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Treatment and prevention of chemotherapy-induced alopecia with PTH-CBD, a collagen-targeted parathyroid hormone analog, in a non-depilated mouse model

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Abstract

Alopecia is a psychologically devastating complication of chemotherapy for which there is currently no effective therapy. PTH-CBD is a collagen-targeted parathyroid hormone analog that has shown promise as a therapy for alopecia disorders. To compare the efficacy of prophylactic versus therapeutic administration of PTH-CBD in chemotherapy-induced alopecia using a mouse model that mimics the cyclic chemotherapy dosing used clinically. C57BL/6J mice were treated with a single subcutaneous injection of PTH-CBD (320 mcg/kg) or vehicle control before or after hair loss developing from three courses of cyclophosphamide chemotherapy (50–150 mg/kg/week). Mice receiving chemotherapy alone developed hair loss and depigmentation over 6–12 months. Mice pretreated with PTH-CBD did not develop these changes and maintained a normal-appearing coat. Mice treated with PTH-CBD after development of hair loss showed a partial recovery. Observations of hair loss were confirmed quantitatively by gray scale analysis. Histological examination showed that in mice receiving chemotherapy alone, there were small, dystrophic hair follicles mostly in the catagen phase. Mice receiving PTH-CBD before chemotherapy showed a mix of normal-appearing telogen and anagen hair follicles with no evidence of dystrophy. Mice receiving PTH-CBD therapy after chemotherapy showed intermediate histological features. PTH-CBD was effective in both the prevention and the treatment of chemotherapy-induced alopecia in mice, but pretreatment appears to result in a better cosmetic outcome. PTH-CBD shows promise as an agent in the prevention of this complication of chemotherapy and improving the quality of life for cancer patients.

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Conflicts of interest

PTH-CBD is patented and exclusively licensed to Biologics MD, LLC. Robert Gensure is Chief Medical Officer of Biologics MD. Robert Gensure, Tulasi Ponnappakkam, Osamu Matsushita, and Joshua Sakon have a stock ownership in Biologics MD. Ranjitha Katikaneni has no conflicts of interest.

Keywords

anagen VI; chemotherapy-induced alopecia; cyclophosphamide; dystrophy; PTH-CBD

Introduction

Alopecia (hair loss) is a psychologically and emotionally distressing side effect of cancer chemotherapeutic drugs [1]. Chemotherapy-induced alopecia (CIA) can result in anxiety, depression, a negative body image, lowered self-esteem, and a reduced sense of wellbeing [2,3]. The incidence and severity of CIA are variable and related to the particular chemotherapeutic protocols [4]. It is estimated that 65–85% of chemotherapy patients experience some degree of alopecia [5,6]. Overall, 47–58% of female cancer patients considered hair loss to be the most traumatic aspect of chemotherapy and 8% would decline treatment for fear of hair loss [2,5].

The mechanism of CIA is unknown and probably specific for each chemotherapeutic agent [7]. There appears to be a direct effect of at least some chemotherapeutics causing damage to the hair follicles [8,9]. There are currently no effective therapies for CIA [10,11], nor are there any satisfactory methods of preventing CIA [12]. The only currently approved prevention for CIA is scalp hypothermia during chemotherapeutic courses to reduce scalp blood flow and reduce delivery, and hence the damage, of the chemotherapeutics to the skin. Clinical reports and testimonials do provide support showing efficacy for this treatment [13–15]. However, the therapy is very expensive (Penguin Cold Cap total cost \$600 US/month plus shipping), and there are concerns that vasoconstriction in the scalp may promote the development of cutaneous scalp metastasis [16].

Parathyroid hormone (PTH) agonists and antagonists have been shown to effectively manipulate the hair follicle response to chemotherapy-induced damage in depilated mice [17]. However, the regrowth of hair required multiple intraperitoneal injections, which may be the result of limited delivery and retention of the drug to the skin. To improve skin delivery and retention, we synthesized fusion proteins linking PTH agonists and antagonists to a collagen-binding domain (CBD) derived from ColH collagenase of *Clostridium histolyticum*. We showed that these compounds are concentrated and retained in the skin after intraperitoneal or subcutaneous administration [18,19]. In a depilated mouse model of cyclophosphamide (CYP)-induced alopecia, a single dose of the agonist, PTH-CBD, restored hair growth over a 30-day period, increasing the number of anagen VI phase hair follicles; CBD-linked PTH antagonists had no evident effect [18]. Although depilation has the advantage of synchronizing the hair follicles and maximizing the chemotherapy-induced damage, depilation itself causes injury to hair follicles as well, which may have affected our experimental results [20,21]. In fact, we confirmed in a pilot study (results below) that PTH-CBD therapy does indeed alter the course of recovery following depilation injury without chemotherapy. During the course of conducting long-term experiments on the effects of PTH-CBD on chemotherapy-induced osteoporosis, we observed that providing three cycles of CYP chemotherapy 1–2 weeks apart, in the absence of depilation, results in long-term hair loss, presumably because spacing the cycles of chemotherapy captures a different set of

hair follicles in the anagen phase each time [22]. We therefore proceeded with a series of studies using this model to test the effects of PTH-CBD agonist in CIA in mice in the absence of depilation.

Methods

Animals

Healthy female mice (C57BL/6J), 3–5 weeks of age, weighing 13–15 g were obtained from Jackson Laboratories (Bar Harbor, Maine, USA) and housed in cages at the animal facility at Ochsner Clinic Foundation under standard living conditions, including a diet consisting of 18% protein purchased from Harlan Company (Barton, Illinois, USA and Madison, Wisconsin, USA). The mice were allowed to acclimatize for a 2-week period before the start of the experiment. Approval for these studies was obtained from the Institutional Animal Care and Use Committee (Ochsner Clinic Foundation, New Orleans, Louisiana, USA).

Chemicals

CYP was obtained from the Ochsner Clinic Foundation Cancer Infusion Center in solution (20 mg/ml) and used within 24 h to ensure full bioactivity. PTH-CBD and PTH(1–33)-CBD were diluted for injection in a collagen-binding buffer vehicle (50 mmol/l Tris HCl, 5 mmol/l CaCl₂, pH 7.5).

Treatment protocols

Pilot study - depilation only—Twenty-one mice were depilated by shaving, followed by waxing using Sally Hanson wax strips (Wal-Mart, Luling, Louisiana, USA). Mice were then divided equally into three groups. (a) Vehicle control: received an injection of vehicle only. (b) PTH-CBD: received a single subcutaneous injection of 320 µg/kg of PTH-CBD. (c) PTH antagonist: received a single subcutaneous injection of 320 µg/kg of PTH(7–33)-CBD. All injections were administered at the time of depilation. Mice were observed for 18 days and then killed.

Study 1: prophylactic effects of PTH-CBD on chemotherapy-induced alopecia

—Thirty mice were divided equally into three groups. (a) No chemo: received injections of vehicle only; (b) chemo: received CYP injections at a dose of 50 mg/kg at 0, 2, and 4 weeks and buffer at week 0. (c) Chemo + PTH-CBD prophylaxis: chemo + PTH-CBD received injections of 50 mg/kg CYP at 0, 2, and 4 weeks and a single injection of 320 µg/kg of PTH-CBD at week 0. All injections were administered intraperitoneally. The mice were observed for 15 months and then killed.

Study 2: treatment effects of PTH-CBD on chemotherapy-induced alopecia

Twenty mice were injected intraperitoneally with three separate doses of CYP (120, 120, and 150 mg/kg) at 0, 2, and 6 weeks. The mice were observed periodically for the general health status, signs of alopecia as well as change in hair color. After 4 months, the mice developed variable hair loss and loss of pigmentation. In addition, we removed hair from a small region on the lower dorsal surface of mice using Nair cream. Mice were divided randomly into two groups of 10 each, a chemotherapy-only group (chemo) receiving a

subcutaneous injection of vehicle and a treatment group (chemo + PTH-CBD therapy) receiving a subcutaneous injection of PTH-CBD (320 mcg/kg) at the site of hair removal. The mice were observed for 21 days and then killed.

Study 3: prophylactic versus treatment effects of PTH-CBD for chemotherapy-induced alopecia—Twenty-four healthy female mice received three doses of CYP by an intraperitoneal injection (150 mg/kg at 0, 1, and 2 weeks). Of these, eight mice (chemo + PTH-CBD prophylaxis) received a single dose of subcutaneous injection of PTH-CBD (320 µg/kg) together with the first course of chemotherapy (week 0). After 12 months, mice receiving chemotherapy alone were divided into two groups, one receiving a single subcutaneous injection of PTH-CBD (320 mcg/kg) (chemo + PTH-CBD therapy) and one receiving vehicle (chemo). The mice were observed for 4 months and then killed.

All the three study protocols have been summarized in Table 1.

Data collection

Photo-documentation—Photo-documentation was obtained to monitor the changes in hair growth. For gray scale analysis, images were captured using the Kodak Gel Logic 100 Imaging System (Eastman Kodak, Rochester, New York, USA) on a Spectroline Bi-O-Vision UV/white light transilluminator (Spectronics Corporation, Westbury, New York, USA).

Photographs were taken with exposure 0.2 s, F-stop 2 mm, and magnification 15 mm to maintain the hair texture in the linear range for analysis. For gray scale analysis, an elliptical region of interest was selected on the dorsal skin of the mice, covering as much of the back of the mouse as possible. This densitometry value was normalized to the average of those obtained from two background region of interests placed on either side of the mouse.

Histology—Mice were killed and skin regions from the nape of the neck to the middle of the back were obtained for histological analysis. The skin was fixed in 10% buffered formalin and processed for routine histology using Hematoxylin and Eosin staining. Skin samples were sectioned along the longitudinal and cross-sectional axes of the hair follicles. The number of anagen VI hair follicles and the number of these hair follicles in each skin layer per high-power field were determined by three independent observers who were blinded to the treatment status of the animals. The high-power fields with maximum follicular density were selected for counting: one with follicles predominantly on long section and one with follicles predominantly on the cross-section selected per slide.

Statistical analysis—Measurements were analyzed by analysis of variance, followed by post-hoc tests, either Bonferroni's or Dunnett's, as indicated for each comparison. The software used for this statistical analysis is GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, California, USA).

Results

Pilot study - depilation only

To determine whether the positive effects of PTH-CBD on hair regrowth in a depilated mouse model of chemotherapy [18] might be partially attributable to positive effects on depilation injury, we examined the effects of our treatments in mice depilated by waxing with no chemotherapy. Vehicle-treated mice showed anagen response on day 9–10 as expected (figure not shown), and by day 18 had a thin coat of hair covering the depilated region (Fig. 1a). PTH-CBD-treated animals had an earlier anagen response at the injection site (day 7), early hair eruption by day 10 as expected, and by day 18 had a thicker coat of hair (Fig. 1a). Antagonist-treated animals had an anagen eruption at the site of injection that was earlier still, on day 5, followed by modest hair eruption over that location (figure not shown). However, further hair growth appeared to have been inhibited such that by day 18, the injection sites appeared relatively bare (Fig. 1a). On histological examination, both vehicle control-treated and agonist-treated animals showed abundant normal-appearing anagen follicles on day 18 (Fig. 1b), as expected following the depilation injury. Antagonist-treated animals had fewer hair follicles at the site of injection, which appeared to be dystrophic, with evident melanocyte clumping (Fig. 1b). These histological changes bear remarkable resemblance to those observed in the depilated CIA animals [18], making it difficult to distinguish drug effects in response to depilation injury versus those in response to chemotherapy injury.

Thus, although depilation has the advantage of synchronizing the hair follicles to provide maximal and more uniform chemotherapy-induced damage, the depilation itself causes an injury response in the hair follicles that can be modulated by treatment with CBD-linked PTH analogs. We therefore proceeded with the following experiments to determine whether our previous findings in the depilated chemotherapy mouse model [18] could be confirmed in a model of chemotherapy alopecia that does not include a depilation step. As the antagonist compounds showed obvious deleterious effects on hair growth, we did not conduct further testing of these compounds in treating chemotherapy alopecia.

Study 1: prophylactic effects of PTH-CBD on chemotherapy-induced alopecia

In a study carried out primarily to evaluate the effects of PTH-CBD on chemotherapy-induced osteoporosis [23], we observed differences in hair loss patterns between treated and control animals. At the termination of the study (15 months after the initial treatment), the group receiving CYP chemotherapy alone (chemo) showed variable hair loss and depigmentation, whereas the group receiving CYP and PTH-CBD (chemo + PTH-CBD prophylaxis) showed normal hair that was indistinguishable from that of mice receiving no chemotherapy (no chemo) (Fig. 2).

Histological examination showed morphological changes in the hair follicles after CYP therapy (chemo), which were more superficially located and showed clumped melanocytes around the bulb, characteristics of the dystrophic anagen and catagen phase [24] (Fig. 3). PTH-CBD pretreatment (chemo + PTH-CBD prophylaxis) led to deeper rooting and reduced melanocyte clumping, thus reversing the dystrophic changes. Hair follicle counts from skin

samples obtained at the end of the study period showed a trend toward greater number of hair follicles in the subcutaneous fat layer (9.3 ± 6.8 vs. 0.6 ± 0.5 , NS), which, on the basis of the anatomical location, can be presumed to be in the anagen VI phase. The greater number of anagen VI hair follicles and deeper-rooted hair follicles are consistent with the observed increases in hair growth. These results provided indications that PTH-CBD pretreatment could prevent the hair loss from CYP chemotherapy.

Study 2: treatment effects of PTH-CBD on chemotherapy-induced alopecia

After observing these prophylactic effects of PTH-CBD in chemotherapy-induced hair loss, we next tested whether PTH-CBD administration can treat CIA if administered after the hair loss develops in this non-depilated mouse model. Mice administered a higher dose of CYP than in the first study developed variable hair loss, thinning, and decreased pigmentation 4 months after chemotherapy (Fig. 4). Twenty-one days after the administration of PTH-CBD (chemo + PTH-CBD therapy), mice showed evidence of partial regrowth and repigmentation of hair, whereas those receiving vehicle alone (chemo) showed continued hair loss (Fig. 4). Skin samples obtained from the site of injection at the termination of the study showed relatively few dystrophic hair follicles in the chemo group, similar to those in the first study (not shown). The PTH-CBD-treated mice (chemo + PTH-CBD therapy) showed more numerous hair follicles with reversal of the dystrophic changes, again similar to those in the first study (not shown). Histological sections of regions of the tail, where the hair coat is normally thin, were indistinguishable between groups (not shown). Thus, it appears that administration of PTH-CBD after hair loss occurs can reverse some of the chemotherapeutic-induced changes, although there is a suggestion of only partial recovery.

Study 3: prophylactic versus treatment effects of PTH-CBD for chemotherapy-induced alopecia

Having observed the beneficial effects of PTH-CBD when administered before or after development of chemotherapy-induced hair loss, we next conducted an experiment to directly compare prophylactic versus therapeutic administration of PTH-CBD on hair growth after CYP administration. Animals receiving chemotherapy alone (chemo) showed evidence of hair loss beginning 6–12 months after treatment, whereas animals treated with PTH-CBD at the time of receiving chemotherapy (chemo + PTH-CBD prophylaxis) showed no evidence of hair loss at any time point (Fig. 5). Animals treated with PTH-CBD after the hair loss had developed (chemo + PTH-CBD therapy) showed partial recovery of hair growth over the next 4 months.

When quantified by gray scale analysis, animals treated with PTH-CBD either prophylactically (chemo + PTH-CBD prophylaxis) or therapeutically (chemo + PTH-CBD therapy) showed significantly greater average light absorption on the dorsum compared with those receiving chemotherapy alone (Fig. 6, $P < 0.05$). As the hair on C57BL/6J is naturally black, this finding indicates a combination of increased hair and/or increased hair pigmentation in the PTH-CBD-treated animals. There was also a nonsignificant trend toward greater light absorption in animals treated with PTH-CBD prophylactically versus those treated after hair loss had developed.

Histological examination of the skin samples from the chemotherapy-only group (chemo) again showed small, dystrophic hair follicles with melanin clumping (not shown). Prophylactic administration of PTH-CBD (chemo + PTH-CBD prophylaxis) resulted in reversal of the dystrophic changes, and mice in the treatment group (chemo + PTH-CBD therapy) showed intermediate histological features (not shown). Hair follicle counts were highly variable, presumably because the hair follicles were not synchronized by depilation. Although analysis by two-way analysis of variance was not significant, there was an apparent trend toward greater number of anagen VI hair follicles in the subcutaneous region with prophylactic PTH-CBD administration (chemo + PTH-CBD prophylaxis) versus chemotherapy alone (chemo) (15.6 ± 12.3 vs. 0.1 ± 0.1 , NS), but not with animals treated with PTH-CBD after developing alopecia (chemo + PTH-CBD therapy) (0.1 ± 0.1 vs. 0.1 ± 0.1 , NS) (Fig. 7). There did appear to be a trend toward a greater number of hair follicles in the dermal layers in the therapy group versus the no chemo group (5.0 ± 2.8 vs. 10.2 ± 0.4 , NS).

Discussion

PTH-CBD is a fusion protein of the active portion of parathyroid hormone and a bacterial collagen-binding domain. This compound was designed to promote the distribution and retention of PTH(1–33) to high collagen-containing tissues with relatively high blood flow, such as bone and skin [19,23]. We have shown previously that PTH-CBD promotes more rapid regrowth of hair in a depilated mouse model of CIA [18]. However, we observed in those studies that depilation itself induces anagen VI phase transition of the hair follicles in control animals not receiving chemotherapy, [20,21] and here, we show that the response to this hair follicle injury is modulated by PTH-linked CBD compounds, raising concerns that the positive effects of PTH-CBD observed previously in the depilated mouse model of CIA may have resulted from improved repair of depilation injury rather than of chemotherapy injury. Interestingly, this study also showed what appear to be previously unknown deleterious effects of PTH antagonists on hair growth, inducing dystrophic changes in hair follicles and inhibiting normal regrowth of hair after depilation. These findings provide evidence that the agonist PTH-CBD and the antagonist PTH(7–33)-CBD do indeed have opposing effects on hair follicles.

We observed in studies of chemotherapy-induced osteoporosis that a chemotherapeutic regimen that more closely mimics standard regimens for cancer therapy in humans also results in long-term hair loss in mice. Presumably, cyclical administration of chemotherapy captures a different set of hair follicles in the anagen phase with each cycle. We therefore proceeded to test the effects of PTH-CBD in this model of CIA in mice in the absence of depilation. When administered prophylactically, a single subcutaneous injection of PTH-CBD prevented CYP-induced hair changes, such as thinning and color change. These changes were grossly apparent in the photo-documentation record; in fact, the chemotherapy-treated mice that received PTH-CBD prophylaxis were indistinguishable from those that did not receive chemotherapy (Fig. 2). Histologically, the mice treated with CYP alone showed hair follicles predominantly in the dystrophic anagen and catagen phases, whereas those pretreated with PTH-CBD showed deeper rooting and reduced melanocyte clumping, thus reversing the dystrophic changes, similar to animals that did not receive chemotherapy (Fig. 3).

When administered as a therapy after hair loss develops from CYP administration, PTH-CBD treatment promoted regrowth of hair, although 21 days after PTH-CBD treatment the hair loss had not completely reversed in most animals. Histologically, the PTH-CBD administration again resulted in more numerous hair follicles and in resolution of the dystrophy induced by CYP treatment. On directly comparing prophylactic versus therapeutic administration of PTH-CBD, we again observed no hair loss after prophylactic PTH-CBD administration; therapeutic administration of PTH-CBD resulted in correction of hair loss that appeared to be less complete. Although PTH-CBD administration resulted in resolution of hair follicle dystrophy when administered either before or after the hair loss event, we only observed apparent increases in anagen VI hair follicles with prophylactic PTH-CBD administration (Fig. 7). There did appear to be a greater concentration of hair follicles in the dermal layers in the therapy group, which appeared to be more mature telogen follicles. Importantly, there was no apparent change in hair growth, nor were there any evident histological changes, in regions of skin (tail, ears) that do not normally have a full coat after PTH-CBD administration.

CYP is an alkylating agent that induces DNA damage, thus preferentially affecting cells that are dividing more frequently. PTH would not be expected to have any direct effects in inhibiting this process; the prevention of hair loss presumably occurs as a result of physiologic antagonism of the chemotherapy-induced hair loss effect. The increase in anagen VI follicles after PTH-CBD treatment is consistent with known effects of PTH in activating WNT signaling and increasing the production of β -catenin [25], which induces hair follicle transition to the anagen phase [26,27]. However, given that CYP primarily damages anagen VI hair follicles [8], it is surprising that prophylactic administration of PTH-CBD provides protection, rather than enhancing chemotherapy damage. We postulate that continued anagen phase induction after this initial damage would result in more rapid replacement of the damaged hair follicles, with an overall net positive effect on hair growth. This would predict that PTH-CBD should be effective in preventing hair loss from a variety of chemotherapeutic agents.

Although various approaches have been available for the prevention and treatment of chemotherapy-induced alopecia, these generally have limited efficacy. Prevention includes the use of scalp tourniquets or scalp hypothermia [28]. Scalp tourniquets decrease the blood flow to the scalp, which in turn reduces the delivery of chemotherapeutics to the hair follicles [29]; however, it also causes significant patient discomfort and would prevent chemotherapeutics from treating tumor cells in the scalp, and is therefore not in widespread use. Scalp cooling is intended to reduce hair loss by reducing blood flow and by decreasing metabolic activity of the hair follicles, decreasing their susceptibility to chemotherapeutic damage [30]. Most studies show decreased hair loss with scalp cooling (WHO grade 0, 1, 2 or 'no wig required'), although most of these are not randomized trials [30]. Specific results depend on the type of chemotherapeutic used, the amount of cooling in different protocols, and the chemotherapeutic protocol utilized [31,32]. Cooling decreases the effectiveness of chemotherapeutics in the scalp, and is contraindicated in patients with extensive hematological malignancies [33]. Other side effects include headaches, uncomfortable sensation, and claustrophobia from the devices, leading to more than 10% of patients discontinuing the treatments in some studies [34,35]. The overall effect of scalp cooling as

an available therapy for CIA is a moderately effective method to prevent hair loss during most chemotherapy regimens [36].

Although there are currently no approved drug treatments for CIA in humans, attempts have been made to utilize existing treatments for other forms of hair loss in chemotherapy patients. Topical minoxidil has been approved for the treatment of androgenic alopecia and alopecia areata, but it has very limited efficacy in CIA [37,38]. Among all the agents that have been evaluated in humans for preventing CIA, only minoxidil was able to reduce the severity or shorten the duration of CIA, but it could not prevent hair loss [33,37]. Although calcitriol was considered a promising agent, it did not prevent or treat CYP-induced alopecia in mice [7,39]. Furthermore, topical administration may result in contact dermatitis [40].

Overall, it appears that PTH-CBD is effective in preventing or reversing hair loss in a non-depilated mouse model of CIA. Prophylactic therapy appeared to be more effective than treatment after hair loss develops. As there are no known or suspected interactions with PTH-CBD on the chemotherapeutic effects of CYP, the reversal of hair loss appears to be the result of the direct effects of PTH-CBD on the hair follicles. Treatment with PTH-CBD results in an increase in the number of anagen VI hair follicles, likely through a mechanism of activation of WNT signaling. With continued study, PTH-CBD may develop as a promising new therapy for CIA.

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Fig. 1.

(a, b) Pilot study – depilation only: (a) photo-documentation. Sevenweek-old female C57BL/6J mice were depilated by shaving, followed by waxing. Mice were then divided equally into three groups and treated subcutaneously with either vehicle buffer, PTH-CBD (320 mcg/kg), or PTH(7–33)-CBD (320 mcg/kg) at the time of depilation. Shown here are photographs of mice obtained at day 18. (b) All mice were killed at the end of the study (day 18). Skin samples from the dorsal region were obtained and were fixed in 10% buffered formalin; longitudinal slices were processed for routine histology using Hematoxylin and Eosin (H&E) staining. CBD, collagen-binding domain; PTH, parathyroid hormone.

Fig. 2.

Prophylactic effects of PTH-CBD: 7-week-old female C57BL/6J mice were treated with either vehicle, cyclophosphamide (CYP) (50 mg/kg/week $\times$ 3 intraperitoneally), or CYP and PTH-CBD (320 mcg/kg subcutaneously). Photographs obtained after 1 year are shown. CBD, collagen-binding domain; PTH, parathyroid hormone.

Fig. 3.

Histology (skin): 7-week-old female C57BL/6J mice were treated with either vehicle, cyclophosphamide (CYP) (50 mg/kg/week $\times$ 3 intraperitoneally), or CYP and PTH-CBD (320 mcg/kg subcutaneously). All mice were killed at the end of the study (15 months). Skin samples from the dorsal (nape of the neck to the middle of the back), ventral, and tail regions were obtained. The skin was fixed in 10% buffered formalin, and longitudinal slices were processed for routine histology, using Hematoxylin and Eosin (H&E) staining. CBD, collagen-binding domain; PTH, parathyroid hormone.

Fig. 4.

Therapeutic effects of PTH-CBD: 7-week-old female C57BL/6J mice were treated with cyclophosphamide (CYP) (120/120/150 mg/kg/2 weeks $\times$ 3). After 4 months, the mice developed variable hair loss and loss of pigmentation. The mice were then divided into two groups and treated with either vehicle buffer or PTH-CBD (320 mcg/kg). The photographs obtained at days 0, 14, and 21 are shown. CBD, collagen-binding domain; PTH, parathyroid hormone.

Fig. 5.

Prophylactic versus therapy: 7-week-old female C57BL/6J mice were treated with cyclophosphamide (CYP) (150 mg/kg/week $\times$ 3 intraperitoneally). Animals received either vehicle, PTH-CBD (320 mcg/kg subcutaneously) at the time of CYP dosing (prophylaxis), or PTH-CBD 1 year after CYP dosing (therapy). Photographs obtained at 16 months are shown here. CBD, collagen-binding domain; PTH, parathyroid hormone.

Fig. 6.

Gray scale analysis: images were captured using the Kodak Gel Logic 100 Imaging System on a Spectroline Bi-O-Vision UV/white light transilluminator. Photographs were taken with exposure 0.2 s, F-stop 2 mm, and magnification 15 mm to maintain the hair texture in the linear range for analysis. For gray scale analysis, an elliptical region of interest (ROI) was selected on the dorsal skin of the mice, covering as much of the back of the mouse as possible. This densitometry value was normalized to the average of those obtained from two ROIs placed on either side of the mouse.

Fig. 7.

Hair follicle counts: quantitative assessment of the hair follicles was performed to determine the number per high-power field (HPF) by two independent observers in a blinded manner. Results are expressed as mean $\pm$ SD.

Table 1

Summary of various chemotherapy-induced alopecia study protocols

Study name/number	Study type	Chemo (CYP) dose/weeks (mg/kg)	PTH-CBD treatment (mcg/kg)	Time of killing
Pilot study	Depilation alopecia	No chemo	320 mcg/kg at depilation	Day 18
Study 1	Chemotherapy-induced alopecia (prophylaxis)	50 mg/kg 0, 2, and 4 weeks	320 mcg/kg at week 0	16 months
Study 2	Chemotherapy-induced alopecia (treatment)	120, 120 and 150 mg/kg 0, 2, and 6 weeks	320 mcg/kg at 4 months	Day 21
Study 3	Chemotherapy-induced alopecia (prophylaxis vs. therapy)	150 mg/kg 0, 1, and 2 weeks	Prophylaxis (320 mcg/kg) at week 0 Therapy (320 mcg/kg) after 12 months	15 months