A Review on the Effects of Chemotherapy Drugs and Cooling alone or in Combination on the Cell Cycle

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Abstract: *When it comes to cell proliferation, this is a process strictly controlled by extracellular signals, such as nutrients, growth factors, temperature, and more. Together, these establish the cell cycle control mechanism, which is the core of cell proliferation. Throughout the current review, a collection of the most pertinent background sources was built, in order to investigate the cytoprotective effect of cooling in opposition to toxicity brought on by chemotherapy, and subsequently another experimental approach was developed to comprehend the impacts of cooling on cell cycle arrest in cell culture. As a result, it is considered that this could show an approach for reaching selective toxicity of chemotherapeutic agents for cells, which in turn can provide a deeper comprehension of the molecular and cellular processes occurring within. With detailed information collected regarding the way that cell cycle events are controlled, it is considered that innovative ways of using this to protect for chemotherapy-induced alopecia will be established.*

Keywords: Cell cycle, Chemotherapy, cooling, Chemotherapy-induced alopecia

1. Introduction

The cell cycle is the fundamental means by which every living entity divides and multiplies. The cell cycle is divided into four main phases: G1 phase (Gap phase 1) where the nucleus has the two complementary strands of DNA; S-phase (synthesis) during which DNA replication takes place, and G2 phase (Gap phase 2) preceding mitosis (M phase). Some cells are either not in an active cycle (post-mitotic) or have become quiescent and entered a resting phase known as G0; during this time when the cell receives a mitotic signal it can re-enter the G1 phase [1]. In order to preserve the stability of the genome in response to cellular damage, there are monitoring mechanisms (checkpoints) that will arrest the cycle at either G1/S or G2/M. In turn, this is able to offer extra opportunities for the cells to activate the repair processes, in order to extract damage prior to DNA replication. Also, if damage is excessive and/or repair cannot occur, cell death by apoptosis can be stimulated [2, 3]. Cell division is a complex, finely tuned mechanism. In each cell cycle phase, the cell monitors both its own growth as well as the external conditions. If these are unfavorable then growth arrest or cell cycle arrest is initiated [4].

One critical period for cell cycle progression exists, which is the G1/S phase boundary [5, 6]. This period is called the restriction point (R point) in the mammalian cell cycle [7]. It is a point in the G1 phase at which the cell committed to the cell cycle and after which extracellular proliferation stimulants are no longer required [1]. Once it progresses through the point of R point, the cell cycle progress to enter S phase and the cells initiate DNA synthesis promptly, then on to G2 phase, and the M phase continuously [8, 9]. If the decision is made that the environment is bad, the cell cannot go through the start, so it will stay in the G1 phase as it is not proceeding to the S phase, or remain in the stationary phase

G0 phase and enter a dormant state [10]. The environment of the cell placement decides whether there are signals to go forward, e.g. differentiation, aging, apoptosis, meiosis, but the main aim of those states' point of branching to those states is also present at this point in the G1 period it is considered [11].

The restriction point may be deregulated in cancer cells, at which point the withdrawal of growth factors no longer induces reversion to a quiescent state and the abnormal cancer cells continue to proliferate ignoring the signal of mitotic arrest coming from surrounding cells [12].

Moreover, the distribution of cells at different stages of the cycle determines cell growth rates and usually S phase percentage reflects the quantity of multiplying cells. Cancers tend to have a higher percentage of S phase cells than that of normal tissues. Furthermore, more aggressive cancer types have a higher proportion of cells in the S phase compared to less aggressive tumours [12-14].

2. Effects of Chemotherapeutic Agents on the Cell Cycle

Since many chemotherapy drugs are only effective on actively dividing cells and not those that are in a quiescent or dormant stage (and out of cycle), cell cycle analysis is important in helping to understand their mechanism of action. Chemotherapeutic drugs primarily target DNA synthesis and the proteins critical for normal mitosis by targeting specific stages of the cell cycle, as explained below and summarised in Figure 1 [15]. When chemotherapy drugs induce DNA damage, either the cell cycle can be arrested temporarily to allow for DNA repair [16] or the cells could be eliminated by apoptosis [17]. This is a decision-making process, where the tumour suppressor p53 plays a vital role.

Many chemotherapeutic drugs interfere with cellular metabolism and trigger apoptosis [18, 19]. It is seen that there are varying levels of stress with different impacts on cells. Mild stress can trigger repair mechanisms, which can aid in cell survival. Low or moderate stress (damage) activates apoptosis, while extremely high stress overwhelms apoptosis and leads to necrosis. An unregulated catastrophic mode of cell death occurs when the cell is intensely damaged or stressed. Chemotherapeutic drug treatment can induce apoptosis, indicating that cells initiate a controlled suicide in which the cell passes through different phases and eventually fragmentation (DNA) in cells is observed, thus apoptosis is a means that can allow an organism to eliminate damaged cells in an orderly fashion [19].

Chemotherapeutic drugs can be classified as cell cycle specific and non-specific drugs. Certain chemotherapeutic agents are meant to impact dividing cells throughout one or more phases of the cell cycle, through bringing on arrest either in G1/S or G2/M phases; for example docetaxel acts on cells in G2 and M phases by exhibiting high affinity to β-tubulin and targeting centrosome organization [20]. By contrast, docetaxel has minimal effects on cells in G1 phase and as a result there is an accumulation of the cells in G2/M phase [20]. Doxorubicin induces G2/M arrest in cell cycle progression [21]. Other drugs, such as cyclophosphamide, may affect dividing cells in all phases of the cycle, and as such are non-cell cycle specific [21].

Figure 1: Chemotherapy drugs and their point of action in the cell cycle

This diagram shows a schematic depiction of the cell cycle and its different stages. This displays the point of action throughout the cell cycle for numerous chemotherapy drugs widely employed in clinical settings. Importantly, even though all drugs are shown as being connected with certain cell cycles stages, usually a phase-overlap exists since certain chemotherapy agents can be aimed at two or greater phases, including overlapping phases, of the cell cycle. On the other hand, others can act on cells regardless of the phase, and are cell cycle independent. G0 means that the cell is out of

cycle, known as quiescence. G1 is where the cell is about to divide and releases a number of proteins necessary for DNA replication. S is where DNA double-stranded breaks are brought on by topoisomerase enzymes, and DNA replication occurs. G2 involves the cell making sure the spindle components are ready for mitosis. M is where the nuclear division occurs, along with chromosome separation, and cytokinesis to bring about the creation of two daughter cells.

3. Effects of hypothermic conditions on cell cycle progression and cytotoxicity

For cells to proliferate they need both stimulation and progression factors [22] to enter the cycle. If one or more of these factors is thermally labile then non-optimal temperatures can create a different rate of loss, higher than that of the enzymatic production, causing different effects in the cell [23]. Cold treatment (cooling) is split into five categories; mild (35-33°C), moderate (31-29°C), low (24-20°C), very low (20°C) and hypothermia (10-4°C) [24, 25].
In 1956, Cheveremont found that in primary embryo skeletal muscle cell cultures after 24h incubation at 16-20°C, the number of cells undergoing mitosis dropped to zero. However, after 3h of warming at physiological temperature (37°C) cells were dividing 2-3 times faster than the non-cooled cells were [26]. The explanation provided for this was that the cells continue to progress through the cell cycle until they arrest at late G2. As might be expected, all phases of the cell cycle of mammalian cells are inhibited at temperatures lower than 10°C (severe hypothermia) [27]. Culturing CHO (Chinese Hamster Ovary) cells at 30-35°C have been shown to demonstrate a reduction in the number of cells in G1 or S phases, whilst maintaining higher cell viability [4]. In addition, after reducing the temperature of cultured cells to 16-20°C, rewarming has been found to increase the rate of cell division by 2-3 fold compared to non-cooled controls [27].

Moreover, previous studies with mouse leukemic and HeLa cells showed prolongation of cell cycle at 25°C-33°C and a temporal dilation of the G1 and S phase. HeLa cells were cultured at different temperatures and this was shown to modify all phases of the cell cycle, whilst M phase was more sensitive than G1, S, G2 phases were to temperature [28]. Furthermore, Al Tameemi *et al*., 2014 showed that cooling during drug exposure may decelerate growth rates and thus protect cells from chemotherapy-induced toxicity [29]. There is some evidence that culturing cells at a reduced temperature can protect from cytotoxic agents modulating (preventing) the effects of chemotherapy drugs on the cell cycle [30].

As a result of difference stressors, such as oxidative stress, osmotic, and heat stress, gene expressions are changes, and the function of a gene product can be changed as well. For the rat model showing lethal hemorrhagic, there was clear evidence of hypothermia, which would u-regulate gene expressions in pro-survival pathways, with a down-regulation seen in metabolic pathways [31].

Normal human fibroblasts which has developed in cell culture displayed mild hypothermia (28°C) being the cause
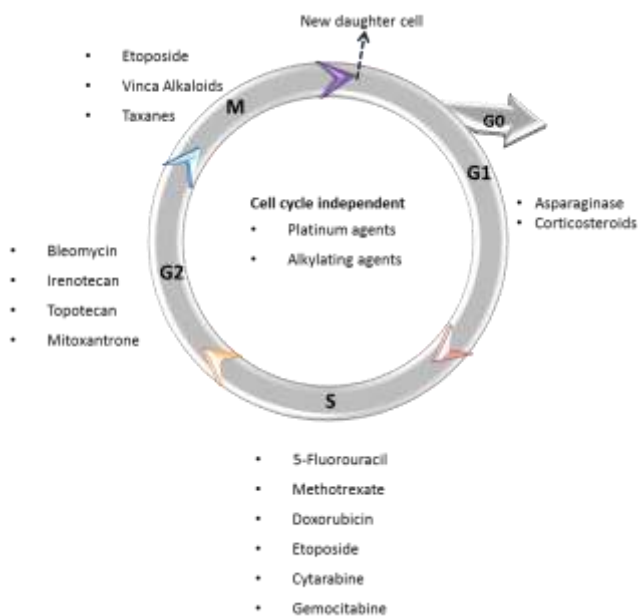
for greater p53 levels and p21 activation. These cells go through a reversible G1/S cell cycle arrest, while cell cycle arrest through p21 is seen to have a protective effect on cells stemming from apoptosis brought on by cytotoxic agents [32, 33]

In addition, p53 has underlined the value of mediating this arrest, wild or defective p53 mouse embryo fibroblasts went through hypothermia. On the other hand, cells with wild-type p53 went through a cell cycle arrest , with the exception of defective p53, which denotes that p53 is important when it comes to mediation [33]. Due to the fact that numerous tumours include p53 mutants, hypothermia is considered to raise the selective toxicity of chemotherapeutic agents for tumour cells [32, 33], and mild hypothermia (or cooling) is employed widely, in order to mitigate hair loss through chemotherapy [29, 34].

Several studies have confirmed that this condition delays cell growth, decreases hair matrix keratinocyte growth rate and shows a delay in the progression of the cell cycle, while maintaining high cell viability. Moreover, cooling induces a decrease in the rates of nutrient consumption and toxic metabolite production, as well as a decrease in protease activity and the production of reactive oxygen species [35].

4. Cooling research aims

Most chemotherapeutic agents trigger apoptosis in dividing cells [36]. Because of the rapid division rate of hair matrix keratinocytes, the hair follicle (HF) represents a target for many chemotherapeutic agents [37].

Chemotherapy-induced alopecia (CIA) is considered to be the most traumatic side effect of cancer treatment, and stress caused by this can have a negative impact on the overall outcomes [38, 39]. Cancer patients going through chemotherapy may lose their hair all over their bodies, but it is the most traumatic for patients to lose the hair on their heads. Scalp HFs are more affected by chemotherapy drugs than other terminal HFs, for example those of the eyebrows, eyelashes, beard, auxiliary and pubic hairs that are variably affected [37]. HFs across various regions of the body has variable durations of anagen. For example, scalp HFs stay in the anagen stage for 2-6 years and ultimately produce long hairs, whereas actively growing eyebrow follicles remain in the anagen stage for between 2-3 months. In the HFs of eye lashes, this stage lasts between 30 and 45 days, producing short hairs [40, 41]. Furthermore, in terms of eyelash HFs, only around 40% of the upper lashes and 15% of the lower lashes are in the anagen phase at any point, whereas for scalp hair, 80-85% are undergoing the anagen phase [40]. Eyebrow and eyelash HFs maintain the slowest hair growth rate for any area of the body. Due to the greater mitotic activity, anagen follicles have the greatest vulnerability to toxicity. Therefore, regions with the greatest proportion of anagen follicles, for example the scalp, are traumatised more severely by noxious events compared with regions comprising a lower proportion of anagen follicles, for example the eyelashes [42, 43].

The only effective treatment for CIA currently available is scalp cooling [44]. The scalp cooling involves Refrigeration unit-fitted machines, designed to circulate liquid refrigerant through a cooling cap during chemotherapy treatments [45]. It has been hypothesised that scalp cooling works by a combination of vasoconstriction, a reduction in the metabolic rate and/or reduced drug uptake by cells in the hair bulb [46].

5. Conclusion

It is widely thought that understanding the processes behind the control of cell cycle progression as a result of drug treatment at physiological temperature and cooling is crucial to comprehending the pathways in charge of the benefits cooling offers against drug toxicity, and specifically in the matter of protection. It can be seen that there are not many research study papers in existence, which look into the reaction seen for a number of temperatures, which can assist in optimising cooling and investigating a number of different temperatures to achieve this. The end goal would be to protect against cell damage brought on through chemotherapy *via* the initiation of a reversible cell cycle arrest. Overall, cooling is seen to be able to achieve short term reversible cell cycle arrest in cell culture, which puts forward the idea cooling has the potential to allow for selective toxicity of chemotherapeutic agents for cells to be reached optimally. Even though there is available clinical evidence on the matter, the processes behind the way cooling acts as a protection are not comprehended to a satisfactory degree, particularly when it comes to looking into the positive effect of cooling and direct protection from CIA. It is suggested that the cytoprotective impact of cooling when dealing with toxicity brought on by chemotherapy is because of its capacity to lower cell proliferation rates.

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