A clinical and biological guide for understanding chemotherapy-induced alopecia and its prevention

Dunnill CJ[1,2], Al-Tameemi W[1] , Collett A[1,2], Haslam IS[1,2,3], Georgopoulos NT[1,2,3*]

[1]Department of Biological Sciences, School of Applied Sciences, University of Huddersfield, Huddersfield, UK.

[2]Institute of Skin Integrity and Infection Prevention, University of Huddersfield, Huddersfield, UK.

[3]Joint senior authors

***Correspondence**

Nik T. Georgopoulos, PhD

Department of Biological Sciences, School of Applied Sciences, University of Huddersfield, Queensgate, Huddersfield, HD1 3DH, UK.

E-mail: N.Georgopoulos@hud.ac.uk

Tel: +44 (0)1484 47 2721, Fax: +44 (0)1484 47 2182

Implications for practice

Chemotherapy-induced alopecia (CIA) represents perhaps the most distressing side effect of chemotherapeutic agents and is of huge concern to the majority of patients. Scalp cooling is currently the only safe option to combat CIA. Clinical and biological evidence suggests improvements can be made, including efficacy in delivering adequately-low temperature to the scalp and patient-specific cap design. The increased use of scalp cooling, an understanding of how to deliver it most effectively and biological evidence-based approaches to improve its efficacy have enormous potential to ease the psychological burden of CIA, as this could lead to improvements in treatment and patient quality-of-life.

Abstract

Chemotherapy-induced alopecia (CIA) is the most visibly distressing side effect of commonly administered chemotherapeutic agents. As psychological health has huge relevance on lifestyle, diet and self-esteem, it is important for clinicians to fully appreciate the psychological burden that CIA can place on patients. Here, for the first time, we provide a comprehensive review encompassing the molecular characteristics of the human hair follicle (HF), how different anticancer agents damage the HF to cause CIA, subsequent HF pathophysiology and we assess known and emerging prevention modalities that have aimed to reduce or prevent CIA. We argue that, at present, scalp cooling is the only safe and FDA-cleared modality available, and we highlight the extensive available clinical and experimental (biological) evidence for its efficacy. The likelihood of a patient that uses scalp cooling during chemotherapy maintaining enough hair to not require a wig is approximately 50%. This is despite different types of chemotherapy regimens, patient-specific differences and possible lack of staff experience in effectively delivering scalp cooling. The increased use of scalp cooling and an understanding of how to deliver it most effectively to patients has enormous potential to ease the psychological burden of CIA, until other, more efficacious, equally safe treatments become available.

Keywords

Chemotherapy-induced alopecia, hair loss, chemotherapy, scalp cooling, side effects, toxicity, hair follicle, cell models, prevention, safety.

1. Introduction to chemotherapy-induced alopecia (CIA)

CIA is an acquired form of hair loss that affects patient quality of life, negatively impacts body image, sexuality and self-esteem, and provides a strong indication of the individual's health status, with most people associating it with cancer [1, 2]. Increasing use of poly-therapies, high-dose taxane administration and an associated increase in cases of permanent CIA are being reported. Though a non-life-threatening condition, CIA is of huge concern to most patients, yet is often viewed as being of minor clinical importance, when the focus is understandably on the treatment of a potentially fatal malignancy. Equally, whilst considerable efforts have been expended in the attempt to ameliorate other side-effects of chemotherapy, the pathobiology of CIA has been heavily overlooked [3].

CIA is often a particular burden for those with young children who report this as the most traumatizing aspect of treatment, as the child becomes emotionally confused and concerned [4]. Consequently, CIA can be one of the most emotionally difficult side-effects, with feedback from female patients showing that losing hair is/would be more difficult to live with than the loss of a breast [5]. Social media and the increased pressure on appearance means patients are likely to feel losing their hair is detrimental to their self-esteem, whilst dealing with a possibly life-threatening disease. These factors could negatively impact on therapeutic outcome, as severe stress and depression [2] is linked to a weakened immune system, an instrumental factor in cancer prognosis [6]. Although most of the research on the emotional effects of CIA has been conducted on females, the available research indicates that, at least for younger males, the impact of CIA is the same as that experienced by females [7]. CIA on females portrays that they have cancer, as most women maintain their hair throughout life. Men commonly undergo androgenic alopecia, however most young males do not, thus males may also be stigmatised as cancer sufferers when CIA occurs.

It is important for clinicians and even patients to fully-appreciate the possible psychological burden of this side-effect and to have a clear understanding of ways available to prevent it [8]. To this end, here we provide an overview of basic human hair follicle (HF) biology, with a focus on those events most relevant to CIA and the processes that occur during hair loss. This includes a description of the known mechanisms by which anticancer agents cause CIA. We discuss the various preventative strategies that have been investigated both in the lab and the clinic, whilst ultimately focussing on the most effective therapy currently available, scalp cooling.

2. The hair follicle (HF)

2.1. Structure and function

Hair is a skin appendage with diverse functions that is important for thermoregulation, protection from solar radiation and sexual dimorphism [9]. In humans, scalp and facial hair is associated with general wellbeing, strong social status, sexual attraction, fashion statement or even to demonstrate political affiliations [10, 11].

The HF is a mini-organ and skin appendage, its primary function is to produce the visible hair shaft [12, 13]. The HF is divided into distinct sections, as detailed in Figure 1. The upper sections of the HF are permanent, with the infundibulum running from the opening of the sebaceous gland (SG) duct to the point where the HF meets the epidermis, providing a funnel-shaped cavity through the epidermis and offering an opening for the hair shaft. The isthmus is located at the lower boundary of the SG at the insertion point for the arrector pili muscle. This region is also commonly described as the bulge, and contains a population of epithelial HF stem cells (eHFSCs), the identity of which has recently been reviewed [14]. The progeny of these stem cells produce the hair bulb matrix keratinocytes which can contribute to formation of the epidermis particularly during wound-healing, and it is damage to these cells that severely impairs long-term hair shaft production [15]. The suprabulbar region contains multiple layers of the outer root sheath (ORS) and inner root sheath (IRS), which form concentric cylinders wrapping the hair shaft itself (Figure 1). Each of these layers has a unique expression of structural and adhesion proteins [16]. The hair bulb contains the matrix keratinocytes, a population of rapidly-dividing progenitor cells that differentiate (specialise) to form the IRS and hair shaft. Matrix cells in the lower part of the hair bulb have a higher mitotic (proliferation) rate than those of the upper part and migrate upwards whilst differentiating [15]. The bulb also contains the HF pigmentary unit (HFPU), within which are found the melanocytes responsible for hair colour.

The HF is primarily epithelial in origin, with the exception of the dermal papilla (DP) and connective tissue sheath (CTS), which are mesenchymal. Inductive signals for HF growth and cycling originate from the DP, an oval mass of specialised fibroblasts embedded in an extracellular matrix with extensive vascularisation [12, 17, 18]. There is a close relationship between the size of the DP and HF, with a larger DP creating a larger HF capable of generating a thicker hair shaft [19]. The CTS surrounds the HF, separating it from the rest of the dermis, and contains nerve endings, vasculature and immune cells (such as mast cells).

2.2. The hair cycle

HF morphogenesis (original/new HF development) occurs antenatally, with the HF beginning a post-natal, life-long cycle through three distinct phases. Following morphogenesis, this hair cycle begins with a regression phase (catagen), followed by a period of relative quiescence (telogen) and finally a long growth phase (anagen). The hair cycle is summarised in Figure 2 and described in more detail below.

2.2.1. Catagen

During the regressive catagen phase, extensive cell death (apoptosis) occurs in the hair matrix keratinocytes, IRS and ORS, greatly reducing the HF volume, with the remnants of the ORS forming the epithelial strand [9]. Structurally, an apoptotic cell undergoes DNA condensation and fragmentation, cytoplasmic condensation, membrane blebbing and formation of apoptotic bodies and is removed in a controlled manner by immunocytes [20]. Apoptosis is crucial in long-term regulation of tissue maintenance, which particularly applies to the HF and its cycling/regeneration; yet exogenous agents can inadvertently induce excessive apoptosis. Many factors can stimulate apoptosis in the HF, including UV radiation, X-rays, extreme temperature, pathogenic toxins, lytic viruses, toxic chemicals and chemotherapeutic drugs [21]. This stimulation of apoptosis can ultimately drive the HF into the regressive catagen phase which stops hair production.

Growth factor-mediated signalling between epithelial and mesenchymal cells orchestrates the creation of the connective tissue that comprises a developing HF and involves diverse signalling pathways, including Wnt, TGF-β/BMP, Hedgehog, epidermal growth factor (EGF), fibroblast growth factor (FGF) and Notch [22, 23] as well as TNF-related signalling events [24, 25]. Catagen-associated apoptosis primarily occurs in the hair matrix keratinocytes, the proximal and central ORS but generally not in the dermal papilla, which expresses high levels of anti-apoptotic Bcl-2 [21]. The compartmentalised expression of pro- and anti-apoptotic factors in the HF is shown in Figure 3. A diverse array of additional molecules have been found to play a role in catagen induction, including FGF-5 [26, 27], IFN-γ [28], substance P [29] and oestrogens [30]. The apoptotic processes within the HF are also controlled by caspases -1, -3, -4, and -7 [21, 31, 32] and can also be triggered by the withdrawal of DP-derived growth factors or by apoptotic signals produced by mast cells located within the CTS [29, 33, 34].

In addition to apoptosis, other events occur during catagen. In particular, the termination of melanogenesis is one of the earliest events and results in the hair shaft becoming less pigmented. The DP becomes condensed and ball-shaped, detaching from the surrounding matrix keratinocytes [35]. The old hair shaft forms the club hair, which comes to reside entirely

in the dermis. Overall, catagen lasts for 7-14 days, with ~2% of scalp HFs estimated to be in catagen at any one time [15].

2.2.2. Telogen

Although traditionally described as a quiescent or resting phase of the hair cycle [16], recent evidence has shown that the HF is highly metabolically and transcriptionally active during telogen [36]. Telogen is referred to as either 'refractory' or 'competent' [37]. In the first state, high levels of DP-derived BMPs, FGF18 and Wnt antagonists prevent any response to anagen-inducing signals. As the levels of these molecules fall, the telogen HF becomes primed to enter anagen, which is described as competent telogen. During telogen, the DP is in close contact with the HF bulge (stem cell region), separated by a shortened epithelial strand known as the secondary hair germ [9]. An estimated 10-15% of HFs are in the telogen phase, which lasts approximately 3-4 months [15, 38].

2.2.3. Anagen

With stimulation of a new anagen phase, the more distal cycling portions of the HF are gradually renewed, the hair bulb ultimately reaches the dermal adipose layer and melanogenesis is at its highest level [35]. HFs remain in anagen for approximately 2-6 years [16], with ~80-85% of scalp HFs in this phase at any given time [15].

3. Chemotherapy drugs and CIA

3.1. Anticancer chemotherapy agents and their action

Since the FDA approved mechlorethamine in 1949 for the treatment of non-small cell lung cancer, >100 chemotherapy agents have been approved for cancer treatment in the US alone [39]. In contrast to surgery and radiotherapy which target the primary tumour, chemotherapy is a systemic treatment and as such it targets both primary and metastasised tumour cells [40]. The principle behind infusing chemotherapeutic drugs is that because a greater number of malignant cells are in the cell cycle (are dividing) at any given time compared with healthy cells, the drug should have a greater impact on malignant cells (by stimulating higher levels of apoptosis). Table 1 provides a list of the main categories of commonly-used anticancer compounds as well as their point of action in the mammalian cell cycle. Chemotherapy agents are routinely administered intravenously but some may be oral or even topical, with their distribution depending on a number of factors, such as blood flow, drug diffusion, protein binding, tissue penetration and lipid solubility. Generally, drugs with extensive tissue penetration or high lipid solubility will tend to exhibit prolonged elimination phases due to slower tissue release [41].

Most agents are administered close to the maximum tolerated dose (MTD) which is quantified relatively to the individual's body surface area; this normalises the dosage accounting for physiological factors such as cardiac output, body fat and size and is expressed as units of mg/m^2 [41]. The frequency and intervals between treatments depend on the cancer type and the treatment regime and thus are quite variable. Clinical evidence demonstrates that most cancers are unlikely to be managed with a single chemotherapy agent and that combinations are more efficient in disease eradication [42]. The advantages of combinations are believed to be that: i) they provide maximal malignant cell death within the range of tolerated toxicity, ii) malignant cells in different phases of the cell cycle are targeted (discussed below), and iii) there is a reduced risk of malignant cell drug resistance development [43]. Chemotherapy is administered in cycles that include rest periods, so that the body has a chance to recover from side-effects (outlined below).

3.2. Cellular and molecular effects of chemotherapy drugs

Cells such as HF matrix keratinocytes, intestinal epithelial cells and bone marrow cells also divide rapidly, and thus chemotherapy drugs cause side-effects in healthy tissues. Bone marrow toxicity causes neutropenia, thrombocytopenia and anaemia, damage to the digestive tract results in mucositis, nausea, vomiting and diarrhoea. Induction of apoptosis in keratinocytes can cause nail bed damage, changes in skin integrity and CIA [40].

Although constant division/cell cycling is one reason why chemotherapy affects cancer cells more than normal cells, cancer cells are also more susceptible to lethal oxidation/reactive oxygen species (ROS). Due to their excessive metabolic rates and abnormally high energy demands, cancer cells operate under conditions of high ROS levels, a state also referred to as oxidative stress; this may in fact represent their 'Achilles heel', as agents that enhance ROS production can selectively trigger more cancer cell death [44]. Many anticancer drugs can increase ROS levels in cancer cells (examples provided below), thus causing them to cross a 'lethal pro-apoptotic threshold'. A range of chemotherapeutic drugs have been shown to induce ROS via various mechanisms, such as phosphorylation of NADPH oxidase (Nox) family members and by directly impacting on the mitochondria, the main site of production of ROS in cells [45].

Agents shown to augment ROS production to apoptotic levels include anthracyclines (e.g. doxorubicin, epirubicin), alkylating agents (e.g. cyclophosphamide), and platinum-based drugs (e.g. cisplatin, carboplatin and oxaliplatin) [46]. Interestingly, it is such agents that induce HF apoptosis at a greater frequency/severity than most other drugs, suggesting a possible

relationship between ROS production and stimulation of HF catagen [47]. Indeed, the high mitotic and melanogenic activity in the hair bulb ensures a high basal level of ROS within this compartment. Whilst the HF is well-equipped to deal with high levels of reactive moieties, it has recently been shown that exogenous sources of ROS will result in hair matrix apoptosis, lipid peroxidation and induction of catagen [48]. Moreover, it has been suggested that oxidative damage of mitochondrial DNA [49] and inhibition of endothelial proliferation in the vascular network surrounding the HF can contribute to CIA [50].

3.3. Chemotherapy-induced HF pathophysiology

The HF is particularly sensitive to chemotherapy induced apoptosis, as >80% of scalp HFs are anagen-phased at any one time [51]. Strikingly, the division rate displayed by HF matrix keratinocytes during anagen can be greater than that of malignant cells [11], thus resulting in susceptibility to chemotherapy agents. High levels of perfusion around the hair bulb by the DP may also make this region of the HF more susceptible to drug-damage.

The severity of CIA depends on the chemotherapy drug, its dose, administration route and treatment schedule. A list of drugs likely to cause CIA and relative severity is provided in Table 1. High intravenous doses usually cause more rapid and extensive hair loss, whereas oral therapy (despite administration at a higher total dosage) is likely to cause less alopecia [52]. CIA extent can be classified using a WHO classification system as: ‘grade 0’ implying no CIA, ‘grade 1’ minor, ‘grade 2’ moderate with wig proposal, ‘grade 3’ severe but reversible with wig proposal, and ‘grade 4’ complete irreversible CIA with wig proposal [53], although other scores/scales are available such as Dean’s scale [54]. The estimated incidence of CIA is >60% for alkylating agents, >80% for anti-microtubular agents, 60-100% for topoisomerase inhibitors and 10-50% for antimetabolites [55]. Although even just a single drug treatment can significantly reduce hair density [56], poly-therapies (consisting of two or more drugs) produce higher incidence and more severe CIA compared to single administrations [53].

In most cases, HF stem cells appear to be largely unaffected by chemotherapy agents, as hair regenerates 3-6 months post-treatment [51, 57]. Although, permanent CIA or incomplete regrowth is rare, an increasing number of cases are being reported, and this is more common in children, thus suggesting that acute damage to HF stem cells may occur [58-60]. In the case of children, permanent diffuse alopecia has been associated with haematopoietic stem cell transplantation [61]. In permanent CIA there is a large decrease in the total number of HFs, but this is not associated with inflammation or fibrosis/scarring [62]. In a study of permanent alopecia, biopsies of the frontal scalp were assessed and showed a reduction in anagen-

phase terminal HFs [63]. Instead, permanent alopecia may be associated with an increase in miniaturised vellus hair [63].

3.3 Experimental models for the study of CIA

As CIA remains an important unmet clinical challenge and since scalp biopsies from patients are difficult to access, there is a clear need to develop robust experimental models to both understand its pathophysiology and to generate avenues for the development of new treatment strategies [11]. Currently available models for studying and understanding CIA together with their advantages and disadvantages are outlined in Table 2. These include animal models (mainly involving the use of new-born rodents), as well as in vitro models.

4. Prevention modalities against CIA

4.1. Pharmacological and biological interventions

Since the 1970s, there have been numerous attempts to prevent CIA by means of mechanical, physical and pharmacological interventions [64-69]. Moreover, several classes of biological and mainly pharmacological agents with different mechanisms of action have been evaluated in animal models of CIA as discussed below.

4.1.1 Drug-specific antibodies

To reduce the severity of doxorubicin-induced alopecia in the new-born rat model, the use of a monoclonal antibody (MAD11) incorporated in liposomes has been explored to neutralise doxorubicin activity. Topical administration of these anti-anthracyclines prevented doxorubicin-induced CIA [70]. Further work explored the antibodies ability to prevent the bone marrow [71], gastrointestinal [72] and mucosal [73] toxicity of doxorubicin with positive outcomes in rats, however no clinical trials to assess this approach for CIA prevention have been reported.

4.1.2 Vasoconstrictors

As changes in DP blood-flow inevitably correlate with the diffusion gradient of drug delivered to the HF, superficial application of topical vasoconstrictors epinephrine or norepinephrine for prevention of CIA was studied in female Sprague-Dawley (albino) adult rats treated with Cytoxan or 1-methyl-1-nitroso-urea (MNU). Vasoconstriction proved highly effective with MNU which has a shorter half-life than Cytoxan, demonstrating the effectiveness of preventing drug

entry to the HF. The effect of lack of blood flow to the human scalp, patient response variability and other possible contraindications are yet to be clinically resolved and there is no evidence, as yet, that this would be advantageous over other approaches (e.g. scalp cooling), however if effective it could be better tolerated [65].

4.1.3 ROS inhibitors/antioxidants

The antioxidant N-acetyl cysteine (NAC), when applied topically in liposomes, protected new-born rats against cyclophosphamide-induced CIA, suggesting that cyclophosphamide stimulates ROS to drive HF apoptosis in matrix keratinocytes [74]. Furthermore, topical application of antioxidants resveratrol or aminothiol PrC-210 reduced CIA in new-born mice treated with Cytoxan [65]. Clinical trials utilising antioxidants for prevention of CIA have not yet been performed.

4.1.3 Hair growth cycle modifiers

Immunosuppressive immunophilin ligands, such as cyclosporine A (CSA), are used in the treatment of autoimmune disease and post-organ transplantation, however these drugs also prolong anagen and inhibit the catagen entry of the hair cycle, resulting in enhanced hair growth in several normal and pathogenic alopecia conditions [75, 76]. Neonatal rats and mice have been used to investigate the effects of CSA on CIA. Topical CSA application locally-protected from alopecia induced by cyclophosphamide, cytosine arabinoside and etoposide [77]. Another immunomodulator, AS101, has been shown to reduce the severity of alopecia in patients treated with a combination of carboplatin and etoposide [68]. Given the strong immunosuppressive nature of CSA, it cannot be developed as an effective CIA treatment, yet enhanced understanding of its mechanism of action may yield information that could lead to development of novel therapies.

Topical minoxidil is used for the treatment of male pattern baldness (androgenetic alopecia); minoxidil modifies hair cycle dynamics by shortening the telogen phase, thus facilitating anagen and encouraging hair growth [78]. In the new-born rat model, local application of minoxidil protected against CIA induced by arabinosyl-cytosine, but showed no protection to doxorubicin and cyclophosphamide-induced CIA [79]. In a clinical study in breast cancer patients, minoxidil was shown to accelerate recovery from CIA, but did not prevent the initial hair loss [78]. Minoxidil appears to be most beneficial for men suffering with androgenetic alopecia, where it accelerates hair-regrowth [80]. Overall, it helps re-growth following CIA, but currently there is no evidence supporting its use in CIA prevention [64].

4.1.4 Cytokines and growth factors

Interleukin 1 (IL-1), which plays a role in the regulation of inflammatory and immune responses to infections, and imuvert, a biological response modifier with immune stimulatory properties derived from the bacterium *S. marcescens*, have both been reported to protect new-born rats from CIA induced by cell cycle-specific agents, namely cytosine arabinoside and doxorubicin, but not from cell cycle-nonspecific agents such as cyclophosphamide [81]. Both imuvert and IL-1 induce the release of multiple cytokines or growth factors and it was suggested that the action of imuvert is via IL-1 [82]. There is also evidence that acidic fibroblast growth factor (aFGF) and EGF protect from CIA but again only if CIA is caused by cell cycle specific agents [81]. Despite the promise of these agents in new-born rat experimentation models, they have not yet been tested in the clinic for CIA prevention.

4.1.5 Cell cycle or proliferation modifiers

As discussed above, rapid cell proliferation in HF matrix keratinocytes during anagen and lack of selectivity in anticancer agents is a primary factor in the pathogenesis of CIA. Hence, one approach to protect against the CIA is to inhibit HF cellular proliferation in order to decrease sensitivity to chemotherapy [83]. An example of this 'protective pre-conditioning' approach is the administration of calcitriol (1,25-dihydroxyvitamin D3) which has multiple effects on keratinocytes, including stimulation of cell differentiation, inhibition of DNA synthesis and G0/G1 cell cycle arrest [84, 85]. Therefore, it is possible that calcitriol, by stimulating terminal keratinocyte differentiation, may alter cell susceptibility to apoptosis. Calcitriol can protect new-born rats from CIA induced by cyclophosphamide, etoposide and combination of cyclophosphamide and doxorubicin [86]. In addition in the adult mouse model, calcitriol could enhance normal pigmented hair shaft regrowth and reduce apoptosis in the hair bulb, however failed to prevent or retard hair loss after administration of cyclophosphamide [87, 88]. A phase I study showed that calcitriol was well-tolerated and 21-subjects showed improved hair retention when treated with taxane therapy [64] but its beneficial effects are most likely limited to taxanes due to the previously mentioned mechanisms of action for calcitriol.

Finally, inhibitors of cyclin-dependent kinase 2 (CDK2), which plays a key role in the transition from G1 to late G2 of the cell cycle, can block progression from late G1 phase into S phase, reduce the sensitivity of HFs to chemotherapy agents and inhibit apoptosis induced by etoposide, 5-fluorouracil, taxol, cisplatin and doxorubicin. In new-born rats, topical application of a CDK2 inhibitor reduced etoposide mediated hair loss by 50% at the site of application and by 33% in CIA induced by combination of doxorubicin and cyclophosphamide [83]. Despite the promise of these findings, such modifiers have not been clinically tested yet.

4.1.6 Inhibitors of apoptosis

Caspase-3 is a key mediator of apoptosis and pathways leading to its activation can be stimulated by a number of chemotherapy agents [89]. Tsuda et al showed that a topical administration of M50054, an inhibitor of caspase-3 reduced CIA induced by etoposide in the new-born rat model [90]. Further experiments have not elucidated whether this would protect against other drugs and no clinical trials have been reported.

4.1.7 Parathyroid Hormones

Parathyroid hormone receptor (PPR) ligands have been shown to have a potential role in the hair cycle by inducing hair regrowth following CIA [91]. The best results have been obtained using cyclophosphamide in mice where it was found that CIA could be reduced, hair re-growth improved and re-pigmentation promoted. This suggests that PPR ligands can be potentially useful as a topical application for preventing/treating CIA, however this may rely on follicles that have not undergone permanent alopecia [92]. Despite initial promise, clinical trial results were disappointing and the first trial was terminated [92]. Understanding the potential issues with pharmacokinetics has led to improved PPR ligands, however there is no information available on the clinical success of these agents to date.

4.2 Physical interventions/non-drug therapies

4.2.1 Scalp tourniquets

Scalp tourniquets are special bands that tightly fit the scalp region to occlude the superficial blood flow and thus reduce the amount of drug delivered to the HFs [93]. Scalp tourniquets are applied when the plasma drug levels are at their peak, i.e. from the last 10 minutes of infusion to 10-minutes after the cessation of drug administration [94]. Tourniquets have achieved a small to moderate degree of rescue from CIA induced by vincristine, cyclophosphamide and doxorubicin. However, it is no longer recommended due to the high pressure applied causing patient discomfort [85, 94].

4.2.2 Scalp cooling

Scalp cooling was introduced in the 1970s [67], with application of cooling throughout the administration of chemotherapy in most cases reducing CIA in patients [95].

A number of hypotheses have been proposed to explain how scalp cooling reduces CIA. Firstly, cooling causes rapid vasoconstriction, which has been shown to significantly reduce blood flow in the scalp. In fact, perfusion can be reduced to 20-40% of normal levels [96] and

this should result in reduced chemotherapeutic drug perfusion through the vasculature of the DP [97]. A second hypothesis is that, the rate of drug diffusion across a plasma membrane is reduced at low temperatures due to lower kinetic energy, whilst membrane lipid fluidity is also lower which will impact on passive diffusion; together these would result in a low proportion of drugs entering HF cells [98]. Thirdly, as cell division is an energy-dependent metabolic process, it is likely that cooling abrogates enzyme-dependent reactions. It has been reported that temperature can particularly affect the G1 and S phases of the cell cycle [99] and this could be especially important for drugs that target specific phases of the cell cycle, such as mitosis-targeting microtubule-destructive drugs. Fourthly, some drugs (e.g. doxorubicin) may enter cells via active transport mechanisms and this would be reduced by cooling. In support of this hypothesis, it has been shown in cell models that doxorubicin-induced damage to DNA is reduced at lower temperatures [100]. Fifthly, a general decrease in the metabolic activity of the cells in the HF could cause a reduction in the cytotoxicity of chemotherapy drugs as a range of cellular processes (such as oxidation) decelerate [97]. In practice, it is likely that a combination of these mechanisms play a role in reducing CIA upon cooling and this may explain the reported efficacy of scalp cooling.

It has been reported that the scalp temperature achieved by cooling is a critical factor in preventing CIA, and dampening the scalp with water improves heat transfer from the head to the cooling source [101]. It has previously been reported that a subcutaneous temperature of 22ºC was a ‘threshold’ temperature necessary for effective cooling, whilst a close relationship exists between epicutaneous and subcutaneous temperatures during cooling, with 22ºC subcutaneous corresponding to an epicutaneous temperature of 19ºC [97]. Interestingly, Komen et al (2016) found that breast cancer patients whose scalp temperature was reduced to 18ºC were the least likely to require a wig following anthracycline treatment; the study also raised the important issue of device fitting, to ensure that all areas of the scalp are cooled effectively, so that adequately low temperatures are achieved [56].

Interestingly, recent laboratory studies have provided support for these clinical observations. It was shown, using a range of *in vitro* models, that cooling can efficiently protect human keratinocytes from chemotherapy drug-induced toxicity [102]. Equally importantly, it was shown that the cooling conditions (temperature) used were also a critical factor in preventing cytotoxicity. These experiments provided for the first time biological evidence that progressive reduction of temperature (26, 22, 18 and 14°C) positively correlated with better protection (rescue) of keratinocytes from drug-induced cell death [102]. It is possible that cooling may have direct cytoprotective effects and at the same time may reduce drug diffusion that renders cells less susceptible to drug toxicity. This is supported by the finding that reducing the scalp temperature below 22ºC does not further decrease blood flow [96], thus any increased

protection by cooler scalp temperatures may not be a result of reduced scalp perfusion. Interestingly, this ‘cut off’ point in the protective effect of cooling has been shown to occur for doxorubicin both at the level of the cell membrane permeability [98] and subsequent DNA damage [100].

Practically, a marked reduction in scalp temperature may lead to an increase in patient discomfort and therefore intolerance, so although ’more cold’ is beneficial, it may not always be feasible. Furthermore, the amount of temperature reduction possible for each person is likely to vary quite considerably due to individual physiological differences/variability [56], however, in most cases, ‘the colder the better’.

4.2.2.1 Scalp cooling using cool caps

Initially, scalp cooling was achieved using crushed ice in plastic bags fixed into position with elasticated bandages [103]. As heat from the head rapidly warmed the ice-packs, these needed to be replaced regularly; this was time-consuming and also meant that temperature increased between replacements [104, 105]. The number of countries and hospitals using scalp cooling increased dramatically following introduction of improved commercially available products. This involved a refrigerated cryogel cap, which is placed in a freezer at -25°C before being fitted to the head (e.g. Penguin cold cap) [104]. However, because of the very low initial temperature these gel-caps are reported to be uncomfortable, and although better than ice-packs, they still thaw rapidly and must be changed regularly to maintain reduced scalp temperature. Thus, several changes are required during chemotherapy perfusion protocols [104] and between replacements scalp temperature unavoidably increases [105].

4.2.2.2 Modern scalp cooling devices

Refrigeration unit-fitted devices designed to circulate liquid refrigerant through a cooling cap are the modern-day choice for scalp cooling. These caps, such as the Paxman (UK) and Dignitana (Sweden) systems, are available in a range of sizes to ensure a suitable fit, as head-sizes and shapes vary [106]. The advantage of these systems is that the coolant achieves a constant, reduced scalp temperature throughout drug infusion without the need for cap replacement. This reduces medical staff time investment and also, because the caps are not cooled to such initially low temperatures (and are not as heavy), they are reported to be more comfortable. Recent studies by Komen et al (2016) have shown that 18°C can be reached at the scalp of patients throughout the course of chemotherapy infusion, and most patients tolerate this intervention very well, with the majority indicating either low or moderate levels of discomfort. Only 1 of 62 patients actually reported a mild headache even when the scalp

cooling device could reduce temperatures down to 10ºC within 30 minutes [56]. Other studies have shown that the drop-out rate due to intolerance is around 3.3% [107], however tolerability varies.

4.2.2.3 Clinical evidence for the efficacy of scalp cooling in cancer patients

Scalp cooling is the only FDA-cleared technique supported by statistically significant and clinical evidence-based efficacy for CIA reduction. Numerous studies have demonstrated that its clinical efficiency can reach ~90% depending on the chemotherapy agent and/or cooling technique used [64, 67].

Auvinen et al (2010) showed that scalp cooling resulted in a significant reduction in CIA with 100% of patients maintaining their hair after doxorubicin treatment, 83.3% after docetaxel, 76.5% after FEC (5-fluorouracil, epirubicin and cyclophosphamide) and 78% after docetaxel or FEC [108].

A larger and prospective multi-centre study conducted by van den Hurk et al (2012) explored the effect of scalp cooling on hair preservation in 1411 chemotherapy patients between 2006 and 2009 [53]. The data was collected by the Dutch scalp-cooling registry, the mean age of the subjects was 53, with 86% having treatment for breast cancer and 96% of these being female. Treatments varied depending on the stage of the cancer and consisted of the following: 5 combinatorial regimes FEC or TAC (docetaxel, doxorubicin and cyclophosphamide), plus several monotherapies (single dose of anthracyclines and taxanes). Patients in the study used the Paxman PSC-1, PSC-2 or ORBIS scalp cooling devices, the median number of chemotherapy and cooling sessions was four [53]. The results were evaluated by questionnaires, with patients scoring their own hair loss according to the WHO scale. The best results were obtained following monotherapy treatments, for instance taxanes such as docetaxel (75mg/cm^2) or paclitaxel (70-90mg/cm^2) with 94% and 81% of patients, respectively, not requiring a wig. The results were less impressive in the case of the TAC combo-therapy, even when used at low doses, as only 8% of patients did not require a wig. Overall, 50% of all 1411 patients surveyed did not use head covering at the time of their last treatment. van den Hurk et al (2010) reported that besides the specific chemotherapy protocol, other factors can have an influence on the use of head cover, such as patient age (generally it is higher in those over 50), gender, ethnicity, and wetting before scalp cooling [53].

Schaffrin-Nabe et al (2015) found out of 226 patients with variable chemotherapy regimens, 146 (88%) had positive results from scalp cooling and did not require a head cover. The worst results were obtained with the highest anthracyline doses or polytherapies or when TAC was administered. Documentation of other variables, however, identified some of the factors other than high drug-dose that affect the success of cooling and these included co-morbidity, current

medications, age, menopause, hair thickness and nicotine intake [107]. Moreover, Komen et al (2016) showed that out of 62 breast cancer patients (median age 60) treated with up to 6-cycles (median 3 cycles) of anthracycline (epirubicin or adriamycin) chemotherapy, 13 (12%) did not require a wig [56]. Cigler et al (2015) evaluated the effects of scalp cooling on 20-patients receiving docetaxel and cyclophosphamide with a total of 4-cycles over 3-week intervals. Scalps were cooled 50 minutes before administration and for 4 hours afterwards. Upon follow up, only 2 out of 20 felt the need to wear a wig, whereas normally the vast majority undergo complete alopecia [54]. Ibrahim et al found that scalp cooling prevented up to 96% of patients requiring a wig after repeated cycles of taxanes or anthracycline and for those that did, it was due to higher doses of anthracycline treatment [109].

More recently, Nangia and colleagues reported the results of the SCALP (Scalp Cooling Alopecia Prevention) clinical trial [110]. This is the first, randomised, multicentre trial (RCT) on scalp cooling (and the first RCT using scalp cooling devices) performed from 2013-2016 and tested the efficacy of cooling on 192 patients, with 119 patients receiving anthracycline or taxane treatment versus 63 receiving no intervention (controls). All patients in the control group needed a wig, whereas 50% of patients receiving scalp cooling did not. This study was terminated on ethical grounds, as the chance of preventing CIA using scalp cooling was so significant [110].

In most studies, the pre-cooling period has been between 5 and 30 minutes to ensure that the scalp is cooled when the drugs reach the HFs [111-113], however recent evidence suggests it should be around 30-minutes [56, 105]. Another equally important consideration during scalp cooling is the period of time necessary to maintain cooling following completion of drug administration (infusion). Routinely, the cap remains in place during the administration of the chemotherapy drugs and also for a period after this, referred to as the post-infusion cooling time (PICT), which allows the drug concentration to drop below toxic levels before the HFs warm up. Although until recently, a 90-min PICT was recommended, van den Hurk et al (2012) specifically examined the effect of PICT in reducing CIA after docetaxel treatment and found that better results were obtained by reducing PICT from 90 min to 45 min [114]. This is presumably because once the plasma concentration of docetaxel drops below toxic levels, the warming of the scalp allows any drug that has accumulated during the course of chemotherapy to be more rapidly ‘flushed out’ of the scalp. This study indicated that some optimisation of cooling protocols might be required to improve the efficacy for different chemotherapy regimens [114, 115]. In line with this, Komen et al (2016) reported that even a 20-min PICT is as effective as the 45-min period [116]. Therefore, both of these studies represent potentially significant improvements in scalp cooling protocols. Shortening the PICT has the additional

advantage of reducing the time that patients would be required to spend in the treatment environment.

Although some concerns have been raised as to whether scalp cooling could be associated with a higher incidence of scalp metastasis, there appears to be no evidence for a link between metastasis and scalp cooling [117]. Studies that have been conducted to specifically address this issue in patients with breast cancer, confirmed that scalp metastasis occurs very rarely, with an incidence between 0.03% and 3% in individuals that did not receive cooling, and this incidence is no different to that for individuals who received scalp cooling for whom the incidence was 0.04–4% [118]. In most cases reported so far, scalp metastases after scalp cooling was not the first metastatic site and thus any that occurred were part of a widespread metastatic disease and not related to scalp cooling. These observations are in accordance with recent studies demonstrating that use of scalp cooling has no effect on the breast cancer patient survival [119]. The lack of any association of scalp cooling with breast cancer metastasis is further supported by a recent, comprehensive systematic review and meta-analysis reporting that scalp cooling does not increase the incidence of these rare scalp metastases [120]. Moreover throughout application of scalp cooling, only the outer part of the scalp to a depth of 2cm is affected, with no alteration of core temperature excluding any risk of hypothermia [101]. However, patients who are at risk of cold-induced urticaria, cold agglutinin disease, cryoglobulinemia, and post-traumatic cold dystrophy should be excluded from scalp cooling [109].

5. Conclusions

Despite the success of adjunct chemotherapy in improving the outcome of cancers such as breast cancer, hair loss still represents a very significant psychological burden for cancer patients. Any intervention that could reduce the side-effects of chemotherapy would be expected to lead to improvements in both the initiation and completion of therapy, in patient quality of life, and possibly survival outcomes. Having provided a review of several biological and clinical aspects of CIA, here we ultimately focused on research demonstrating that scalp cooling is currently the only available safe and effective option for CIA reduction/prevention. Despite the well-established ~50% success rate of scalp cooling, clinical and biological evidence suggests that further improvement can be made. Improvements relating to changes in PICT have clearly demonstrated this. Another important aspect is the efficacy in delivering adequately low temperature to the scalp, and improving clinical staff expertise in fitting the cap, as well as the possibility of patient-specific cap design could prove important in increasing the currently-reported efficacy of scalp cooling. Finally, an improved understanding of the biological mechanisms of cooling may not only inform the cap design or temperature of choice, but also provide novel avenues for enhancing the capacity of scalp cooling to protect from CIA.

Conflict of interest

The authors declare no conflict of interest.

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Figure captions

Figure 1 Structure of the HF

The schematic illustrates the organisation and structure of the human HF, including key areas of the organ, such as the bulge region, the outer route sheath (ORS) and inner root sheath (IRS), and the hair bulb that includes the hair matrix keratinocyte compartment and the dermal papilla region. Reprinted with permission from [121].

Figure 2 The hair ‘cycle’

Schematic diagram of the three main phases of hair cycle: the growth phase (anagen), the dystrophic phase (catagen), an extremely shortened resting phase (telogen) and the ‘shedding’ of the hair (exogen). In anagen, the hair bulb is located deep inside the skin and hair grows towards the skin surface. The dermal papilla survives catagen and moves upward to the lowermost portion of the bulge, which then forms the secondary germ at its base during telogen. In telogen, the hair falls out and the hair bulb relocates down again as the new hair grows. At their cycle end, telogen HFs can be activated through a) mechanical depilation, b) pharmacologically, and c) by specific signalling factors (e.g. Wnt signalling), which stimulates a return to anagen and the generation of the new lower follicle and hair shaft. As the new hair grows in, the old hair is shed during exogen. The duration of each phase depends on the type, site and specific genetic programming of the follicle.

Figure 3 Molecular regulators of apoptosis in the HF

The diagram illustrates the expression pattern of pro-apoptotic (e.g. Fas, p53, Bax) and anti-apoptotic (Bcl-2, survivin) molecules in the different HF compartments.

Tables

Table 1 List of the main categories of commonly-used anticancer compounds

The table lists the main categories of commonly-used anticancer compounds, their point of action in the cell cycle and the likelihood of causing CIA [122, 123]. Note: the likelihood to cause CIA relates to the clinical administration of each drug as a monotherapy.

	Usually causes CIA	Occasionally causes CIA	Unlikely to cause CIA
DNA replication (S phase)	***Topoisomerase inhibitors*** doxorubicin, epirubicin, daunorubicin, irinotecan, topotecan, etoposide, teniposide	amsacrine	–
	Alkylating agents cyclophosphamide, ifosfamide	busulfan, melphalan, lomustine	carmustine, procarbazine, streptozocin
	Antimetabolites –	cytatarbine, gemcitabine, 5-fluorouracil (5-FU)	6-mercaptopurine (6-MP), methotrexate, hydroxyurea, mitoxantrone, fludarabine, raltitrexed, capecitabine, idarubicin
	Platinum-based heavy metal alkylators –	–	cisplatin, carboplatin

Mitosis (M phase)	***Anti-cancer antibiotics*** –	–	mitomycin C
	Anti-microtubule agents docetaxel, paclitaxel, vindesine, vinorelbine	vincristine, vinblastine	–

Table 2 Currently available models for studying and understanding CIA

Model information	Advantages	Disadvantages	References
New-born/young rodents ➢ hair is depilated from the rodents causing all HFs to enter anagen ➢ 7-8 days old rats have spontaneous hair growth for around a week	✓ can experiment on hair growth arising from the anagen phased follicle ✓ has a level of consistency	× HFs are not matured × new born rats lack pigmentation thus melanogenesis cannot be studied × only shows how chemotherapy drugs affect anagen × in humans each follicle in a unique phase, whereas in the rodent they are all in anagen	[124, 125]
Adult C57BL6 mouse ➢ adult mice with fully grown hair/mature/telogen phased HF have their hairs depilated	✓ mature HF can be recognised by pigmentation ✓ has a level of consistency ✓ can experiment hair growth arising from the anagen phased follicle	× in humans each HF in a unique phase, whereas in the rodent following depilation, they are all in anagen × anagen in humans lasts years as opposed to weeks in the mice	[126]
Nude mouse human skin graft ➢ human scalp skin is grafted onto nude mice, hair sheds within a month and then regrows	➢ unique physiology of the human HF is better maintained ✓ can experiment hair growth arising from the anagen phased follicle	× the xenograft HF cycle is not yet well characterised × wound healing-, reinnervation-, and reperfusion-related phenomena are absent factors during normal in vivo scalp HF cycling	[35, 127-129]

Ex vivo Cultured human HFs ➢ anagen phased HFs are taken from the scalp and grown in the laboratory (in vitro)	✓ HFs are human ✓ HFs are in anagen ✓ experiments can be well controlled	× human HFs are difficult to obtain (need specialist clinicians and volunteers) × HFs can spontaneously enter catagen due to stress and/or structural damage	[130, 131]
In vitro keratinocytes ➢ normal or immortalised skin cells, and normal HF keratinocyte cultures are grown in the laboratory (in vitro)	✓ like human matrix keratinocytes cell are highly proliferative (relevant) ✓ experiments can be extremely well controlled and repeated systematically ✓ molecular mechanisms can be studied in detail	× cell monolayers studied compared with the highly-structured, differentiated HF tissue × immortalised (not primary) cell lines have genetic mutations	[102]

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