How do chemotherapeutic agents damage the ovary?

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BACKGROUND: Chemotherapy treatment in premenopausal women is associated with an increased risk of premature ovarian failure (POF) but the exact mechanism through which this occurs is uncertain. In this review we examine the current evidence for the direct action of chemotherapeutic agents on the ovary and discuss possible molecular pathways through which follicle loss may occur.

METHODS: A systemic search of the databases, PubMed and Google Scholar, was made for all English language articles through to 2011 in each subject area discussed.

RESULTS: POF results from the loss of primordial follicles but this is not necessarily a direct effect of the chemotherapeutic agents. Instead, the disappearance of primordial follicles could be due to an increased rate of growth initiation to replace damaged developing follicles. Likewise, the loss of oocytes need not necessarily be a direct result of damage: evidence suggests that chemotherapy drugs can also induce oocyte death indirectly via damage to somatic cells. Specific molecular mechanisms and likely ovarian targets are discussed for some of the anti-cancer drugs most commonly used to treat premenopausal women. Finally, we consider current and prospective methods of preserving fertility.

CONCLUSIONS: It is likely that different chemotherapeutic drugs act through a range of mechanisms and on different target cells. More research into the cellular mechanisms underpinning chemotherapy-induced follicle loss could lead to the generation of treatments specifically designed to prevent POF.
Introduction

Advances in chemotherapy treatments are leading to ever-increasing survival rates among cancer patients. For many childhood cancers, the 5-year survival rate is now >70% (Thomson et al., 2002), with survival rates in adult cancers also increasing. The most common malignancy in adult women is breast cancer, affecting one in nine women, with an estimated 25% of these women premenopausal at diagnosis (Stearns et al., 2006): the 5-year survival rate for women treated for breast cancer in the UK is now >80% (ONS, 2010). Given this success, clinical concern in good-prognosis malignancies can now also focus on the long-term adverse effects of chemotherapy. The main problems associated with chemotherapy in female survivors include early menopause and an increased infertility rate in women who maintain ovarian activity after chemotherapy (Letourneau et al., 2012a). Early onset of the menopause can result in reduced quality of life (Letourneau et al., 2012b), and also has associated risks including osteoporosis (Bruning et al., 1990), cardiovascular disease (jeanes et al., 2007) and psychosocial problems, such as depression (Carter et al., 2005).

Various terms are employed in the literature to cover the early onset of menopause caused by cessation of ovarian function. Here, we use the term premature ovarian failure (POF), which we define as amenorrhea due to the premature depletion of functional ovarian follicles, in women <40 years (Goswami and Conway, 2005). The alternative term of primary ovarian insufficiency is sometimes employed to encompass the additional observation that apparent ovarian failure (i.e. amenorrhea with elevated FSH concentrations) can in some cases be temporary or intermittent, not precluding future menses or even pregnancy (Welt, 2008; Cooper et al., 2011). The terms acute ovarian failure and chemotherapy-related amenorrhea are also used in the literature but often with less clear definitions and have therefore been avoided in our review.

Whilst an end-point of POF following chemotherapy has been well established, the precise mechanism by which this occurs is less clear, yet without this knowledge it may be difficult to design effective treatment to protect against POF. The aim of this review is to discuss current evidence for the direct action of chemotherapy drugs on the ovary and to examine pathways by which follicle loss may occur.

Methods

For this review, journal databases, primarily PubMed but also Google Scholar, were searched using keywords including chemotherapy, POF, doxorubicin, cyclophosphamide, cisplatin, irinotecan, etoposide and ovarian function, for all English language articles through to 2011. Reference lists of key papers were examined for relevant articles.

Results

Chemotherapy and POF

The risk of developing POF following chemotherapy is dependent on various factors. Some chemotherapy regimens are considered more gonadotoxic than others, with particularly strong evidence that alkylating agents (e.g. cyclophosphamide) are highly ovotoxic. Meirow (2000) showed that alkylating agents caused ovarian failure in 42% of women treated, whilst those treated with platinum agents or plant alkaloids had no significant increased risk of POF. Byrne et al. (1992) found that women under the age of 20 years treated with alkylating agent chemotherapy were nine times more likely to develop early menopause than control patients. It has been estimated that 60–80% of women who are treated with cyclophosphamide, methotrexate and 5-fluorouracil will develop POF (Bines et al., 1996; Lower et al., 1999). Not surprisingly, dosage of the treatment used is also important and several studies have shown early menopause to occur in a dose-dependent fashion (Chiarelli et al., 1999). In mice treated with cyclophosphamide, an increase in primordial follicle loss was seen with increasing drug dose (Meirow et al., 1999).

In addition, age of the patient at treatment is key, as older women have a much higher reported incidence of acute POF, occurring during or immediately following treatment (Petrek et al., 2006; Letourneau et al., 2012a). Whilst chemotherapy damage to follicles can occur at all ages, the age-related difference is likely to be a result of older women having a smaller primordial follicle reserve at the start of treatment compared with young women (Meirow and Nugent, 2001), so that the loss from that already reduced follicle pool is more likely to induce POF by the end of treatment (Meirow et al., 2010).

Clinically, administration of chemotherapy can have two distinct effects on ovarian function; the first is immediate, the second longer term. The immediate effect, occurring during treatment, is a temporary one that induces amenorrhea and results from loss of the growing follicle population. However, provided that sufficient primordial follicles remain in the resting pool upon the cessation of treatment, the population of growing follicles will then be replenished, and menses resume. In contrast, later ovarian failure is a result of loss of the primordial follicle pool, and results in POF. Where there is only partial loss of primordial follicles, this longer term effect may not manifest itself until years or even decades after treatment, when the patient then undergoes premature menopause. Where the reduction in the primordial follicle pool is near complete, the effect is acute, and the patient undergoes POF shortly after treatment. This ‘two-hit’ effect is clearly demonstrated by the prospective study of Petrek et al. (2006) who found that in women treated with doxorubicin and cyclophosphamide, 84% became amenorrhoeic by the end of treatment, but with a steady recovery during the following 9-month period, such that almost half of the patients were menstruating by the end of the study. Importantly though, resumption of menstrual cycles can be short lived before permanent amenorrhoea occurs (Sklar, 2005), while Partridge et al. (2007) found that temporary amenorrhoea at the time of treatment is an indicator of early menopause.

Age has been a commonly used surrogate for the ovarian reserve, but attention is now turning to hormonal indicators of ovarian reserve, including anti-Müllerian hormone (AMH). AMH is produced by the granulosa cells of growing follicles from the primary to the antral stage (Weenen et al., 2004; La Marca et al., 2009) and serum levels of this hormone can be used to reflect the number of small...
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Growing follicles present in the ovary, thus inferring the number of primordial follicles present (Kelsey et al., 2012; Nelson et al., 2011). AMH falls rapidly during chemotherapy, and to a greater extent than either oestradiol or inhibin B (Anderson et al., 2006; Decanter et al., 2010; Rosendahl et al., 2010). AMH concentrations before treatment have been investigated as a predictor of ongoing ovarian function after chemotherapy for patients with breast cancer (Anderson et al., 2008; Anderson and Cameron, 2011): in the multivariate analysis AMH remained a significant predictor while age did not (Anderson and Cameron, 2011), indicating that AMH is a sufficiently accurate index of the ovarian reserve and of greater power than age. Recently, we have shown that AMH is detectable in girls of all ages, and falls rapidly during cancer treatment in both pre-pubertal and pubertal girls; both the fall during treatment and recovery thereafter varies with risk of gonadotoxicity (Brougham et al., in press). While these result needs to be confirmed in other populations, they suggests that AMH may be of value in individualizing advice on the likely effects of chemotherapy on the ovary, as well as assessing gonadal toxicity both in individual women and in research contexts.

Importantly, missing from almost all publications are the long-term data needed to establish the extent to which women resuming menstrual cycles are likely to undergo POF later (Bines et al., 1996; Partridge et al., 2007; Letourneau et al., 2012a). In the case of younger women, such studies would need to follow them for decades, which to date has only been carried out in two studies of childhood cancer survivors. In one of these studies, the reproductive outcomes for 830 cancer survivors were analysed to determine the risk of developing POF following either irradiation or chemotherapy treatment (Chiarelli et al., 1999). The long-term follow-up in this study was between 5 and 30 years and showed no overall increase in risk of premature menopause in patients treated with chemotherapy. However, the patients who were treated only with chemotherapy were the patients who had the fewest years of follow-up (45% had <10 years of follow-up and were therefore aged <30 years when the study was conducted) and so the extent of the ovarian insult resulting from the chemotherapy treatment may not have become evident yet. The Childhood Cancer Survivor Study also followed a large number of patients diagnosed between 1970 and 1986, and found that the incidence of POF was significantly higher in childhood cancer survivors than in sibling controls and rose progressively with time throughout the duration of follow-up (Sklar et al., 2006).

Potential targets in the ovary

By the time of birth, the human ovary has a finite supply of oocytes, derived from proliferation and maturation of primordial germ cells. Primordial follicles form from ~17 weeks of gestation, each primordial follicle consisting of an immature oocyte in meiotic arrest surrounded by a few flattened somatic (granulosa) cells. These follicles constitute the resting pool of germ cells, and some will remain at this stage for the full duration of the female reproductive lifespan. Primordial follicles are continuously being recruited out of the resting pool and activated to grow, with recruitment greater when the number of growing follicles is also high, i.e. at younger ages (Fig. 1A). The loss of primordial follicles from the resting pool is continuous until none remain: the presence of less than a thousand is associated with the menopause (Wallace and Kelsey, 2010). Although recently challenged (Johnson et al., 2004), the vast majority of evidence points to the number of follicles held within the primordial follicle pool being finite. Once activated, both the oocyte and surrounding somatic cells undergo sequential stages of growth and development, characterized by somatic cell proliferation and oocyte growth, without resumption of meiosis until this is reactivated by the ovulatory LH surge. Of the follicles recruited from the resting pool, few make it through to the pre-ovulatory stage: instead the vast majority of follicles become atretic and die at some point during antral development.

Chemotherapy drugs act by a range of mechanisms but with particular cytotoxicity to dividing cells. This has clear relevance to an examination of which aspects of ovarian function are likely to be most sensitive to these agents. Oocytes and somatic cells will have different vulnerabilities to cytotoxic agents, with the oocytes non-dividing, although rapidly growing in developing follicles, while the somatic cells of such follicles have a high degree of proliferation. Given this, different ovarian cell types may have differential sensitivities to the various classes of drug.

Primordial follicles: direct or indirect target?

The reproductive lifespan of a woman depends upon the number of primordial follicles remaining in her ovaries, with POF resulting from the loss of that primordial pool. While chemotherapy treatment may induce POF by directly killing primordial follicles, this need not be the case. Instead, reduction of the primordial pool can arise indirectly, via the loss of activated, growing follicles (Meirow et al., 2010). The commitment of activated follicles to either ovulation or atresia means that their loss will not directly contribute to determination of the reproductive lifespan. Growing follicles, however, produce factors, such as AMH (Durlinger et al., 1999), which regulate the rate of follicle activation: thus acute loss of the growing follicle population is thought to result in increased recruitment of primordial follicles into the growing pool. Repeated cycles of chemotherapy treatment may, therefore, have marked effects on the size of the remaining primordial pool by indirect as well as direct means (Fig. 1).

Which follicle classes are at risk?

At any one time, there are follicles within the ovary at various stages of maturation. It is possible that specific stages are more susceptible to chemotherapy-induced damage than others. There are relatively little data on this to date (Table I), with the focus of most experimental studies examining the primordial follicle population. Öktem and Oktay (2007b) found that ovarian biopsies from patients treated with chemotherapy had significantly lower primordial follicle counts than untreated controls. A study by Yucebilgin et al. (2004) also found that primordial follicle counts decreased following the administration of paclitaxel or cisplatin to rats in vivo. Cyclophosphamide and its metabolites caused a decrease in healthy primordial follicles and small primary follicles in both mouse and rat ovaries (Desmeules and Devine, 2006; Petrillo et al., 2011). However, as discussed above, it is possible that the loss of primordial follicles is, in part at least, a secondary effect (Fig. 1B). More mature follicle classes are also vulnerable to damage by chemotherapeutic agents. Pre-antral follicles have been shown to be susceptible to chemotherapy with a deterioration in follicle quality following treatment both in vitro and in vivo (Raz et al., 2002; Abir et al., 2008). The rapid falls in serum concentrations of inhibin B and AMH during chemotherapy (Anderson et al., 2006;
Decanter et al., 2010; Rosendahl et al., 2010) also indicate loss of follicles at the pre-antral and early antral stages. Injection of mice with doxorubicin leads to a significant reduction in the population of secondary follicles compared with controls (Ben-Aharon et al., 2010).

As described above, the loss of the production of inhibitory substances by growing follicles could lead to accelerated depletion of the primordial reserve, with more primordial follicles undergoing growth initiation to replace damaged growing follicles.

Germ or somatic cells?
There are numerous cell types in the ovary which may be potential targets for damage by chemotherapeutic agents. It is often assumed that chemotherapy acts directly on the oocyte within immature follicles, initiating cell death and therefore germ cell loss. There is, however, limited evidence available for this (Table I), and it need not always, or indeed even often, be the case. Following follicle formation, oocytes are held within immature follicles in meiotic arrest and as chemotherapeutic agents are designed to act upon dividing cells, it is perhaps more likely that the common primary targets of chemotherapy drugs are the mitotically-active somatic cells of the ovary. Granulosa cells surround the oocyte and proliferate during follicle maturation. Given the bidirectional communication between the oocyte and the granulosa cells, with each regulating the growth and maturation of the other (Matzuk et al., 2002), damage to granulosa cells will result in indirect damage to the oocyte, leading to germ cell loss (Fig. 1Bi). Any damage to granulosa cells which compromises their ability to proliferate could also have a latent effect on primordial follicles, becoming apparent only when follicle growth is initiated; however, this is perhaps a less likely mechanism of follicle damage given that primordial follicles are in an inactive, resting state. It is also important to consider the possibility of oocyte damage becoming apparent only later. Such delayed cellular dysfunction does occur in chronic onset chemotherapy-induced cardiomyopathy (Shakir and Rasul, 2009). With the oocyte, the consequences of latent damage could, of course, only become apparent at the resumption of meiosis or following fertilization. Reassuringly, there is increasing evidence that potentially mutagenic chemotherapy doses to the gonads are not associated with an increased incidence of genetic defects in children of cancer survivors (Winther et al., 2011).

Doxorubicin has been demonstrated to cause apoptosis in mature ovulated murine oocytes (Perez et al., 1997; Jurisicova et al., 2006) but any direct action on immature oocytes within follicles is less clear. Nor
is it evident whether doxorubicin is more toxic to oocytes or granulosa cells when whole follicles are exposed to the drug. In a recent study, doxorubicin caused DNA damage and apoptotic cell death in both oocytes and granulosa cells of human primordial follicles in vitro (Soleimani et al., 2011). A model in which human fetal ovary pieces were xenografted into severe combined immunodeficient mice, with the mice then treated with cyclophosphamide, found that the oocytes showed evidence of apoptosis before the pregranulosa cells,
potentially indicating that the oocyte is more susceptible to damage than the granulosa cells (Oktem and Oktay, 2007a). However, this model used fetal ovarian tissue so that the only follicle class present was primordial and there may still have been mitotically active germ cells present. Human ovarian slices treated in vivo with cyclophosphamide show an increase in damaged granulosa cell nuclei and follicular basement membranes following treatment (Raz et al., 2002). A follow-on in vivo study of ovarian biopsies from women following chemotherapy found similar results (Abir et al., 2008). Ben-Aharon et al. (2010) examined ovaries from mice treated with doxorubicin and identified terminal deoxynucleotidyltransferase-mediated dUTP nick-end labelling (TUNEL)-positive staining (indicating DNA damage) first in the granulosa cells, with TUNEL-positive oocytes appearing over time.

There is also the possibility that ovarian stromal tissue is susceptible to chemotherapy, which could in turn adversely affect follicle health. An ultrastructural study of ovarian biopsies from 10 girls who had undergone treatment for childhood leukaemia found moderate to severe signs of stromal fibrosis and capillary changes (Marcello et al., 2002; Tingen et al., 2009). Apoptosis involves several characteristic morphological changes to a cell, including nuclear condensation, cell shrinkage and membrane blebbing as well as the fragmentation of DNA. Necrosis, which some studies have indicated as the mechanism of cell death implicating in these instances. One such signalling cascade is the ceramide pathway: suppression of oocyte apoptosis occurs when one of the components (acid ceramidase) is disrupted, or upon the addition of sphingosine-1-phosphate (S1P; Morita et al., 2000). How therapeutically useful this interference could be is in the future remains to be seen.

Given the array of mechanisms of action of different chemotherapeutic agents, it is likely that the various drugs each induce specific pathways so that the mechanism of cell death in the ovary following chemotherapy treatment may vary quite widely depending on the drug administered (Fig. 2). Below, we examine in detail the action of several of the drug types most commonly used to treat cancers in premenopausal women.

Doxorubicin (anthracycline)
Doxorubicin is an anthracycline which is often used to treat lymphomas, leukaemia, breast cancer and sarcomas. Its precise mechanism of action is unclear, though it is thought to intercalate with DNA and prevent its replication and transcription (Jurisicova et al., 2006), partly through inhibition of topoisoerase II. There is some evidence in cardiomyocytes that doxorubicin has an affinity for cardiolipin (Pointon et al., 2010), a ubiquitously expressed protein on the inner membrane of mitochondria. Doxorubicin may interfere with the electron transport chain through this interaction, leading to a release of cytochrome c into the cytosol. This in turn activates the caspase family of proteins and so causes apoptosis and cell death. In the cell nucleus, there is evidence that doxorubicin up-regulates p53 protein expression, a DNA repair protein which initiates apoptosis in the presence of high levels of DNA damage (Smith and Suresh Kumar, 2010). Doxorubicin can also cause DNA double-strand breaks leading to activation of ataxia telangiectasia mutated protein kinase (ATM), a DNA-repair protein which may initiate apoptotic cell death in the presence of high levels of DNA damage (Soleimani et al., 2011). In cardiomyocytes, doxorubicin induces an increase in reactive oxygen species (Tan et al., 2010), which can result in endoplasmic reticulum stress and thereby initiation of apoptosis via caspase 12.

Although doxorubicin was once considered to be only weakly ootoxic, recent evidence indicates that this may not be the case (Letourneau et al., 2012a). Doxorubicin could affect the ovary by any, or indeed all, of the above mechanisms but these should all primarily affect mitotically and metabolically active cells, both characteristics of granulosa cells rather than oocytes (Downs and Utecht, 1999). It can be hypothesized, therefore, that granulosa cells are likely to be preferentially targeted by doxorubicin.

Cyclophosphamide (alkylating agent)
Cyclophosphamide results in intra-strand and inter-strand cross-linking of DNA, which interferes with cell division. Effects of alkylating agents, such as cyclophosphamide, have been studied in the granulosa cells of rat ovaries. As with doxorubicin, treatment with cyclophosphamide also has a mitochondrial effect, as it induces a reduction in mitochondrial transmembrane potential and an accumulation of cytochrome c in the cytosol, again leading to activation of the caspase family and apoptosis (Zhao et al., 2010). Cyclophosphamide has also been shown to induce a large up-regulation of the proapoptotic Bax protein. Bax is largely found in the cell cytosol, however, during cell death it inserts into the outer membrane of the mitochondria. This could be how cyclophosphamide causes the reduction of mitochondria transmembrane potential, leading to activation of the apoptotic cascade. Recently, cyclophosphamide metabolites were found to induce the expression of H2AX (a marker of double-strand DNA breaks) predominantly in oocytes but also in the granulosa cells of mouse ovaries cultured in vitro (Petrillo et al., 2011).

Owing to the facts that cyclophosphamide has a direct effect on cell division through DNA cross-linking and also appears to have an effect on the mitochondria to induce an apoptotic cascade, it can be hypothesised that this drug will, as with doxorubicin, preferentially target the more metabolically active granulosa cells.

Potential mechanisms of cell death
The ovary is normally the site of a large amount of cell death, with the majority of follicles within the ovary undergoing cell death through atresia. The exact cellular mechanism which underlies follicle atresia is currently unclear, with some studies suggesting apoptosis (Tilly, 1996; Durlinger et al., 2000) and others disputing this (de Bruin et al., 2002; Tingen et al., 2009). Apoptosis involves several characteristic morphological changes to a cell including nuclear condensation, cell shrinkage and membrane blebbing as well as the fragmentation of DNA. Necrosis, which some studies have indicated as the process involved in atresia, involves characteristic changes including increased membrane permeability and nuclear degeneration (de Bruin et al., 2002). Another mechanism of cell death implicated in atresia is autophagy, which involves large portions of the cell cytoplasm being enveloped in double-membrane structures and then degraded (Choi et al., 2010). One of the main problems in trying to dissect these molecular pathways is the considerable cross-talk between different signalling cascades.

Whether the cell death mechanisms which are instigated by chemotherapeutic agents are similar to the process of atresia remains to be seen. There is some evidence that apoptosis is involved in oocyte loss following chemotherapy (Perez et al., 1997), and specific apoptotic signalling pathways have been investigated in these instances. One such signalling cascade is the ceramide pathway: suppression of oocyte apoptosis occurs when one of the components (acid sphingomyelinase) is disrupted, or upon the addition of sphingosine-1-phosphate (S1P; Morita et al., 2000). How therapeutically useful this interference could be is in the future remains to be seen.
Figure 2 Potential mechanisms by which chemotherapy agents may cause cell death. Chemotherapeutic drugs may act at the level of the nucleus to cause DNA damage or interfere with DNA transcription and replication. They can also act on the mitochondria to induce the release of cytochrome c into the cytoplasm. These pathways all interconnect and lead to cell death, often through the caspase family of proteins, which are associated with apoptosis. (A) General pathway of action and (B) pathway of action for individual drugs.
Cisplatin (platinum-containing compound)

Cisplatin causes DNA damage by the formation of inter-strand and intra-strand DNA adducts. These adducts interfere with cellular transcription and replication. If they are not efficiently dealt with by the cell then there is activation of signal transduction pathways culminating in the initiation of apoptosis (Siddik, 2003). Recent evidence has implicated a non-receptor tyrosine kinase called Abl in cisplatin-induced cell death in immature oocytes (Gonfloni, 2010). Abl is thought to act as a sensor of DNA damage and when activated has a downstream effect on TAp63-α, a homologue of p53 which is expressed in the oocyte. Cisplatin administration to neonatal mouse ovaries causes an accumulation of Abl and TAp63-α in the oocyte, leading to oocyte death (Gonfloni et al., 2009). When Abl is inhibited pharmacologically using imatinib (a blocker of Abl activity), oocyte death in response to cisplatin is significantly reduced. TAp63 has also been shown to be essential for the induction of oocyte death from DNA damage induced by ionizing radiation, indicating an important role of TAp63 in protecting the germline from DNA damage (Suh et al., 2006). As with doxorubicin and cyclophosphamide, however, cisplatin can also activate an intrinsic mitochondrial pathway leading to a release of cytochrome c into the cytosol resulting in subsequent activation of the caspase pathway and thus apoptosis. Cisplatin also causes endoplasmic reticulum stress (Mandic et al., 2003), with subsequent activation of caspase 12 and apoptosis. Nonetheless, cisplatin’s primary action appears to be induction of DNA damage.

Studies using ionizing radiation to induce DNA damage show that oocytes are more susceptible than somatic cells, dying earlier following a low level of exposure (Adriaens et al., 2009). This makes sense from an evolutionary perspective, as it will likely act as a quality control mechanism to protect the germline. It can be hypothesized, therefore, that unlike doxorubicin and cyclophosphamide, cisplatin may preferentially target the oocyte, or through its effects on mitochondria, both germ and somatic cells.

Irinotecan and etoposide (topoisomerase inhibitors)

Topoisomerase enzymes (I and II) bind to DNA and allow it to unwind during DNA replication. There are several chemotherapeutic drugs which act as inhibitors of these enzymes, such as irinotecan (whose active metabolite SN38 inhibits topoisomerase I) and etoposide (which inhibits topoisomerase II). As these drugs act by preventing DNA replication, their primary action is likely to be on the proliferating granulosa cells. As well as interfering with DNA replication, irinotecan has been shown to upregulate apoptosis by inducing the expression of Fas Ligand (FasL) in granulosa cells of large follicles (Utsumoniyi et al., 2008). The Fas/FasL pathway has been implicated in granulosa cell apoptosis during the process of follicle atresia and has also been linked to p53-mediated apoptosis (Kim et al., 1999). In hepatocellular carcinoma cells, irinotecan administration leads to an upregulation of p53 and an increase in the expression of Bax and caspase 9 (Takeba et al., 2007). Irinotecan may therefore, like doxorubicin and cyclophosphamide, preferentially target granulosa cells to induce follicle loss.

Whilst etoposide can interfere with DNA replication as discussed above, it also can induce double-strand DNA breaks in cells which triggers apoptosis through the activation of ATM and the subsequent phosphorylation of histone H2AX (Tanaka et al., 2007). Double-strand DNA breaks are the commonest form of DNA damage induced by ionizing radiation. Etoposide may act in a similar way and, as discussed above for cisplatin, preferentially target the oocyte through this mechanism.

Protecting the ovary from damage

Owing to the increasing number of women who are affected by POF as a result of chemotherapy treatment, preservation of fertility is a question of growing importance. The longest established option currently available to women for fertility preservation is embryo cryopreservation, with other possibilities, such as cryopreservation of oocytes and ovarian tissue, emerging more recently. These techniques, which have been extensively reviewed elsewhere (e.g. Anderson and Wallace, 2011; Donnez and Dolmans, 2011), essentially involve the removal of gametes from the woman before gonadotoxic treatment, with subsequent cryopreservation or vitrification. As such, they are primarily fertility-preservation treatments, unlikely to maintain ovarian function for an extended period. The ideal solution would be if the ovary could be protected from the toxic effects of the chemotherapeutic drugs, thus also alleviating the non-fertility symptoms associated with premature menopause. These possibilities are examined further below.

GnRH agonists

The administration of GnRH agonists has long been investigated as a potential strategy to protect the ovary from the effects of chemotherapy (Waxman et al., 1987). GnRH agonists cause suppression of the hypothalamic–pituitary–gonadal axis and return the ovary to a more quiescent state. It has been proposed that an ovary in this state would be less vulnerable to the damaging effects of chemotherapeutic agents. This hypothesis has been based on observations that prepubertal girls appear less susceptible to chemotherapy-induced ovarian damage than older patients. It is more likely, though, that younger patients are less affected as they have a larger follicle reserve. This means that any depletion in the ovarian reserve after chemotherapy will not manifest until many years later, rather than the prepubertal ovarian environment being in itself protective. The mechanisms by which GnRH agonists may be able to protect the ovary are unclear. One hypothesis is that GnRH restricts the blood flow to the ovary, allowing less of the chemotherapeutic agent access to the ovarian reserve, with a rat model system having shown that ovarian vascular permeability and density decreased following GnRH agonist treatment (Kitajima et al., 2006). Another potential mechanism is that GnRH agonists act directly on the ovary. This seems unlikely to be responsible for a long-term protective effect as GnRH receptors are only present in pre-ovulatory follicles and the corpus luteum (Choi et al., 2006). GnRH agonists have a protective effect against doxorubicin acting directly on granulosa cells in vitro (Imai et al., 2007) but these granulosa cells were taken from mature follicles. Whilst it has been shown that GnRH agonists cause an inhibition of follicular recruitment (Ataya et al., 1989), it is not clear if there is a direct effect on smaller follicle classes. Several clinical studies have investigated the efficacy of GnRH agonists in protecting the ovarian reserve but most studies had small patient numbers and were often not randomized (Beck-Fruchter et al., 2008; Blumenfeld and von Wolff, 2008). The recently published PROMISE-GIM6 study, a large
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References

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Authors’ roles

All authors contributed significantly to the manuscript, its preparation and to final approval of the version to be published. S.M., R.A.A. and N.S. were primarily responsible for the search, selection and assessment of articles, and for writing and revising the manuscript. C.G. and W.H.W. contributed to the concept, data interpretation and manuscript revision.

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Concluding remarks

Chemotherapeutic agents often have a negative impact on the reproductive potential of young women but the exact mechanisms through which this occurs are uncertain. Although POF results from the loss of primordial follicles, this is not necessarily a direct effect of the chemotherapeutic agents but could be due to primordial follicles undergoing growth initiation to replace damaged developing follicles. Evidence to date suggests that chemotherapy drugs can target the oocyte directly, or can induce oocyte death indirectly via damage to somatic cells. It is likely that drugs from different classes act through a range of mechanisms, possibly with different target cells; any preventative treatment will have to take this into account. Owing to the current limitations of options to both preserve fertility and delay menopause, more research into potential cellular mechanisms of drug action could allow the generation of treatments specifically designed to prevent follicle loss.

Pharmacological intervention

The ideal way of protecting the ovary from chemotherapy treatments would be to directly and specifically block any damage caused. Since evidence to date suggests that different drugs have different actions on the ovary, any protective treatments may need to be tailored specifically to the drug regimen used. As discussed above, the administration of the tyrosine kinase inhibitor imatinib in conjunction with cisplatin led to a reduction in the loss of primordial follicles in neonatal mouse ovaries when compared with cisplatin treatment alone (Gonfloni et al., 2009).Whilst this is a promising candidate for a fertility-protecting treatment, more research is needed to determine its usefulness. Indeed, one study has indicated that imatinib could itself compromise ovarian function during treatment (Zamah et al., 2011). Also, if imatinib is interfering with the cell death mechanisms induced by cisplatin, care would be required to ensure that it did not reduce the efficacy of cisplatin as an anti-tumour drug.

Another possible pharmacological target is the ceramide pathway. Ceramide is a sphingosine-based lipid signalling molecule which, when generated in cells, can trigger apoptosis (Morita and Tilly, 2000). Studies which have manipulated the ceramide pathway by adding S1P, a downstream anti-apoptotic metabolite of ceramide, have reported that mature oocytes are protected from doxorubicin-induced cell death in vitro (Perez et al., 1997). In vivo, rodents which are treated with S1P have a decrease in the primordial follicle death following chemotherapy (Hancke et al., 2007) with no discernable genomic damage in the offspring of irradiated rodents given such ovarian protection (Paris et al., 2002). An examination of an S1P analogue against the effect of radiotherapy in primates indicated a protective effect on ovarian function (Zelinski et al., 2011): whether this protective effect can be replicated in humans, or following chemotherapy treatment, is yet to be seen but these data in a closely related species provide substantial grounds for optimism that protective effects in women may be feasible.
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