at causing hair regrowth in dogs with alopecia X.

Abstract

An oestrogen receptor pathway that regulates the telogen–anagen hair follicle transition in mice has been described. The purpose of this study was to investigate whether fulvestrant, a pure oestrogen receptor antagonist, would cause hair regrowth in Pomeranian dogs with hair cycle arrest (alopecia X). Eleven Pomeranian dogs with hair cycle arrest were randomly assigned to receive two intramuscular injections of either 10 mg kg^{-1} fulvestrant (n = 6) or an equal volume of saline (n = 5) 1 month apart. Complete blood count, chemistry panel, and urinalysis were monitored prior to the first injection and monthly for 2 months. Dogs were evaluated each month for degree of hair growth, percentage of body affected, and quality of new hair growth. Three control dogs received fulvestrant after the completion of the study. In addition, one control dog and one treatment dog received two subcutaneous injections of 20 mg kg^{-1} fulvestrant 1 month apart. No dogs that received 10 mg kg^{-1} fulvestrant had any evidence of hair regrowth. The control dog that received 20 mg kg^{-1} fulvestrant had substantial hair regrowth 1 month after the first injection. No adverse effects from the treatment were noted. Fulvestrant does not appear to be a feasible treatment for dogs with hair cycle arrest (alopecia X) when administered intramuscularly at 10 mg kg^{-1}. A higher dose of fulvestrant requires more investigation but may be cost-prohibitive.

Accepted 05 October 2006

Hair cycle arrest (also known as alopecia X, adrenal hyperplasia-like syndrome, growth hormone responsive alopecia, and others) is a common condition seen in Pomeranian dogs and other breeds.[1] The pathomechanism of the hair loss is not known, but appears to be related to an abnormality in the hair follicle's ability to cycle properly.[1] Once the hair is in telogen, new hair growth does not occur. Little is known about the hair cycle and what controls it in dogs. Of recent interest is the recognition of an oestrogen receptor pathway that regulates the telogen–anagen hair follicle transition in mice.[2–4] Oestrogen has been shown to be produced by the hair follicle itself,[5] and oestrogen receptors have been identified in canine hair follicles.[6] Interestingly, melatonin and trilostane, two drugs used to treat hair cycle arrest,[7,8] have been shown to block the activation of oestrogen receptors in human breast cancer cells.[9,10]

Continued research investigating more potent oestrogen receptor blockers for treating women with breast cancer has resulted in the synthesis of an injectable pure oestrogen receptor antagonist, fulvestrant (Faslodex®, AstraZeneca Pharmaceuticals LP, Wilmington, DE, USA) without known agonist effects. This drug has a high affinity for oestrogen receptors α and β, resulting in their downregulation.[11,12] This product has been tested in dogs, mice, rats, monkeys, and people and has shown no adverse effects other than those related to anti-oestrogen activity such as a reduction in fertility.[11,13] The purpose of this study was to investigate whether fulvestrant would cause hair regrowth in Pomeranian dogs with hair cycle arrest.

Eleven Pomeranian dogs with hair cycle arrest were entered into the study. The study was approved by the University of Tennessee, Knoxville Institutional Animal Care and Use Committee. There were four intact males, four castrated males, two intact females, and one spayed female dog. Hypothyroidism and hyperadrenocorticism were ruled out prior to enrolment. With the exception of the intact females, all dogs had been part of a previous study in which biopsies had been obtained to look for α-oestrogen receptor α in Pomeranian dogs with hair cycle arrest.[7] Dogs were randomly assigned to receive two

intramuscular injections in the caudal thigh of either fulvestrant (10 mg kg^{-1}) or a similar volume of saline, 1 month apart. This resulted in six dogs receiving fulvestrant and five dogs receiving saline. The owners were blinded as to which treatment each dog received. Because of the viscosity of the fulvestrant, the investigator was not blinded to treatment. Complete blood count, chemistry panel, and urinalysis were monitored prior to the first injection and monthly for 2 months. Dogs were evaluated for degree of hair growth, percentage of body affected, and quality of new hair growth before the first injection and once a month for 2 months. Continued monitoring after that time was accomplished by telephone communication and e-mail. In addition, owners of dogs in the control group were offered to have their dogs treated with fulvestrant at the completion of the study and three dogs received this treatment. Three months after the completion of the study, one dog originally treated with 10 mg kg^{-1} fulvestrant and one control dog received two subcutaneous injections of 20 mg kg^{-1} fulvestrant, 1 month apart.

A total of nine dogs received two intramuscular injections of 10 mg kg^{-1} fulvestrant (six dogs from the initial randomized portion of the study and three control dogs). There was no evidence of new hair growth in any of these dogs. Owners of two dogs that received fulvestrant felt that the areas where the hair coat was present from the beginning were thicker than at the start of the study but this was not perceived by the investigator. Of the two dogs subsequently treated with two subcutaneous injections of 20 mg kg^{-1} fulvestrant 1 month apart, one regrew a substantial amount of hair in the alopecic areas (Fig. 1) 1 month after the first injection. This dog, an intact female, had been in the control group and had not received any fulvestrant prior to this time. The other dog had originally been treated with 10 mg kg^{-1} fulvestrant and showed no evidence of hair regrowth at the increased dose. There were no adverse effects from the fulvestrant injections. Complete blood count and chemistry panel values were unchanged during the course of the treatments. Post-treatment urinalyses were only performed on three dogs (two treated with saline, one treated with fulvestrant) because of the difficulty in obtaining samples. While no abnormalities were detected, meaningful interpretation is not possible. One dog treated with fulvestrant whose alkaline phosphatase and alanine aminotransferase were mildly increased at the beginning of the study remained increased throughout the study. There was no swelling or pain at the injection sites.

Control of the hair cycle is complex and just now being elucidated. Telogen hair follicular papilla express bone morphogenic protein (BMP-4) and oestrogen receptor α, both are important molecules for maintenance of hair follicles in telogen.[14] It is theorized that oestrogen stimulates BMP-4 expression, which is associated with downregulation of certain molecules such as sonic hedgehog homologue, resulting in hair growth inhibition. When BMP-4 activity is reduced by oestrogen receptor antagonists or noggin, these molecules are no longer suppressed, leading to anagen initiation.

The oestrogen receptor is a likely target in dogs with hair cycle arrest since both melatonin and trilostane have been shown to block oestrogen receptors in human breast cancer cells[9,10] and both can induce hair growth in some dogs with hair cycle arrest.[7,8] In addition, gonadectomy may result in hair regrowth in dogs with hair cycle arrest. In mice, stimulation of anagen following gonadectomy appears to be mediated via the oestrogen receptor pathway because anagen can be blocked by treating the mice with topical oestrogen post-surgery.[3]

Fulvestrant binds to the oestrogen receptor in a competitive manner with similar affinity to oestradiol. This

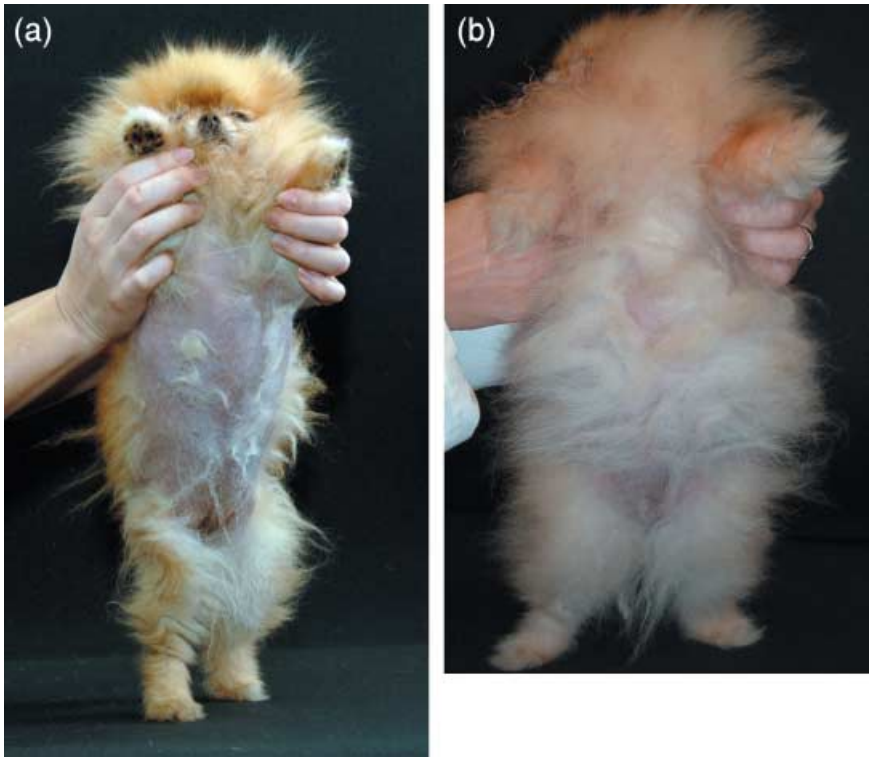

Figure 1. The ventral abdomen of an intact female Pomeranian dog. (a) Before fulvestrant treatment. (b) One month following the second injection of fulvestrant (20 mg kg^{-1} subcutaneous).

product was chosen over other anti-oestrogens because it is the only pure anti-oestrogen as compared to tamoxifen, which has both oestrogen-antagonist and -agonist responses.[3,11] In addition, it appears to be very safe in dogs. Initial studies with dogs and rats showed that fulvestrant is well absorbed and widely distributed after intramuscular injection, and is eliminated mostly in the faeces.[13] Metabolism of the drug is similar in humans, dogs, and rats with primary clearance by the hepatobiliary route.[13] No increased toxicity has been seen when administered to patients with mild hepatic impairment. Multiple-dose toxicity studies in dogs of up to 12 months duration using doses fourfold higher than that of people showed only anti-oestrogenic side-effects.[13] In people, injection reactions are the most common adverse event consisting of transient pain and inflammation.[13] In the present study, fulvestrant was well tolerated in all dogs with no injection reactions observed by the owners.

Unfortunately, fulvestrant failed to cause hair regrowth in any of the dogs in this study after intramuscular injections of 10 mg kg^{-1}. It is possible that the dose given was too small. The dose was chosen based on extrapolation from the human dose of 185 mg m^{-2} and was the same as that used in monkey studies (10 mg kg^{-1}).[15] The cost of 250 mg per 5 mL fulvestrant is approximately $1000US. It was hoped that a Pomeranian dog could be treated with one or two doses of approximately 0.5 mL, which would provide approximately 10 doses per vial. Only one of the two dogs treated with subcutaneous injections of 20 mg kg^{-1} fulvestrant had hair regrowth. More dogs would need to be treated with this dose in order to determine the success rate to justify the increased expense. It is also possible that the route of administration was inappropriate. In mice studies the intraperitoneal injection of fulvestrant at a dose equal to that used topically was ineffective in causing hair growth.[1,3] Additionally, for anagen initiation the hair follicle may need stimulation from another molecule, such as noggin or insulin-like growth factor in conjunction with blocking the oestrogen receptor,[14] which may fail to be expressed in sufficient concentrations in dogs with hair cycle arrest (alopecia X).

References

1. Frank LA. Growth hormone-responsive alopecia in dogs. Journal of the American Veterinary Medical Association 2005; 226: 1494–7.
2. Oh H, Smart RC. An oestrogen receptor pathway regulates the telogen-anagen hair follicle transition and influences epidermal cell proliferation. Proceedings of the National Academy of Sciences of the United States of America 1996; 93: 12525–30.
3. Smart R. C., Oh, H, Chanda, S et al. Effects of 17-β-estradiol and ICI 182 780 on hair growth in various strains of mice. Journal of Investigative Dermatology Symposium Proceedings 1999; 4: 285–9.
4. Chanda S, Robinette CL, Couse JF et al. 17β-Estradiol and ICI-182780 regulate the hair follicle cycle in mice through an oestrogen receptor-α pathway. American Journal of Physiology, Endocrinology, and Metabolism 2000; 278: E202–E210.
5. Schweikert HU, Milewich L, Wilson JD. Aromatization of androstenedione by isolated human hairs. Journal of Clinical Endocrinology and Metabolism 1975; 40: 413–7.
6. Bratka-Robia CB, Egerbacher M, Helmreich M et al. Immunohistochemical localization of androgen and oestrogen receptors in canine hair follicles. Veterinary Dermatology 2002; 13: 113–8.
7. Frank LA, Donnell RL, Kania SA. Oestrogen receptor evaluation in Pomeranian dogs with hair cycle arrest (alopecia X) on melatonin supplementation. Veterinary Dermatology 2006; 17: 252–8.
8. Cerundolo R, Lloyd DH, Persechino A et al. Treatment of canine alopecia X with trilostane. Veterinary Dermatology 2004; 15: 285–93.
9. Kiefer T, Ram PT, Yuan L et al. Melatonin inhibits oestrogen receptor transactivation and cAMP levels in breast cancer cells. Breast Cancer Research and Treatment 2002; 71: 37–45.
10. Puddefoot JR, Barker S, Glover HR et al. Non-competitive steroid inhibition of oestrogen receptor functions. International Journal of Cancer 2002; 101: 17–22.
11. Howell A, Osborne CK, Morris C et al. ICI 182 780 (Faslodex™). Development of a novel, 'pure' antiestrogen. Cancer 2000: 89: 817–25.
12. Robertson JF, Nicholson RI, Bundred NJ et al. Comparison of the short-term biological effects of (Faslodex) versus Tamoxifen in postmenopausal women with primary breast cancer. Cancer Research 2001; 61: 6730–46.
13. Bross PF, Baird A, Chen G et al. Fulvestrant in postmenopausal women with advanced breast cancer. Clinical Cancer Research 2003; 9: 4309–17.
14. Botchkarev VA, Kishimoto J. Molecular control of epithelial–mesenchymal interactions during hair follicle cycling. Journal of Investigative Dermatology Symposium Proceedings 2003; 8: 46–55.
15. Wakeling AE, Dukes M, Bowler J. A potent specific pure antiestrogen with clinical potential. Cancer Research 1991; 51: 3867–73.

Résumé Chez la souris, un récepteur aux oestrogènes a été rapporté comme important pour la régulation de la transition entre la phase télogène et la phase anagène. Le but de cette étude était de déterminer si le fulvestrant, un antagoniste purdes récepteurs aux oestrogènes pouvait provoquer une repousse pilaire chez des Pomeranians à arrêt du cycle folliculaire (alopécie X). Onze Pomeranians présentant un arrêt du cycle ont été divisés en deux groupes au hasard et ont reçu soit deux injections intramusculaires de fulvestrant à 10 mg kg^{-1} (n = 6) soit un volume équivalent de soluté salé à un mois d'intervalle. Une numération formule, une biochimie sanguine et des analyses urinaires étaient réalisées avant la première injection puis tous les mois pendant deux mois. Les chiens ont été évalués tous les mois pour la repousse pilaire, le pourcentage d'atteinte cutanée et la qualité des poils. Trois chiens du groupe contrôle ont été traités avec le fulvestrant après la fin de l'étude. En outre, un chien du grouep contrôle et un chien du groupe traité ont reçu deux injections de 20 mg kg^{-1} de fulvestrant à un mois d'intervalle. Le chien du groupe contrôle qui a reçu 20 mg kg^{-1} de fulvestrant a présenté une repousse substantielle un mois après la première injection. Aucun effet secondaire n'a été observé. Le. Fulvestrant n'apparaît pas être un traitement pratique chez les chiens présentant un arrêt du cycle à la dose de 10 mg kg^{-1} par voie intramusculaire. L'intérêt d'une dose supérieure mérite des études supplémentaires, mais le coût pourrait être prohibitif.

Resumen Previamente se ha descrito una ruta intracelular del receptor de estrógenos que regula la transición de fase telógena a anágena en el ratón. El propósito de este estudio fue investigar si fulvestrant, un antagonista puro del receptor de estrógeno, podría causar crecimiento del pelo en perros de raza Pomerana

con arresto del ciclo folicular (alopecia X). Once perros Pomeranos con arresto en el ciclo folicular se asignaron al azar para recibir dos inyecciones intramusculares de 10 mg/kg de fulvestrant (n = 6) o un volumen equivalente de suero salino (n = 5) con un mes de separación entre inyecciones. Se controló la evolución de los animales mediante recuento de sangre, bioquímica sanguínea y urianálisis antes de la primera inyección y mensualmente durante dos meses. Los perros se evaluaron cada mes en relación con el crecimiento de pelo, porcentaje del cuerpo afectado, y calidad del nuevo pelo. Tres perros control recibieron fulvestrant tras completar el estudio. Además un perro control y un perro tratado recibieron dos inyecciones subcutáneas de 20 mg/kg de fulvestrant con un mes de separación. Ninguno de los perros que recibió una dosis de 10 mg/kg presentó crecimiento de pelo. El perro control que recibió 20 mg/kg presentó crecimiento de pelo sustancial un mes después de la primera inyección. No se observaron efectos adversos con el tratamiento. Fulvestrant no parece ser un tratamiento adecuado para perros con arresto del ciclo folicular (alopecia X) a la dosis de 10 mg/kg. Una dosis mas elevada de fulvestrant requiere más investigación pero el coste puede ser prohibitivo.

Zusammenfassung Ein Signalweg des Östrogenrezeptors, der den Übergang vom telogenen in das anagene Haarfollikel bei Mäusen reguliert, wurde bereits beschrieben. Das Ziel dieser Studie war es herauszufinden, ob Fulvestrant, ein reiner Östrogenrezeptorantagonist, das Nachwachsen der Haare bei Pomeraniern im arretierten Haarzyklus (Alopecia X) bewirken würde. Elf Pomeranier im arretierten Haarzyklus wurden zufällig eingeteilt, um zwei intramuskuläre Injektionen im Abstand von einem Monat von entweder 10 mg kg^{-1} Fulvestrant (n = 6) oder demselben Volumen von physiologischer Kochsalzlösung (n = 5) zu erhalten. Blutstatus, Blutbiochemie und Harnanlyse wurden vor der ersten Injektion und dann monatlich zwei Monate lang überwacht. Bei den Hunden wurden monatlich das Ausmaß des Haarwachstums, der Prozentsatz des Körpers, der davon betroffen war, sowie die Qualität des neugewachsenen Haares, evaluiert. Drei Kontroll-Hunde erhielten Fulvestrant nach Beendigung der Studie. Zusätzlich erhielten ein Kontroll-Hund und ein zu behandelnder Hund zwei subkutane Injektionen von 20 mg kg^{-1} Fulvestrant im Abstand von einem Monat. Keiner der Hunde, die 10 mg kg^{-1} Fulvestrant erhalten hatten, zeigten Evidenz für ein Nachwachsen der Haare. Der Kontrollhund, welcher 20 mg kg^{-1} Fulvestrant bekommen hatte, zeigte einen Monat nach der ersten Injektion beachtliches Haarwachstum. Nebenwirkungen wurden bei der Behandlung keine festgestellt. Fulvestrant scheint für Hunde im arretierten Haarzyklus (Alopecia X) keine brauchbare Therapie zu sein, wenn es intramuskulär bei einer Dosis von 10 mg kg^{-1} verabreicht wird. Für die Verwendung einer höheren Dosis von Fulvestrant bedarf es weiterer Untersuchungen, es könnte aber unerschwinglich teuer sein.