Chemotherapy and the adult gonad: a review[1]

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With survival in some cancers now a possibility, significance is attached to the details of survival. A holistic approach to the patient takes account of the sequelae of treatment consequent to cure. A high frequency of sterility is an important consideration when embarking upon the treatment of curable malignancies that affect younger populations, such as Hodgkin's disease, childhood leukaemia, non-Hodgkin's lymphoma, choriocarcinoma and testicular teratoma.

Fertility at presentation of malignancy

At presentation, a high proportion of patients with a variety of malignancies are sterile. In one series of women with breast cancer, only 17% were observed to have a secretory endometrium (Grattarola 1964). In groups of women in whom there is a high risk of development of breast cancer, anovulatory cycling with low levels of luteal phase progesterone occurs (Bulbrook *et al.* 1978). In a review of men with lymphoma and teratoma, oligospermia at presentation was noted in 33% of both groups. Following 'freezing' for artificial insemination, additional defects post-thawing were seen, sperm having reduced motility in a further 33% of patients. Thus, 66% of these patients had quantitative or qualitative defects in sperm (Sanger *et al.* 1980).

Histological changes following cytotoxic chemotherapy

The histological effects of chemotherapy and radiotherapy to the testis include loss of germinal epithelium, with relative survival of Leydig and Sertoli cells. In women, fibrosis results, producing a streak ovary. The timing of events in the testis has been observed in the mouse subsequent to intraperitoneal injection of nitrogen mustard (Landing *et al.* 1949). Within the first 24 hours germinal cell nuclear chromatin condenses; its cytoplasm exhibits eosinophilia and hyaline change. These events are most marked in spermatogonia and primary spermatocytes. Next, germinal epithelium becomes disrupted, with sperm lying free and disorientated within the tubular lumen. At 48 hours, abnormal germ cells are observed with multinuclear giant cells present. Finally, cell lysis occurs. In this experimental model, recovery ensues over a period of 6 weeks. Initially there is Sertoli and Leydig cell survival, then increasing numbers of spermatogenic precursors are present.

Hormonal changes consequent to cytotoxic chemotherapy

As expected, the endocrinology mirrors the histological change. In men, with preservation of Leydig cells, testosterone (T) and luteinizing hormone (LH) are normal. With destruction of germ cells, follicle-stimulating hormone (FSH) is raised and prolactin (PRL) may be elevated (Chapman *et al.* 1979*b*). This relates to a poorly-understood feedback loop, linking testicular and adrenal androgen production to increased PRL (Vermeulen *et al.* 1977). In women, gross ovarian destruction produces low levels of 17 β oestradiol (E_2) and progesterone (P). FSH and LH are raised. PRL may be marginally elevated (Chapman *et al.* 1979*a*). As a consequence of gonadal ablation women become infertile and are at risk of premature osteoporosis if not given hormone replacement. Men become sterile (Chapman *et al.* 1981).

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Effects of cytotoxic chemotherapy upon the gonad

Single-agent chemotherapy

A summary of the effects of chemotherapy upon the gonad is dogged by the paucity of data. This is because studies are limited to a few curable diseases. As a general principle, alkylating agents are regarded as more toxic than antimetabolites. This conclusion is drawn from multiple rather than single-agent studies. The first note of an effect of chemotherapy upon the gonad was made in 1956 when Louis and colleagues commented upon the amenorrhoea observed in all 4 premenopausal patients treated with busulphan for chronic myeloid leukaemia. These women received approximately 10 mg busulphan daily and all were menopausal 3 months into therapy. From early studies with chlorambucil, dose-toxicity correlates were first established in malignant disease. In patients with lymphoma, spermatogenesis was noted to be affected after more than 400 mg of this alkylating agent had been given (Richter *et al.* 1970). With total dosages of less than 2600 mg, recovery of spermatogenesis was observed at between 5 and 24 months (Cheviakoff *et al.* 1973). Scheduling of chlorambucil is now completely different and the relevance of these conclusions to current therapy is unknown.

The most extensively studied single agent with regard to gonadal toxicity is cyclophosphamide. Knowledge about its effects has been extended by observations on its use in non-malignant conditions. The use of this compound in renal failure and rheumatoid arthritis has produced information regarding the significance of duration of therapy, drug dose, and patient age to the development of sterility. In a study of 8 males with glomerulonephritis, given cyclophosphamide at dosages varying between 2 and 5 mg/kg/24 hours, those 4 men treated for less than 60 days were noted to have normal sperm counts 6 to 10 years after treatment; but 4 men in whom treatment extended between 89 and 489 days were azoospermic (Etteldorf *et al.* 1976). In observations of 31 men treated for nephrotic syndrome, and given cyclophosphamide at dosages between 50 and 100 mg/day azoospermia was noted in some men from 3 weeks into treatment. The rapidity of effect implies that all generations of germ cells are destroyed by this compound. Testicular biopsies were performed in these men, and the histology as expected showed survival of Sertoli cells only. In 10 of this group, serial seminal analysis was performed after treatment. Active spermatogenesis occurred in only 2 men, at 3 and 19 months after cessation of therapy (Fairley *et al.* 1972). In 22 women with either rheumatoid arthritis or glomerulonephritis, 17 were observed to become amenorrhoeic between 1 and 33 months into treatment. The onset of amenorrhoea occurred within 4 months in those women aged over 35 years and at up to 33 months in younger women. Of 13 women observed off therapy, only one developed a return of her menses (Warne *et al.* 1973). In women with breast cancer treated with prophylactic adjuvant cyclophosphamide, the dose of chemotherapy causing amenorrhoea is inversely proportional to the age of the patient. Women aged over 40 years all become amenorrhoeic after receiving less than 5 g, whilst those patients aged between 30 and 40 years become amenorrhoeic after receiving 9 g of cyclophosphamide (Koyama *et al.* 1977).

Of those women treated with adjuvant chemotherapy for breast cancer using cyclical melphalan, a greater survival advantage was noted for premenopausal women. This advantage was initially presented as being the result of a chemically-induced menopause: 69% of women aged less than 39 years at treatment were disease-free at 4 years, whilst 61% of women aged between 40 and 49 years were free of disease. Of the younger group 22% were rendered amenorrhoeic and of the older women 73% became amenorrhoeic (Fisher *et al.* 1979). A later study with adjuvant cyclophosphamide, methotrexate and 5-fluorouracil related survival to total dose of adjuvant chemotherapy received rather than to menopausal status (Bonadonna & Valagussa 1981). Certainly, this is a more sensible explanation of a muddled point.

Procarbazine, used in the management of Hodgkin's disease, was given parenterally to 55 rhesus and green monkeys, 26% of whom developed neoplasia observed at post-mortem. An additional finding was of universal gonadal degeneration occurring within a dose range 1.8 to

100 g (Sieber *et al.* 1978). Patients with Hodgkin's disease receive an average of 1.5 g of procarbazine during each cycle of chemotherapy.

However, experimental animal models do not duplicate human behaviour, as small studies with adriamycin show. A single dose of 35 mg/m^2 sterilizes mice. However, 2 patients given full-dose adriamycin (i.e. 450 mg/m^2) during the treatment of acute myeloid leukaemia were observed to have return of spermatogenesis 6 months after the termination of chemotherapy (da Cunha *et al.* 1979).

Of single agents, mustine is probably the compound most toxic to the gonad. Early studies showed that 80% of women become amenorrhoeic one to two months after a single dose of this alkylating agent (Sobrinho *et al.* 1971). Vinblastine is less toxic and the amenorrhoea occurring during single-agent chemotherapy may be reversible. In one very small series of 3 women, menses returned in one patient 2 months after the cessation of therapy (Sobrinho *et al.* 1971).

Drugs that might be dismissed as being non-toxic to the gonad are of consequence to germ cell function. Prednisone is a component of many commonly-used chemotherapeutic regimens. At daily dosages of greater than 10 mg it produced significant reductions in sperm counts (Mancini *et al.* 1966). The survival advantage produced by this steroid is controversial: in Hodgkin's disease, American and British data conflict as to its benefit (Jacobs *et al.* 1976, British National Lymphoma Investigation 1975).

Combination chemotherapy

The effects on the gonad of multiple-agent chemotherapy have been described in breast cancer, acute lymphatic leukaemia (ALL), Hodgkin's disease (HD), and non-Hodgkin's lymphoma (NHL). Patients with NHL may receive CVP. This involves the receipt of six 3-weekly cycles of cyclophosphamide, vincristine and prednisone. In one study, reversible sterility was observed in 75% of male patients; recovery of spermatogenesis occurred over a 34-month follow-up period (Roeser *et al.* 1978). In a smaller cohort of similarly treated men, only 30% were noted to have active spermatogenesis, and recovery occurred within two years of treatment (Sherins & DeVita 1973).

Women with breast cancer are effectively treated with CMF (cyclophosphamide, methotrexate, 5-fluorouracil). With this regimen, 13 of a group of 15 women with a mean age of 42 became menopausal within 10 months of initiation of chemotherapy (Rose & Davis 1980). A difference is seen in the rapidity of onset of a chemical menopause with multiple-agent regimens that are more effective in inducing a remission. Adriamycin given with cyclophosphamide to younger women induces anovulatory cycling with oligo- or polymenorrhoea 3 months into chemotherapy, and this proceeds to a chemical menopause. Amenorrhoea is complete at 2 months in perimenopausal women (Schultz *et al.* 1979). With adriamycin, cyclophosphamide and 5-fluorouracil a clear age advantage in fertility preservation is seen. In one study, the mean age of women rendered amenorrhoeic by treatment was 45 years, whilst those women with retention of menses had a mean age of 33 years (Samaan *et al.* 1978).

A common choice of chemotherapeutic regimen for the treatment of advanced Hodgkin's disease includes mustine, vinblastine, procarbazine and prednisone (MVPP), and mustine, vincristine, procarbazine and prednisone (MOPP). These two regimens produce sterility in approximately 80% of all patients, male and female. There is a much greater likelihood of preservation of fertility if the patient is treated when younger than 30 years (Waxman *et al.* 1982). Details of any long-term prospect of recovery of fertility are poorly elucidated.

These studies of alkylating agents contrast with multiple-agent chemotherapy using mainly antimetabolites in acute lymphatic leukaemia. These patients proceed to normal fertility post-treatment (Shalet *et al.* 1981, Siris *et al.* 1976). Of 31 ovarian examinations performed upon girls dying of acute leukaemia, only 7 showed atrophic change (Himelstein-Braw *et al.* 1978).

Alternative futures

If one is concerned with the details of survival, there are two alternatives. The first involves the

modification of treatment to less toxic regimens; the second, gonadal protection during chemotherapy. A similar idea, developed independently in America and the UK, involves the use of superactive analogues of gonadotrophin-releasing hormone (GnRH). These analogues were initially used to induce puberty in the hypogonadal and found to be ineffective (Brook & Dombey 1979). In the pubescent rat subject to their long-term use, secondary sexual characteristics were observed to regress (Tcholalakian *et al.* 1978). The reason for this regression is that these analogues bind more effectively than endogenous GnRH to pituitary receptors and, after initial stimulation, render them unresponsive: the normal response is to the pulsatile release of GnRH (Fraser *et al.* 1980). With their prolonged use gonadotrophin levels fall, reaching a nadir within 8 days (Nilius *et al.* 1978). In women using the d ser^6 substituted analogue, anovulatory cycling occurs (Bergquist *et al.* 1979). In men with the d trp^6 substituted analogue, oligospermia is seen after 6 weeks of continued use (Linde *et al.* 1981).

The hypothesis that the down-regulated gonad is protected from the effects of chemotherapy has been examined by Glode *et al.* (1981). A test group of mice was pretreated with the d leu^6 substituted GnRH analogue before receiving cyclophosphamide in dosages toxic to the testis. In comparison to the control groups, minimal loss of germinal epithelium occurred. Human studies are proceeding in the UK.

As has been demonstrated, cytotoxic drugs cause gonadal destruction. Now that patients are effectively treated for malignant disease, this side effect of treatment is important.

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