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Expert Review

Protection Against Chemotherapy-Induced Alopecia

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Purpose. The goal is to provide an overview on the advances in protection against chemotherapy-induced alopecia (CIA).
Materials and Methods. The four major parts of this review are (a) overview of the hair follicle biology, (b) characteristics of CIA, (c) state-of-the-art animal models of CIA, and (d) experimental approaches on protection against CIA.
Results. The hair follicle represents an unintended target of cancer chemotherapy. CIA is a significant side effect that compromises the quality of life of patients. Overcoming CIA represents an area of unmet needs, especially for females and children. Significant progresses have been made in the last decade on the pathobiology of CIA. The pharmacological agents under evaluation include drug-specific antibodies, hair growth cycle modifiers, cytokines and growth factors, antioxidants, cell cycle or proliferation modifiers, and inhibitors of apoptosis. Their potential applications and limitations are discussed.
Conclusion. Multiple classes of agents with different action mechanisms have been evaluated in animal CIA models. Most of these protective agents have activity limited to a single chemotherapeutic agent. In comparison, calcitriol and cyclosporine A have broader spectrum of activity and can prevent against CIA by multiple chemotherapeutic agents. Among the three agents that have been evaluated in humans, AS101 and Minoxidil were able to reduce the severity or shorten the duration of CIA but could not prevent CIA.

KEY WORDS: alopecia; chemotherapy; hair follicle.

INTRODUCTION

The three major and frequent toxicities of cytotoxic cancer chemotherapy are bone marrow suppression, gastrointestinal disturbances and alopecia. The incidence of alopecia is 65%. Alopecia negatively affects a patient's perception of physical appearance, body image, sexuality and self-esteem, and deprives patients of the privacy of having cancer (1–4). Chemotherapy-induced alopecia (CIA) is considered one of the most negative factors in cancer patient care. The National Coalition for Cancer Survivorship cites CIA as one of the most emotionally upsetting aspects of coping with cancer. Female patients are particularly affected; a survey shows that 47% patients consider CIA the most traumatic side effect of chemotherapy, and 8% would reject chemotherapy due to fear of CIA (5,6). Alopecia also results in reduced social interactions in school-age children and teenagers (7–9). The negative psychological impact of CIA may have additional undesirable biological consequences, as depression lowers immune function and is associated with cancer progression (10).

The recent advances in the understanding of cancer biology have led to the discoveries of novel agents that target tumor-specific molecular lesions, e.g., tyrosine kinase inhibitors that attack the faulty signaling pathways or anti-angiogenic agents that attack the tumor blood supply. These newer agents are primarily cytostatic and are usually given in combination with the traditional cytotoxic chemotherapeutic agents (e.g., paclitaxel and doxorubicin in breast cancer). Hence, chemotherapy-induced toxicities such as CIA remain formidable problems in the management of cancer patients. Furthermore, the recent demonstration of survival extension by combinations of chemotherapy and molecular targeting agents has led to the view that the emphasis of cancer treatment is shifting from cure to long-term maintenance, which further highlights the importance of the quality of life issue (11).

Substantial efforts have been expended and, consequently, multiple drugs have been developed, to manage chemotherapy-induced bone marrow suppression and gastrointestinal disturbances. In comparison, the development of CIA treatment is lagging and no effective treatments for CIA are available at present.

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ABBREVIATIONS: Ara-C, cytosine arabinoside; CIA, chemotherapy-induced alopecia; EGF, epidermal growth factor; FGF, fibroblast growth factor; IRS, inner root sheath; ORS, outer root sheath; PTH, parathyroid hormone; PTHrP, PTH-related protein.

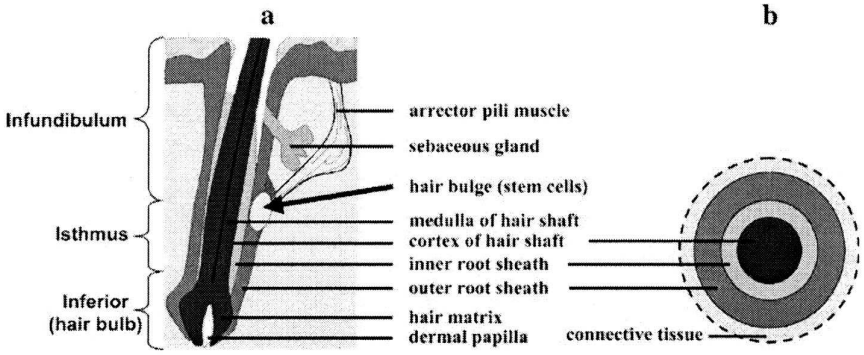

Fig. 1. Hair follicle structure. Microscopic anatomy of a hair follicle in its mature anagen phase. (a) Longitudinal view. *From top to bottom* with respect to outer skin surface, a hair follicle is divided into three segments, infundibulum, isthmus and inferior (hair bulb). (b) Cross-section. *From central to peripheral*, the basic units of the follicle are hair shaft, inner root sheath (*IRS*), and outer root sheath (*ORS*). The bulge in ORS (*arrow*) near the insertion site of arrector pili muscle is the proposed location for stem cells.

The purpose of this review is to discuss the advances in CIA. "Hair Follicle Biology" provides an overview of the hair follicle biology. "Chemotherapy-Induced Alopecia" describes the characteristics and mechanisms of CIA. "Animal Models of CIA" summarizes the two animal models, i.e., neonatal rat model and adult black mouse model, for studying CIA. "Experimental Approaches for Treating CIA" discusses the potentials and limitations of the experimental approaches on treating CIA.

HAIR FOLLICLE BIOLOGY

Hair Follicle Structure

Hair follicle structure is related and changes according to the stages of its growth cycle (12). Figure 1a shows the longitudinal view of a hair follicle in anagen, the active growth phase. An anagen hair follicle can be divided into three functional sections, i.e., infundibulum, isthmus, and inferior (also called bulbar) segments from top to bottom, with respect to the outer surface of skin. Hair bulb is located in the inferior segment, and contains hair matrix and dermal papilla. Matrix cells in the lower part of hair bulb have a high mitotic rate, while the matrix cells in the upper part have low mitotic rate and can differentiate into cells that make up the inner root sheath (IRS). The pigment-producing melanocytes are located in the hair bulb matrix. Dermal papilla consists of an oval mass of fibroblasts and is totally encapsulated by the matrix epithelium. The size (diameter) of dermal papilla correlates with the size of hair follicle and the size of the produced hair shaft; e.g., larger dermal papilla correlates with larger hair follicles and produces thicker hair shafts. The bulge in the outer root sheath (ORS) near the insertion of arrector pili muscle is the location of epithelial stem cells that serve as a reservoir for hair bulb matrix, epidermal and sebaceous gland cells (13–16). When the hair bulge is damaged, irreversible permanent alopecia may occur. Sebaceous gland, hair follicle and hair shaft collectively represents a pilosebaceous unit.

Figure 1b shows the cross-section of a hair follicle, which, from side to side, consists of several enclosed epithelial cylinders. The follicular sheath, consisting of basement membrane and connective tissue, surrounds the whole hair follicle. The basement membrane is known as a hyaline (or vitreous or glassy) membrane and shows strong periodic acid-Schiff-positive staining. Inside the basement membrane of the hair follicle, the innermost cylinder is the hair shaft and the outermost cylinder is ORS that separates the follicle from the dermis. The middle cylinder, IRS, molds the shaft. IRS consists of three layers. The outermost layer of IRS, Henle's layer, is tightly attached to ORS. The innermost cuticle layer of the IRS is interlocked with the cuticle of the hair shaft surface. This allows the IRS and hair shaft to move together during the period of growth. At the level near or slightly below the sebaceous gland duct, IRS breaks down and the shaft is separated from IRS. The cortex cells in a hair shaft are densely packed and are usually heavily pigmented.

Hair Growth Cycle

The process of hair shaft formation involves a complex mechanism of matrix cell proliferation, keratinization and differentiation in IRS and ORS. From the perspective of hair growth, a follicle consists of two distinct portions. The upper part, which is approximately one-third of the length of a follicle in anagen phase, remains stable throughout the life cycle. The lower and remaining part is the portion that participates in the follicular growth cycle and undergoes shortening and lengthening (17).

Figure 2 outlines the growth cycle of a hair follicle. Under normal circumstance, the growth cycle consists of three main phases: anagen, catagen and telogen. Anagen is the period for the regeneration of the lower, cycling portion of a follicle, and for the production of a hair shaft. At the initiation of anagen, the epithelial cells at the base of a follicle divide and maintain a constant high mitotic rate. This process is accompanied by the production of proteolytic enzyme facilitating the downward growth penetrating the dermis (18,19); this results in an epidermal finger and the

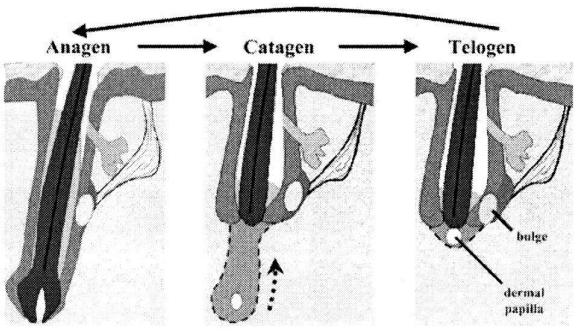

Fig. 2. Hair growth cycle. A hair growth cycle consists of anagen, catagen and telogen. In anagen, matrix cells in hair bulb have high mitotic activity and hair shaft is produced. Catagen is the apoptosis-driven degenerative process, when the lower two segments of a hair follicle (isthmus and inferior segments) are diminished and when the dermal papilla moves upward through the contractile activities of ORS and to a location in close proximity to the hair bulge throughout the telogen phase. Telogen is the resting stage. During transition from telogen to anagen, stem cells in the hair bulge are activated by dermal papilla, divide into matrix cells and start a new hair growth cycle.

establishment of ORS (12,20). Once the early anagen follicle reaches its destined depth, the bulbar epithelial cells reverse their growth direction, progress upward, form IRS and differentiate into other cylindrical layers of a hair follicle. The maintenance of ORS throughout anagen is due to the proliferation of cells residing in ORS and is independent of the proliferation of cells in bulbar matrix (21). During the anagen phase, hair follicle and hair shaft are extended. Hair length is determined by the duration of anagen and not by the follicle length.

Catagen is the degenerative phase, when the isthmus and hair bulb regress. Similar to anagen, catagen is a highly controlled process, which involves the separation of dermal papilla from hair bulb, loss of the layered differentiation of the lower parts of a follicle, cessation of bulbar epithelial cell division, and shrinkage of the lower follicle secondary to apoptosis. In the early stage of catagen, the basement membrane surrounding the hair bulb loses its structure as ORS collapses; this phenomenon serves as a marker of the initiation of the catagen phase. Catagen hair follicles are also recognized by the cessation of pigment production by melanocytes; the proximal end of a hair shaft is usually not pigmented when a hair follicle stops growing. During catagen, a hair follicle moves upward and IRS is shortened, corresponding to a loss of the lower one-half to two-thirds of its length from hair bulb to hair bulge. At the end of catagen, ORS cells in the most superficial portion of the bulge, or keratogenous zone, undergo terminal differentiation or tricholemmal keratinization. This creates an irregular corona around the hair shaft that interlocks with the proximal part of ORS cells, thereby anchoring the hair shaft to the follicle during the next phase, telogen (22). The telogen hair shaft has a brush-like base attached to the ORS sac and is often called club hair. During telogen, the hair follicle is stable with no obvious structural changes. The hair shaft is usually retained in the hair follicle during catagen and telogen but can be easily extracted by vigorous grooming.

The growth cycle may be followed by shedding of a hair shaft, an event termed exogen. Exogen is often associated with the formation of a new hair shaft from the next growth cycle. In humans, club hairs are shed at the onset of anagen when new hair grows. In rodents such as mice, club hair shafts can be retained in hair follicles, and a single hair follicle may possess several club hairs with only one growing (23). After telogen ends, the follicle reenters anagen and a new hair growth cycle starts.

Hair Follicle Stem Cells

The dynamic and cyclical changes, involving well-controlled processes of degradation and regeneration, in a hair follicle suggest the presence of stem cells in this self-renewing tissue. The search for stem cells and their locations have been guided by the expected properties of stem cells, i.e., (a) relatively undifferentiated, (b) high proliferative potential, (c) responsible for tissue maintenance and self-renewal, (d) slowly cycling, and (e) located close to a population of rapid dividing cells and in a stable, well-protected structure or position (13). The high mitotic rate of hair matrix cells in the hair bulb had led to the first hypothesis that stem cells are located in or close to hair bulb (21,24,25). Subsequent studies, by measuring the potential of hair follicle regeneration after systematic removal of various components of a hair follicle surgically or by radiation, provided evidence that stem cells were not located in the hair bulb (26–29). Another reason to exclude hair bulb as the location for stem cells is the cyclic degeneration of this unit, which would have led to the destruction of stem cells as opposed to being the sanctuary for these cells.

Several lines of evidence indicate that hair follicle stem cells are located in the upper part of ORS, namely hair bulge (14,16,30–33). First, the upper ORS, in contrast to the lower part, is stable throughout the growth cycle. Second, results of chase-labeling experiments indicate that label-retaining cells are located in hair bulge. Third, cells that express typical markers for epithelial stem cells (i.e., β1-integrin and CD34) and share common gene expression patterns with embryonic stem cells and stem cells from hematopoietic and neural systems are located in hair bulge. Fourth, cells in hair bulge are responsible for hair follicle regeneration and tissue renewal in the whole skin epithelium including sebaceous gland and epidermis. According to the "Bulge Activation Hypothesis", stem cells in the hair bulge are activated by dermal papilla during the late telogen phase or early anagen phase, thereby initiate a downward growth and divide into transient amplifying cells to form the hair matrix. In the catagen phase, the dermal papilla moves upward through the contractile activities of the ORS to remain in close position to the hair bulge during the telogen phase (13).

Hair Growth Pattern

There are two types of hair growth, i.e., synchronous or asynchronous. In most mammals, hair growth occurs in a wave pattern, or synchronous growth. For example, hair follicles in mice and rats follow a wave pattern of hair growth in their first cycle after birth, which starts at the head progressing to the tail and starts at the abdomen progressing

to the back (34). Because of the wave pattern, during which hair follicles communicate with each other, a group of hair follicles at a specific area are usually in the same stage of hair growth cycle. In general, only about 10% of hair follicles in adult mice or rats are in anagen and the period of anagen phase usually lasts for 1 to 2 weeks (34).

The normal hair growth cycle in human scalp occurs in a mosaic pattern, or asynchronous growth, where the growth cycle in individual hair follicles is independent of the neighboring hair follicles. The two animals that show similar asynchronous hair growth are Guinea pigs and Angora rabbits (35).

CHEMOTHERAPY-INDUCED ALOPECIA

Reports of CIA began to appear in the medical literature in the late 1950s (36). In humans, CIA usually begins at 1–3 weeks and is complete at 1–2 months, after initiation of chemotherapy (37). Alopecia becomes visibly noticeable after the loss of 50% or more of existing hair. CIA is usually reversible after chemotherapy; hair regrowth typically occurs after a delay of 3–6 months (2,38). In 65% of cases, the new hair shows graying and/or changes in hair structure and texture. After CIA, the hair growth rate may also be significantly reduced.

Multiple classes of anticancer drugs induce alopecia (37). The frequencies of alopecia for the four major drug classes are: over 80% for antimicrotubule agents (e.g., paclitaxel), over 60% for alkylators (e.g., cyclophosphamide), 60–100% for topoisomerase inhibitors (e.g., doxorubicin), and 10–50% for antimetabolites (e.g., 5-fluorouracil plus leucovorin). The severity of CIA depends on the drug and its administration route, dose, and treatment schedule (see "Animal Models of CIA"). Combination therapy consisting of two or more agents usually produces higher incidence of and more severe CIA compared to single agent therapy.

Radiation can also induce alopecia, but only when the head is within in the radiation field. Radiation-induced alopecia is more variable and less predictable compared to CIA (39,40).

ANIMAL MODELS OF CIA

Research into overcoming CIA has been hampered in part due to the lack of clinically relevant models. Although *in vitro* systems such as cell cultures and histocultures can be used to study the effects of chemotherapeutic agents on hair follicle, *in vivo* models are preferred due to the complexity of hair growth and cycling (37,41–44).

An interesting experimental model is the grafting of human scalp skin containing hair follicles onto the skin of a mouse; the transplanted hairs are shed within a month, and the regrowth of new hair begins within several months after transplantation (45). This model has been used to study the biology of, and the effects of chemotherapy on, human hair follicles (46–48). Areas that require attention are that the transplantation procedure may affect the physiology of hair follicles, and that the microenvironment in the skin such as growth factors and cytokines may be altered during surgery and storage.

The more commonly used models in CIA studies are mice and rats (without human skin grafts). As discussed in "Hair Follicle Biology," the major difference in hair growth on human and rodent skin/scalp is the growth pattern, i.e., mosaic vs wave. In the latter, the entry of hair follicles into anagen follows a wave, beginning from the head and moving towards the tail, e.g., neonatal rats; and hair loss in CIA follows the same wave pattern. Some animals, e.g., Guinea pigs and Angora rabbits, exhibit the mosaic hair growth pattern as in humans, but are not commonly used due to either failure to induce CIA (Guinea pigs) or incomplete hair loss (Angora rabbits) (35).

CIA occurs when hair follicles are in anagen (38). About 90–95% of hair follicles in human scalp are in the anagen phase lasting 2–6 years, <1% are in the catagen phase lasting 2–3 weeks, and <10% are in telogen phase lasting 3–4 months (49). To mimic the human situation, animal models of CIA typically involve procedures that cause the hair follicles to enter the anagen growth phase. Two approaches have been used. The first approach is to use neonatal rats that show spontaneous anagen hair growth. The second approach is to synchronize the hair follicles in adult mice by depilation. These two models have been commonly employed to study the pathology of CIA in hair follicles and the protection against CIA. The following sections review the establishment and characteristics of these two CIA animal models.

Neonatal Rat Model

The neonatal rat CIA model was first introduced by Hussein *et al.* (38,50); the 7- to 8-day-old rats show spontaneous anagen hair growth for about 1 week. CIA was observed after treatment (intraperitoneal injection) with a cell cycle S-phase specific antimetabolite cytosine arabinoside (Ara-C), a cell cycle nonspecific alkylator cyclophosphamide, and an anthracycline and topoisomerase II inhibitor doxorubicin. The nature and severity of CIA were drug-specific (50–54). Doxorubicin (2 mg/kg/day for 7 days or 3 mg/kg/day for 4 days) induced hair loss that was confined to the head, proximal neck, and injection site. Ara-C (50–75 mg/kg/day for 5–7 days), cyclophosphamide (single dose of 50 mg/kg) or etoposide (three doses of 1.5 mg/kg/day) induced whole body alopecia in 1 week after the first dose. On a cellular level, doxorubicin induced severe apoptosis in hair bulb (51).

A major advantage of the neonatal rat model is the relative ease of assessing the effects of CIA; visible hair loss typically starts from the head region and progresses to involve the entire body in approximately 2 days. One drawback of this model is that the hair follicles are in the first hair growth cycle after birth, which involves the establishment of the entire hair follicle structure. In contrast, a normal hair growth cycle involves only the generation of only the lower parts of a hair follicle. Another drawback is that the neonatal Sprague Dawley rats have white fur. The lack of pigmentation in hair shafts limits the study of the effect of chemotherapy on melanocytes. Another potential confounding factor is that the levels of growth factors and cytokines in the hair follicles of neonatal rats may differ from the levels in developmentally mature animals. This, in turn, may alter the response of hair follicles to drugs.

Adult Mouse Model

The adult, black C57BL/6 mouse model was developed by Paus *et al.* (44). In these mice, skin melanocytes are confined to hair follicles and the stage of the hair growth cycle is indicated by the skin color (pink during telogen and black during anagen). As these are adult animals, their hair follicles have gone through several postnatal growth cycles and the hair shafts are pigmented, as would be the case for hair follicles in human scalp. These properties of the C57BL/6 mouse model represent potential advantages over the neonatal rat model. In adult mice, the induction of hair follicles to enter anagen phase is achieved by depilation, occurring in about 8 to 9 days after the procedure (43,44).

CIA studies using the adult mouse model have been focused on cyclophosphamide. These studies have provided significant insight on the follicular pathology. A single dose of cyclophosphamide (150 mg/kg injected intraperitoneally), given on day 9 after depilation, induced premature catagen development, dystrophic follicles, and complete alopecia in 6 days (44). On a cellular level, cyclophosphamide induced massive apoptosis of keratinocytes and melanocytes in hair bulb of anagen hair follicles, without affecting dermal papilla fibroblasts (55,56). On a molecular level, cyclophosphamide treatment caused upregulation of the death receptors Fas/Apo-1 and the mitochondrial pro-apoptotic protein Bax in hair follicles (57,58).

Depending on the position of a hair follicle in the hair growth cycle after CIA, the recovery of hair follicles from cyclophosphamide CIA and the subsequent hair regrowth may use one of the two pathways, i.e., dystrophic anagen and dystrophic catagen (44,59,60). In dystrophic anagen, hair follicles remain in anagen phase after CIA and produce functionally impaired hair shaft (e.g., loss of pigment) during regrowth. In dystrophic catagen, the anagen phase is terminated and followed by a catagen phase during CIA, and the regrowth of a hair shaft occurs during the new anagen phase of the subsequent hair growth cycle, in which normal pigmented hair shafts are produced.

EXPERIMENTAL APPROACHES FOR TREATING CIA

The major approaches to prevent or minimize CIA are by physical (scalp tourniquets, hypothermia or cooling) and pharmacological means. Scalp tourniquets, used to occlude the superficial blood flow to scalp and reduce the amount of drug delivered to hair follicles, is the earliest approach (61), but is no longer recommended due to patient discomfort. Scalp hypothermia or cooling, accomplished by using ice or temperature-controlled devices, is to decrease the drug uptake in scalp and/or alter the drug metabolism in hair follicular cells, and is under clinical evaluation (2,38,62–64). Both scalp tourniquets and hypothermia are impractical when the chemotherapy is administered as a continuous infusion over a long duration (37). The remainder of this section is focused on the pharmacological approach. At present, there are no approved drug treatments for CIA in humans. Several strategies have been evaluated and have shown promising results in animal models. These protective agents are usually applied prior chemotherapy. They are summarized in Table I, and discussed below.

Drug-specific Antibodies

Topical administration of liposomes loaded with antibody to doxorubicin reduced the severity of doxorubicin CIA in neonatal rats (52). The drawback of this strategy is the limited usefulness in combination chemotherapy where the antibody is not likely to protect against CIA from other drugs in the combination.

Hair Growth Cycle Modifiers

Cyclosporine A, an immunosuppressive immunophilin ligand, induces active growth and inhibits regression of hair follicles, and promotes hair growth in experimental alopecia models. The use of Cyclosporine A in CIA originated from its common side effect of hypertrichosis, a direct effect on hair follicles unrelated to immunosuppression (65–67). In other cell types, cyclosporine A, through inhibition of T-cell receptor signal transduction pathways by binding to calcineurin, inhibits G_0 to G_1 cell cycle transition and cell proliferation (68,69). In neonatal rats, topical application of cyclosporine A prevented CIA by cyclophosphamide, Ara-C and etoposide (70). In adult mice, topical or systemic application of cyclosporine A prevented the progression of damaged hair follicles into telogen, retarded hair loss and induced rapid hair regrowth, in cyclophosphamide CIA (44,60).

Another immunomodulator, AS101, protected against Ara-C CIA in rats and reduced the severity of CIA in human patients treated with a combination of carboplatin and etoposide (71). The protection by AS101 may be mediated through macrophage-derived factors and related to prostaglandin E2 secretion (71).

Minoxidil, introduced in 1970s to treat hypertension and male baldness, shortens telogen phase and causes hair follicle to enter anagen, thereby stimulates hair growth. Minoxidil also prolongs the duration of anagen and enlarges miniaturized follicles irrespective of the underlying causes. Several possible molecular mechanisms of the action of Minoxidil on hair growth have been proposed, including opening of potassium channels by its sulphated metabolite, stimulation of keratinocyte proliferation, inhibition of collagen synthesis and production, stimulation of vascular endothelial growth factor (VEGF) and prostaglandin synthesis (72). Topical minoxidil is approved for treating androgenic alopecia and alopecia areata in humans. With respect to CIA, local injection of minoxidil protects against Ara-C CIA but not cyclophosphamide CIA in neonatal rats (53). The same study also shows that topical application of 2% Minoxidil did not offer protection against CIA by Ara-C, possibly due to the inability of Minoxidil to penetrate hair follicles. In multiple clinic trials, topical Minoxidil (2% solution) shortens the duration of, but cannot prevent CIA in breast cancer patients receiving adjuvant chemotherapy (73) or gynecologic cancer patients receiving cyclophosphamide, doxorubicin and *cis*-platinum (74), is not effective in prevention of doxorubicin CIA in female patients with different types of solid tumors (75), and fails to induce significant hair re-growth in busulphan and cyclophosphamide-induced permanent alopecia (76).

Table I. Summary of Experimental Pharmacological Approaches for CIA

Strategies	Compound	Neonatal rats					Adult mice		Humans	Reference
		Ara-C	Cph	Dox	Etop	Pac	Cph	Dox		
Antibody	Dox monoclonal	ND	ND	√	ND	ND	ND	ND	ND	(52)
Hair growth cycle modifiers	Cyclosporine A	√	√	ND	√	ND	$√^1$	ND	ND	(44, 60, 70)
	AS101	√	ND	ND	ND	ND	ND	ND	$√^2$	(71)
	Minoxidil	√	×	ND	ND	ND	ND	ND	$√^3$	(53, 73)
Cytokines and growth factors	Imuvert	√	×	√	ND	ND	ND	ND	ND	(38, 50)
	Interleukin 1	√	×	ND	ND	ND	ND	ND	ND	(38, 50)
	EGF	√	×	ND	ND	ND	ND	ND	ND	(54)
	FGF1	√	×	ND	ND	ND	ND	ND	ND	(54)
	FGF7	$√^1$	×	ND	ND	ND	ND	ND	ND	(84)
Antioxidant	NAC	ND	√	ND	ND	ND	ND	√	ND	(88, 89)
	NAC + Imuvert	√	√	ND	ND	ND	ND	ND	ND	(89)
Cell cycle or proliferation modifiers	Vitamin D_3	ND	√	√	√	√	×	ND	$×^1$	(55, 97–102)
	PTHrP and receptor (agonist, antagonist)	ND	ND	ND	ND	ND	$√^1$	ND	ND	(104)
Apoptosis inhibitors	p53 inhibitors	ND	ND	ND	ND	ND	√	ND	ND	(105, 107)
	Caspase-3 inhibitors	ND	ND	ND	√	ND	ND	ND	ND	(108)

Dox Doxorubicin, *Pac* paclitaxel, *Cph* cyclophosphamide, *Etop* etoposide, *NAC N*-acetylcysteine, *ND* no data.
√ indicates protection (i.e., prevention of hair loss). $√^1$ indicates partial protection (i.e., retarded hair loss or enhanced hair regrowth but no prevention). $√^2$ indicates reduced severity in patients treated with a combination of carboplatin and etoposide. $√^3$ indicates shortened duration of CIA in breast cancer patients receiving adjuvant chemotherapy. × indicates no prevention. $×^1$ indicates no protection against CIA by a combination of 5-fluorouacil, doxorubicin, and cyclophosphamide in breast cancer patients.

Cytokines and Growth Factors

Hair follicles express receptors for multiple cytokines and growth factors, e.g., fibroblast growth factors (FGF), transforming growth factors, insulin-like growth factors, epidermal growth factors (EGF), interferon and interleukins (17). The development and growth cycle of hair follicles are affected by more than one of these proteins, possibly through autocrine and/or paracrine mechanisms (17,77,78).

Imuvert, a biological response modifier from the bacterium Serratia Marcescens, and interleukin 1 protected rats from CIA by Ara-C but not cyclophosphamide. Both agents can induce the release of multiple cytokines or growth factors. The protection by Imuvert is presumably mediated through interleukin 1 (38,50).

EGF protected against Ara-C CIA but had no effect on cyclophosphamide CIA, in neonatal rats (54). EGF, at concentrations between 1 and 25 ng/ml, significantly inhibits the uptake of ^{3}H-thymidine by hair matrix cells maintained in organ cultures (79).

Several proteins in the FGF family play a role in hair follicle biology and/or offer protection against CIA. FGF1 or acidic FGF shows similar effects as EGF. FGF2 or basic FGF has little effect on DNA synthesis in hair matrix cells but delays the first hair cycle initiation and development in mice (79–81). FGF2 is a natural mitogen for melanocyte and can sustain melanocyte growth and survival (82). FGF7 or keratinocyte growth factor stimulates the proliferation of keratinocytes in hair follicles, partially protects neonatal rats against Ara-C CIA and increases hair follicle survival following lethal irradiation (83–85). FGF5, expressed just before the end of anagen phase, may trigger catagen onset; mice lacking FGF5 have extended anagen and longer hair length (86,87).

Antioxidants

Antioxidant *N*-acetylcysteine, an analog and precursor of glutathione, when administered topically or parentally, protected neonatal rats from cyclophosphamide CIA and protected adult mice from doxorubicin CIA (88,89). The combination of *N*-acetylcysteine and Imuvert offered protection against CIA by a combination of Ara-C and cyclophosphamide in the neonatal rat model (89).

Cell Cycle or Proliferation Modifiers

The rapid cell proliferation in hair follicles during anagen renders hair follicles susceptible to the toxicity of chemotherapy. Hence, one approach to protect against CIA is to inhibit cell proliferation in order to decrease the sensitivity of hair follicular cells to chemotherapy.

Calcitriol, 1,25-dihydroxyvitamin D_3, has multiple effects on keratinocytes, i.e., inhibits DNA synthesis, causes cell cycle arrest at the G0/G1 interphase, and induces differentiation. Calcitriol also abolishes the expression of Ki67, a marker of cycling cells, and inhibits the growth of multiple other cell types (90–96). In neonatal rats, calcitriol reduced CIA by cyclophosphamide, etoposide and a combination of cyclophosphamide plus doxorubicin (97–99). In adult mice, calcitriol enhanced the regrowth of normal pigmented hair shafts, reduced apoptosis in hair bulb, but did not prevent or

retard cyclophosphamide CIA (55,100). Calcitriol has the distinction in that it is the only experimental agent that has been studied and shown to protect against CIA by paclitaxel, a major agent used to treat cancers in women (breast, ovarian) (101). Calcitriol, while it was considered the most promising agent for treating CIA (97), caused contact dermatitis and failed to protect against CIA by a combination of 5-fluorouacil, doxorubicin and cyclophosphamide in breast cancer patients (102).

Parathyroid hormone (PTH)-related peptide (PTHrP) is responsible for malignancy-associated hypercalcemia, and participates in the regulation of keratinocyte proliferation and differentiation (103). PTH and PTHrP inhibited proliferation and induced terminal differentiation in cultured human keratinocytes, whereas PTH antagonists stimulated epidermal proliferation. In adult mice treated with cyclophosphamide, PTH antagonists and PTHrP antagonists reduced apoptosis in hair bulb matrix and delayed the onset of CIA, whereas PTH agonists and PTHrP agonists enhanced the apoptosis in cells in the hair bulb but accelerated hair regrowth after CIA; neither agonist and antagonists of PTH or PTHrP prevented CIA (104).

Inhibitors of Apoptosis

The transcription factor and tumor suppressor protein p53 induces apoptosis in response to a variety of cellular stress signals. p53 is also involved in chemotherapy-induced apoptosis in hair follicles; p53-deficient mice did not show hair loss nor apoptosis of keratinocytes after cyclophosphamide administration (105–107). These observations led to the evaluation of inhibitors of p53 signaling or functions for treating CIA. The three potential drawbacks of this approach are the inherent limitations associated with p53 inhibition, the inability to inhibit apoptosis mediated via p53-independent pathways, and the inability to overcome CIA due to apoptosis-independent drug effects (e.g., antiproliferation).

M50054, 2,2′-methylenebis, an inhibitor of caspase-3 activation, inhibited etoposide-induced apoptosis in human U937 monocytic leukemic cells. In neonatal rats, topical application of M50054 reduced CIA by etoposide (108).

In summary, multiple classes of agents with different action mechanisms have been evaluated in animal CIA models. Most of these protective agents have activity limited to a single chemotherapeutic agent. In comparison, calcitriol and cyclosporine A have broader spectrum of activity and can prevent against CIA by multiple chemotherapeutic agents. These two agents share a common property of being able to perturb the cell cycle progression of hair follicular cells or other cell types, suggesting cell cycle block as an attractive broad-based approach for protecting against CIA. Among the agents that have been or are being evaluated in human patients, i.e., AS101, minoxidil and calcitriol, the first two agents were able to reduce the severity or shorten the duration of CIA but could not prevent CIA.

PERSPECTIVES

CIA is a significant side effect that compromises the quality of life of cancer patients. Overcoming CIA represents an area of unmet needs, especially for females and children. Significant progresses have been made in the last decade on the pathobiology of CIA, and several experimental and pharmacological approaches to overcome this side effect are under evaluation. In view of the fact that cancer is usually treated with combinations of chemotherapeutics, an effective CIA treatment would likely require agents that are effective for different chemotherapeutics with different action mechanisms. An approach would be to combine the different strategies discussed in "Experimental Approaches for Treating CIA". Secondly, the protection should be selective to hair follicles (e.g., topical application), such that the antitumor efficacy of chemotherapy is not compromised.

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