Testicular function following chemotherapy

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Testicular dysfunction is a common long-term sequela of cytotoxic chemotherapy used in the treatment of many malignancies. The degree to which testicular function is affected is dose- and agent-dependent. The impact on germinal epithelial function of standard multi-agent regimens used in the treatment of lymphomas has been widely studied. Procarbazine-containing regimens result in azoospermia in the vast majority of patients, but much lesser degrees of long-term gonadotoxicity are apparent with the newer forms of chemotherapy. High-dose chemotherapy used as preparation before bone marrow transplant is also associated with irreversible germinal epithelial failure in the majority of men. Treatment of testicular cancer with cisplatin and carboplatin regimens leads to temporary azoospermia in most men, with a recovery to normospermia in 80% by 5 years. There is also evidence of mild Leydig cell impairment in a proportion of men treated with cytotoxic agents, although the clinical significance of this is not clear. Several methods of preserving testicular function during potentially sterilizing treatment have been considered. At present, sperm banking remains the only proven method, although hormonal manipulation to enhance recovery of spermatogenesis and cryopreservation of testicular germ cells are possibilities for the future.

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Introduction

Cytotoxic chemotherapy has improved the survival rates in many conditions, particularly haematological and testicular malignancies. Treatment is, however, associated with significant morbidity in many patients, and testicular dysfunction is amongst the most common long-term side effects of therapy in men. Germinal epithelial damage resulting in oligo- or azoospermia has long been a recognized consequence of treatment with chemotherapeutic agents, and there is also evidence of Leydig cell impairment following treatment. Testicular damage is drug-specific and dose-related (da Cunha et al., 1984; Watson et al., 1985; Meistrich et al., 1989; Pryzant et al., 1993). The chance of recovery of spermatogenesis following cytotoxic insult, and also the extent and speed of recovery, are related to the agent used and the dose received.

Impact of cytotoxic chemotherapy on testicular function in adult men

Testicular damage caused by cytotoxic drugs was first described in humans in 1948, when azoospermia was reported in 27 of 30 men following treatment with nitrogen mustard (Spitz, 1948). Many other drugs, particularly alkylating agents, have subsequently been shown to be gonadotoxic, and the agents most commonly implicated are listed in Table I. The germinal epithelium is far more sensitive to the effects of cytotoxic drugs than the Leydig cells, and, whilst complete azoospermia is not uncommon following therapy, evidence of Leydig cell impairment is usually limited to raised LH concentrations with normal or low normal testosterone concentrations (Howell et al., 1999). There are some studies, however, which have demonstrated a reduction in testosterone concentrations after treatment with gonadotoxic agents (Whitehead et al., 1982; Chatterjee et al., 1994), and there is some evidence to suggest that the Leydig cell impairment following chemotherapy may be clinically important (Holmes et al., 1994; Howell et al., 2000a,b). Most clinical studies have focused on either cyclophosphamide given alone for immunologically mediated disease, or combination chemotherapy used in the treatment of haematological malignancies and testicular cancer (Table II).

An analysis of 30 studies was published which examined gonadal function after various chemotherapy regimens, and which included a total of 116 males who had been treated with...
cyclophosphamide alone (Rivkees and Crawford, 1988). Gonadal function and/or histology was assessed by a number of different methods; semen analysis, basal LH and FSH concentrations and testicular biopsy being the most commonly used. Some 52 of the 116 patients (45%) had evidence of testicular dysfunction following treatment. The incidence of gonadal dysfunction correlated with the total dose of cyclophosphamide, occurring in over 80% of post-pubertal patients who received more than 300 mg/kg.

**Lymphomas**

The impact on testicular function of chemotherapy used in the treatment of lymphomas, especially Hodgkin’s disease (HD), has been widely reported. Several studies have reported azoospermia with raised FSH concentrations in over 90% of men following cyclical chemotherapy with MVPP (mustine, vinblastine, procarbazine and prednisolone) (Chapman et al., 1979; Whitehead et al., 1982). Testosterone concentrations are usually within the normal range, but LH concentrations are often near the upper limit of normal or frankly raised, suggesting a degree of Leydig cell impairment (Howell et al., 1999).

In an attempt to reduce the gonadotoxic effect of MVPP by halving the dose of alkylating drug and reducing the procarbazine dose, a hybrid combination of chlorambucil, vinblastine, prednisolone, procarbazine, doxorubicin, vincristine and etoposide (ChlVPP/EVA) has been used. However, in a direct comparison with MVPP, hybrid chemotherapy was found to have the same effect on gonadal function (Clark et al., 1995). The combination of MVPP with courses of ABVD (adriamycin, bleomycin, vinblastine and dacarbazine; BEAM = BCNU, etoposide, cytosine arabinoside and melphalan; CBV = cyclophosphamide, BCNU and etoposide; ChlVPP/EVA = chlorambucil, vinblastine, prednisolone, procarbazine, doxorubicin, vincristine and etoposide; CHOP = cyclophosphamide, vincristine and prednisolone; COPP = cyclophosphamide, vincristine, procarbazine and prednisolone; MACOP-B = mustine in place of vinblastine; MOPP = mustine, vinblastine, procarbazine and prednisolone; MVPP = mustine, vinblastine, procarbazine and prednisolone; VACOP-B = vinblastine, doxorubicin, prednisolone, vincristine, cyclophosphamide and bleomycin; VAPEC-B = vincristine, doxorubicin, prednisolone, etoposide, cyclophosphamide and bleomycin; VEEP = vincristine, etoposide, epirubicin and prednisolone).

In one study (Viviani et al., 1985), a total of 53 men was treated with combination chemotherapy for HD. Of 29 men treated with MOPP (similar to MVPP but with vinblastine replaced by vincristine), 28 were azoospermic, a median time of 6 months after the completion of therapy. Twenty-one of these men were re-tested 18 to 58 months after the initial analysis, and in only three was any recovery of spermatogenesis seen. The impact of ABVD was considerably less however, with a normal sperm count in 11 of 24 patients and oligospermia in a further five. Furthermore, full recovery of spermatogenesis occurred within 18 months of the first evaluation in all 13 men in whom the sperm count was repeated.

Other chemotherapy regimens utilized for the treatment of lymphomas have also been investigated. The use of COPP (cyclophosphamide, vincristine, procarbazine and prednisolone), which includes the gonadotoxic agent cyclophosphamide in addition to procarbazine, is associated with marked gonadal dysfunction. Azoospermia was found in all 92 patients following treatment with six or more cycles of COPP, along with significant rises in gonadotrophin concentrations compared with pretreatment values (Charak et al., 1990). The median follow-up in this study was six years, with 17% of patients treated more than 10 years previously, suggesting that germinal epithelial failure is likely to be permanent. The impact of COPP chemotherapy on Leydig cell function was investigated further (Bramswig et al., 1990). These authors found raised basal LH concentrations in 18 of 75 (24%) patients and, in addition, raised gonadotrophin-releasing hormone (GnRH)-stimulated LH concentrations in 65 of 75 (88%), suggesting that subtle Leydig cell impairment is common following this treatment.
Chemotherapy regimens used for the treatment of non-Hodgkin’s lymphoma (NHL) are generally less gonadotoxic than those used for HD. In one study of 71 patients treated with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone)-based chemotherapy, all men were rendered azoospermic during treatment, but by 5 years 67% had recovered to normospermic levels, with a further 5% oligospermic (Pryzant et al., 1993). The reduced incidence of permanent infertility in men treated for NHL compared with HD patients is probably related to the absence of procarbazine in the standard regimens used for NHL (Bokemeyer et al., 1994), although the reduction in the dose of alkylating agents may also be important. The absence of procarbazine and alkylating drugs is also the likely explanation for the reduced toxicity of ABVD that has been reported (Viviani et al., 1985). Other regimens not containing procarbazine, which have been used to treat NHL and HD, have also been shown to be less gonadotoxic. VAPEC-B (vincristine, doxorubicin, prednisolone, etoposide, cyclophosphamide and bleomycin; Radford et al., 1994), VACOP-B (vinblastine, doxorubicin, prednisolone, vincristine, cyclophosphamide and bleomycin; Muller and Stahel, 1993), MACOP-B (mustine in place of vinblastine; Muller and Stahel, 1993), and VEEP (vincristine, etoposide, epirubicin and prednisolone; Hill et al., 1995) have all been associated with normal post-treatment fertility in the vast majority of men. Thus it is clear that, in common with cyclophosphamide, the dose of procarbazine is important in determining the long-term effects on gonadal function (Whitehead et al., 1982; da Cunha et al., 1984; Bramswig et al., 1990; Bokemeyer et al., 1994).

**Bone marrow transplant**

The success of bone marrow transplant, or peripheral stem cell rescue following marrow ablation, has improved over the past decade, and this form of therapy is increasingly being used in a number of malignancies. Only limited data are available on the impact of high-dose chemotherapy on testicular function. Most of the studies have included patients who also received total body irradiation (TBI), and as this is also gonadotoxic it is difficult to assess the gonadal damage that is specifically a consequence of the chemotherapy. A cohort of 68 men was treated with one of four different high-dose chemotherapy regimens: cyclophosphamide, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and etoposide (CBV) \( n = 20 \); busulphan and cyclophosphamide \( n = 23 \); cyclophosphamide and TBI \( n = 15 \); or high-dose melphalan \( n = 10 \) for HD, NHL, acute myeloblastic leukaemia (AML), acute lymphoblastic leukaemia (ALL) and myeloma (Howell et al., 1999). In addition to TBI in 21 patients, most had received prior chemotherapy, 19 with MVPP or ChlVPP/EVA hybrid (chlambucil, vinblastine, procarbazine, prednisolone, etoposide, vincristine and doxorubicin) which are known to result in gonadal damage in a high proportion of patients. At a mean of 7.5 years after treatment, 60 of 68 patients (88%) had germin al epithelial damage indicated by a raised FSH concentration, and in addition 33 patients (49%) had a LH concentration at or above the upper limit of normal, suggesting a degree of Leydig cell impairment. There were no significant differences in mean LH and FSH concentrations between the different treatment groups. The majority of men with normal FSH concentrations had received busulphan and cyclophosphamide (6 of 8), suggesting that this regimen may be less toxic to the germinal epithelium. However, none of these patients had received TBI or procarbazine-containing chemotherapy, whilst all men in the CBV group had received prior treatment with either MVPP or ChlVPP/EVA hybrid, and the majority of the men in the other two groups had received TBI. It is probable that these differences in additional therapy account, at least in part, for the apparent variation in gonadotoxicity between the groups.

Another group (Sanders et al., 1996) reported findings in 155 men treated with cyclophosphamide (200 mg/kg) or busulphan and cyclophosphamide (busulphan 16 mg/kg, cyclophosphamide 200 mg/kg) as preparation for bone marrow transplant (BMT). After an average of 2 to 3 years post-transplant, 67 of 109 who received cyclophosphamide (61%), but only eight of 46 (17%) patients treated with busulphan and cyclophosphamide, had recovery of testicular function defined by normal LH, FSH and testosterone concentrations with evidence of sperm production. The only prospective study to examine testicular function following high-dose treatment reported on 13 men who received either BEAM (BCNU, etoposide, cytosine arabinoside and melphanal) \( n = 11 \) or melphalan and single fraction TBI \( n = 2 \) (Chatterjee et al., 1994). All had previously received multi-agent chemotherapy, and four had abnormal semen parameters before transplantation. All patients were azoospermic 2–3 months post-transplantation, associated with raised FSH concentrations. LH concentrations increased and testosterone concentrations decreased after transplantation, indicating that Leydig cell impairment was apparent in addition to germ cell failure.

**Testicular cancer**

Other patients in whom the effects of chemotherapy on testicular function have been widely investigated are those with testicular cancer (Hansen et al., 1990; Palmieri et al., 1996; Lampe et al., 1997). In an attempt to delineate which abnormalities result from cytotoxic chemotherapy, several of these studies also examined pretreatment testicular function, or compared chemotherapy-treated patients with those who underwent orchidectomy alone. One group (Lampe et al., 1997) analysed data relating to 170 patients with testicular germ cell cancers, who underwent treatment with either cisplatin- or carboplatin-based chemotherapy. Forty patients (24%) were azoospermic before treatment, and a further 41 (24%) were oligospermic. At a median of 30 months after the completion of chemotherapy, only 64% of those who were normospermic before therapy remained normospermic, while 54 (32%) of the total cohort were azoospermic and 43 (25%) were oligospermic. The probability of recovery of a normal sperm count was found to be higher for those men with a normal pretreatment sperm count, those who received cisplatin- rather than carboplatin-based therapy, and in those treated with more than four cycles of chemotherapy. Recovery continued for more than 2 years, with the calculated chance of spermatogenesis at 2 years being 48% and at 5 years 80%. Several authors have compared testicular function in patients following chemotherapy with that of patients treated with orchidectomy alone (Hansen et al., 1990; Palmieri et al., 1996; Lampe et al., 1997). All have demonstrated greater testicular dysfunction in the cytotoxic-treated groups, with evidence of germinal epithelial damage indicated by raised FSH concentrations and/or reduced sperm counts. In addition, mild Leydig cell impairment, as indicated by raised LH concentrations in the presence of a normal testosterone
concentration, was found in 59 to 75% of men following chemotherapy, compared with 6 to 45% in those following orchidectomy alone.

Similar results have been demonstrated in patients treated with cisplatin-based chemotherapy for osteosarcoma (Meistrich et al., 1989; Siimes et al., 1990) and lung cancer (Aasebo et al., 1989). The majority of patients treated with cytotoxic chemotherapy for leukaemia, however, do not have persistent gonadal dysfunction. In one study (Wallace et al., 1991), long-term germinal epithelial dysfunction was found in only six out of 36 (17%) patients treated during childhood for ALL, although the period of follow-up in this study was considerable and the majority of patients had evidence of germinal epithelial damage on testicular biopsy immediately after chemotherapy, a median time of 10.7 years earlier.

Impact of age and pubertal status

It has been suggested that prepubertal boys are less susceptible to the effects of chemotherapy than adult men, but there is no convincing evidence to support this argument. Testicular function has been assessed in patients treated for HD during childhood with CHVPP (chlorambucil, vinblastine, procarbazine and prednisolone). Testicular dysfunction, as indicated by raised gonadotrophin concentrations, was found in a significant proportion of a cohort of 46 male patients treated with CHVPP (Mackie et al., 1996), with 89% and 24% having raised FSH and LH concentrations respectively. Cyclophosphamide alone (Sanders et al., 1988), and combined with busulphan (Michel et al., 1997), does not prevent normal progress through puberty, but germinal epithelial dysfunction is affected in some, indicated by azoospermia and/or raised FSH concentrations. Following high-dose cyclophosphamide in combination with TBI, testicular dysfunction was more common, with 17 of 25 patients having raised FSH concentrations and 10 of 25 having raised LH concentrations (Sanders et al., 1988). Similarly high rates of toxicity were also reported in a small group of boys treated with cyclophosphamide and TBI before puberty (Leiper et al., 1987), with raised LH concentrations in five out of six, and raised FSH concentrations in all six that had reached pubertal age. Thus, the rates of testicular dysfunction observed following therapy in childhood are not significantly different to those seen in adults.

Clinical importance of Leydig cell impairment

Whilst the clinical impact of germinal epithelial damage and azoospermia is clear (Table II), the clinical relevance of the mild Leydig cell impairment, which occurs in a proportion of patients, is not. The mechanism of Leydig cell impairment following chemotherapy is not known. There is no histological evidence of Leydig cell abnormalities on testicular biopsy after cytotoxic therapy. Chemotherapy may have a direct toxic effect on the Leydig cell, but there is also some evidence that germinal epithelial damage may indirectly affect Leydig cell function. Azoospermia following germinal cell damage causes a reduction in testicular volume and testicular blood flow (Wang et al., 1983). The testosterone output of the testes is a product of the veno-arterial concentration difference of testosterone and the venous outflow from the testes, and is thus reduced by any reduction in testicular blood flow. Whilst a modest reduction in blood flow may be corrected by an increase in intratesticular testosterone concentration, there is evidence from an animal model that more marked reductions in blood flow cannot be overcome (Satchell and Galil, 1983). In addition, as arterial flow into the testes is reduced, the stimulatory effect of LH may be diminished. Damage to the germinal epithelium and a reduction in testicular volume may also affect Leydig cell function by causing structural changes within the testis or alterations in the paracrine control of Leydig cell function (Huhtaniemi and Toppari, 1995; Carreau, 1996).

A number of symptoms which occur in overt hypogonadism (fatigue, sexual dysfunction and altered mood) also occur more commonly in patients following cytotoxic chemotherapy (Howard et al., 2000). In addition, reduced bone mineral density (BMD), which is associated with testosterone deficiency, has also been demonstrated in cytotoxic-treated men, and bone density has been shown to correlate with testosterone concentrations in these patients (Holmes et al., 1994; Howell et al., 2000a). Thus, there is some evidence to suggest that Leydig cell impairment following chemotherapy may be clinically important (Holmes et al., 1994), and testosterone replacement may therefore be beneficial in a proportion of these patients. Testosterone and gonadotrophin concentrations should be measured following chemotherapy, and men with a raised LH concentration in the presence of a frankly low or low/normal testosterone concentration should be considered for androgen replacement.

Transmissible genetic damage

In addition to impairment of steroidogenesis and sperm production, there has been concern that cytotoxic chemotherapy may also result in transmissible genetic damage. Animal studies have demonstrated untoward effects in offspring of animals treated with cytotoxic agents, but no clear evidence for this has been reported in humans. Increased aneuploid frequency has been observed in human spermatozoa following chemotherapy for HD (Monteil et al., 1997; Robbins et al., 1997), and an increase in chromosomal abnormalities demonstrated several years after treatment for testicular cancer (Genesca et al., 1990). However, data concerning the outcome of pregnancies have not shown any increase in genetically mediated birth defects, altered sex ratios or birthweight effects in offspring of cancer survivors (Robbins, 1996). This may be as a result of selection bias against genetically abnormal spermatozoa or conceptuses, or because existing epidemiological studies are not sufficiently powered to detect small differences. On the evidence thus far, however, it is reasonable to conclude that patients treated with cytotoxic chemotherapy, who remain fertile, are not at increased risk of fathering children with genetic abnormalities.

Prevention of testicular damage

The deleterious effect of chemotherapy on germinal epithelial function has initiated a search for possible strategies to preserve fertility in men undergoing therapy. Cryostorage of semen has become standard practice, and should be offered to all men before undergoing potentially sterilizing therapy. Improvements in the techniques used to store semen (Royère et al., 1996) and advances in the field of assisted reproduction, such as intracytoplasmic
sperm injection (ICSI), have increased the chance of successful pregnancies using cryopreserved spermatozoa. However, there are some limitations to this method of preserving fertility. First, it is not a feasible option for prepubertal patients. Furthermore, testicular function in adult males with malignant disease is often impaired before treatment (Chapman et al., 1981), resulting in poor sperm quality or difficulty providing adequate semen for storage. This reduces the prospects for successful utilization of stored semen following therapy. In addition, the process of freezing and thawing semen further reduces the sperm quality and chances of fertilization. As a result, methods for protecting, or enhancing the recovery of normal spermatogenesis following gonadotoxic therapy have been pursued.

The belief that prepubertal boys have a lower rate of permanent chemotherapy-induced gonadal damage (Rivkees and Crawford, 1988) has led many investigators to propose that suppression of testicular function in adult men (i.e. inducing a prepubertal state) will provide a degree of protection against cytotoxic therapy. Irrespective of the validity of the hypothesis, data derived from animal models have been encouraging, but there is at present no convincing evidence of similar success in humans. Enhanced recovery of spermatogenesis has been demonstrated in procarbazine-treated rats by the administration of the GnRH analogue Zoladex for 2 weeks before chemotherapy and during chemotherapy (Ward et al., 1990). Increased stem cell survival was evident by 50 days, and at 90 days the sperm count was close to normal values and significantly higher than in procarbazine-only-treated rats. Similar protective effects of hormonal treatment have been described following the use of testosterone (Delic et al., 1986), testosterone and oestradiol (Kurdoglu et al., 1994), GnRH and testosterone (Pogach et al., 1988), and GnRH and the antiandrogen flutamide (Kangasniemi et al., 1995; Meistrich et al., 1995), following gonadal insult with procarbazine, cyclophosphamide or radiotherapy. Others (Pogach et al., 1988) have suggested that testosterone administered after treatment with procarbazine enhanced the recovery of spermatogenesis. More recently, it has been confirmed that treatment with either testosterone or Zoladex following irradiation with 3.5 Gy markedly improves the recovery of spermatogenesis, even if treatment is delayed for 10 weeks after irradiation (Meistrich and Kangasniemi, 1997). The enhanced recovery of spermatogenesis in rats treated with GnRH-agonist, evaluated by the repopulation index (the percentage of tubules that contain three or more spermatogonia that had reached type B stage or greater), is illustrated in Figure 1. The same group had previously shown that spermatogenesis did not occur after a similar dose of irradiation despite the presence of type A spermatogonia in the seminiferous tubules (Kangasniemi et al., 1996); they postulated that the role of hormonal treatments in the ‘protection’ of germinal epithelial function, may be to enhance recovery of surviving A spermatogonia and to facilitate their differentiation to more mature cells, rather than to protect them from damage during cytotoxic therapy or radiotherapy. They suggested that a reduction of intratesticular testosterone is the mechanism by which hormone therapy stimulates recovery of spermatogenesis.

In humans, attempts to reproduce the protective effects seen in animals have been unsuccessful. Several groups have used GnRH analogues, with and without testosterone, to suppress testicular function during MOPP (Johnson et al., 1985) or MVPP (Waxman et al., 1987) chemotherapy for lymphoma, and cisplatin-based chemotherapy for teratoma (Ortin et al., 1990). None has demonstrated any significant protective effect of these therapies in terms of maintenance of spermatogenesis or increasing the rate of recovery. However, none of the studies involved the continuation of gonadal-suppressive therapy for a significant period of time after the completion of chemotherapy. The most recent animal data suggest that hormonal treatment may enhance recovery of spermatogenesis from surviving stem cells rather than protect them from damage during cytotoxic insult. Thus, suppression of gonadal function with a GnRH analogue or testosterone for a fixed time after the completion of chemotherapy may prove more successful in reducing the impact of these treatments on fertility.

This approach relies on enhancing recovery of sperm production, and therefore a prerequisite for its success is the survival of stem cells during the gonadotoxic insult. There are, however, few data regarding testicular histology after chemotherapy. Following cisplatin-based chemotherapy, spontaneous recovery of spermatogenesis occurs in most patients, although there is often a latent period of azoospermia which may last several years. The eventual recovery of spermatogenesis, however, implies the survival of A spermatogonia. Following chemotherapy for HD with procarbazine-containing regimens, and high-dose chemotherapy, recovery to oligo- or normospermia is much less common. Testicular biopsies taken after standard chemotherapy (MVPP and COPP) for HD have shown complete germinal aplasia with a Sertoli cell-only pattern (Chapman et al., 1979, 1981; Charak et al., 1990; Ortin et al., 1990; Das et al., 1994). Thus, although the recovery of spermatogenesis in a minority of these patients indicates that some germ cells survive in some patients, the absence of histological evidence of this at biopsy in many men suggests that all spermatogonia may be eradicated during chemotherapy.

Figure 1. The effect of GnRH-agonist treatment on repopulation indices in rats irradiated with 3.5 Gy. GnRH-agonist treatment was started immediately after irradiation and continued for 3, 6 or 10 weeks; results were compared with animals receiving no GnRH-agonist treatment (0). All rats were killed 10 weeks after irradiation, except for one group which was treated for 10 weeks and killed at 16.5 weeks (10+6). *P<0.02; **P<0.005. Adapted (with permission American Society of Andrology) from Meistrich and Kangasniemi (1997).
Hormonal manipulation after treatment to enhance the recovery of spermatogenesis is therefore likely to be of most benefit in those patients in whom the testicular insult is less severe, as it is in these patients in whom there is significant preservation of a spermatogonia. The success of this approach in those patients who have undergone more gonadotoxic therapy will depend on whether any stem cells remain intact, and complete ablation of the germinal epithelium, as may occur in many men treated for HD, is likely to be irreversible.

Results from recent animal experiments have also indicated another possible method of preserving testicular function during gonadotoxic therapy. In 1994, it was demonstrated that stem cells isolated from a donor mouse could be injected into the seminiferous tubules of a sterile recipient mouse and result in the initiation of spermatogenesis (Brinster and Zimmermann, 1994). More recently, the same group has demonstrated that spermatogenesis can be achieved in previously sterile mice following cryopreservation then injection of donor stem cells into the testis. Potentially, therefore, stem cells could be harvested from the human testis before the start of sterilizing therapy, freeze-stored, and re-implanted at a later date, with a subsequent return of spermatogenesis. A clinical trial testing this hypothesis is currently underway in adults: 11 men have had testicular tissue harvested shortly before commencing treatment with sterilizing chemotherapy for HD or NHL. In each case, a 0.5-cm³ piece of testicular tissue has been subjected to enzymatic digestion to produce a single-cell suspension which, following equilibration in cryoprotectant, has been stored in liquid nitrogen (Radford et al., 1999). Five men have now successfully completed chemotherapy, and thawed testicular suspension has been re-injected into the donor testis. Results of follow-up semen analyses and hormonal evaluation are awaited with great interest.

Summary

Treatment with cytotoxic chemotherapy and radiotherapy is associated with significant testicular damage. Alkylating agents, such as cyclophosphamide and procarbazine, are the most common agents implicated. The vast majority of men receiving procarbazine-containing regimens for the treatment of lymphomas, or high-dose chemotherapy, are rendered permanently infertile. Cisplatin-based chemotherapy for testicular cancer results in temporary azoospermia in most men, with a recovery of spermatogenesis in about 50% after 2 years and in 80% after 5 years. There is also evidence of Leydig cell impairment in a proportion of these men, although the clinical significance of this is not clear. Several methods of preserving testicular function during potentially sterilizing treatment have been considered. At present, sperm banking remains the only proven method, although hormonal manipulation to enhance recovery of spermatogenesis and cryopreservation of testicular germ cells are possibilities for the future.

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