The radiosensitivity of the human oocyte

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BACKGROUND: We determined the best model available for natural follicle decline in healthy women and used this to calculate the radiosensitivity of the human oocyte. METHODS: Ovarian failure was diagnosed in six patients with a median age of 13.2 years (range 12.5–16.0) who were treated with total body irradiation (14.4 Gy) at 11.5 years of age (4.9–15.1). We previously estimated the dose of radiation required to destroy 50% of the oocytes (LD50) to be <4 Gy. This estimate is an oversimplification, because decay represents an instantaneous rate of temporal change based upon the remaining population pool, expressed as a differential equation: dy/dx = −y(0.0595 + 3716/(11780 + y)], with initial value y(0) = 701 200. RESULTS: Solving the differential equation, we have estimated the number of follicles left after irradiation given as sol(51 ± s + r), where r equals age at treatment, s equals age at diagnosis of ovarian failure, and 51 years is the average age of menopause. The surviving fraction of oocytes as a percentage is 100 times this value divided by sol(r). The mean surviving fraction for the six cases is 0.66%. We obtain a function, g(z), which decreases in value from 100% at zero dosage to mean value at dosage z = 14.4 Gy. We have g(z) = 10^mx+c, where c = log10(100) = 2, and m = [log10(0.66) − c]/14.4. Solving g(z) = 50 gives an LD50 of 1.99. CONCLUSIONS: Based on new data and a revised mathematical model of natural oocyte decline, we have determined the surviving fraction of oocytes following irradiation and estimate the LD50 of the human oocyte to be <2 Gy.

Key words: fertility/human oocyte/ovarian failure/radiotherapy

Introduction

Females treated for cancer with abdominal, pelvic or total body irradiation (TBI) may sustain permanent damage to the ovary and loss of primordial follicles, resulting in impaired fecundity and a premature menopause. An understanding of the dynamics of ovarian follicle decline will facilitate our ability to predict the impact of treatment on ovarian function and to manage fertility issues in these young women more scientifically.

The human ovary is endowed with a fixed pool of primordial follicles, maximal at 5 months of gestational age, which steadily declines throughout life, culminating in the menopause at an average age of 51 years. Follicle depletion, as a result of atresia and recruitment towards ovulation, leads to premature exhaustion of the follicle pool and menopause long before death, in contrast to other mammals. A number of mathematical models have been proposed in humans to describe the rate of follicle decline based on a series of data describing the number of follicles present at different ages in humans (Block, 1952, 1953; Baker, 1963; Gougeon, 1984; Richardson et al., 1987).

For any given age, the size of the follicle pool can be estimated based upon a mathematical model of decline. The rate of oocyte decline represents an instantaneous rate of temporal change, based upon the remaining population pool. Therefore, reduction of the follicle pool as a consequence of cytotoxic therapy will result in premature exhaustion of the pool, and advance the onset of the menopause. In order to predict the age of menopause in patients who have experienced radiotherapy to a field that includes the ovaries, the extent of the radiation-induced damage to the follicle pool must be determined.

We have previously estimated the dose of radiation required to destroy 50% of primordial follicles (LD50) to be <4 Gy (Wallace et al., 1989a). For a given dose of radiotherapy, the surviving fraction can be determined and the age of menopause predicted by applying a mathematical model for decay. In this way, patients can be counselled appropriately with regard to their reproductive life span and their window of opportunity for fertility (Wallace et al., 2001).

Methods

Natural decline of the ovarian follicle pool

Data estimating the number of ovarian follicles available at different ages in humans are found in a number of studies, and such data have been used to construct complex mathematical models of follicle decline (Block, 1952, 1953; Baker, 1963; Gougeon, 1984; Richardson et al., 1987).

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The application of such a model will enable the number of follicles present to be predicted as a mathematical product of the initial follicle store and the rate of subsequent attrition for any given age. It is estimated that during embryo development, approximately seven million germ cells are formed in the ovarian rudiment, but only two million are present at birth and 300 000 by menarche (Block, 1952; Baker, 1963). The precise number of oocytes remaining at menopause is unclear. Ovarian follicles have been counted in the ovaries of 43 females aged 6–44 years, following accidental death, and the number of follicles present at menopause was predicted, using linear extrapolation, to be 2200 (Block, 1952). This is likely to be an overestimate, as further studies of follicle numbers present in the ovaries of pre-, peri- and post-menopausal women have demonstrated that <1000 ovarian follicles remain in peri-menopausal women, indicating that follicle decline accelerates in the decade preceding menopause (Richardson et al., 1987). With only an estimated 400 ova- lutions occurring during the reproductive period, this progressive reduction is attributable to follicle death by apoptosis.

Evidence for temporal decline has been well characterized in rodents, and early mathematical models were constructed based on a model of negative exponential rate of decay: \( y = A \exp(-bx) \), where \( A \) is the number of follicles present at birth; or, following a logarithmic transformation to give a model for linear decay: \( \log(y) = \log(A) - bx \), where \( x \) refers to age, \( y \) to the number of follicles and \( b > 0 \) is the rate of exponential decay (Faddy et al., 1983).

The rate of decline \( b \) was then determined by applying simple regression analyses of logged number of follicles against age. Each model can be described as \( y(x) = f(x) \) for \( x \) between zero (birth) and the estimated age of menopause, where \( y(x) \) is the estimated number of follicles present at age \( x \), and \( f(x) \) is a function used to obtain \( y(x) \). The proposed mathematical models vary depending upon the chosen subset of data describing follicle numbers at different ages. Richardson et al. provided the mathematical model (Richardson et al., 1987):

\[
\log_{10}(y) = \frac{6.13 \pm 0.33 - 0.06 \pm 0.01x}{x}
\]

Based on one source of data (Block, 1952), and:

\[
\log_{10}(y) = \frac{5.94 \pm 0.37 - 0.04 \pm 0.01x}{x}
\]

Based on another source of data (Gougeon et al., 1984).

We previously described the function \( \log_{10}(y) = 6.3 - 0.06x \), based on two sources of data (Block, 1952; Baker, 1963).

The above models are constructed using data describing a population aged 6–44 years. Therefore, it is with some reservation that these models have a wider application from birth to menopause. Furthermore, none of the above models make use of more than two data sources, and a larger data set is likely to produce a more accurate model. Construction of the above models is based on the assumption that ovarian follicle decline follows simple exponential decay; however, it is now recognized that the loss of ovarian follicles is more complex. A graphical representation of ovarian follicle number, expressed logarithmically against age, suggested that ovarian follicle decline is bi-exponential with ‘broken-stick’ regression (Faddy et al., 1992). An increase in the rate of exponential decline appeared to occur at age 38 years, corresponding to a follicle pool of 25 000. Following these observations, Faddy et al. proposed a more comprehensive model of piecewise exponential decay (Faddy et al., 1992) based on a least squares fit to data from three other authors (Block, 1952, 1953; Gougeon, 1984; Richardson et al., 1987):

\[
\log_{10}(y) = 952000 - 0.097x \quad \text{for } 0 < x < 37.5
\]

and:

\[
\log_{10}(y) = e^{19.02 - 0.237x} \quad \text{for } 37.5 < x < 51
\]

Although data from Baker were not used (Baker, 1963), the value at birth of 952 000 is in line with his results, and assumes a population of 1000 follicles at a menopause occurring at age 51 years. However, this model fails to concord with the distribution of menopausal ages described by Trelour (Trelour, 1981). Furthermore, biologically, this abrupt change when the oocyte population falls to 25 000 is unlikely; more plausibly, the change is likely to represent an instantaneous rate of temporal change based on the remaining population pool, which is expressed mathematically as a differential equation. Faddy and Gosden provided a revised model (Faddy and Gosden, 1996) obtained by incorporating Trelour’s data into a least squares analysis of the four quantitative studies in terms of the differential equation:

\[
dy/dx = -y[0.0595 + 3716/(11780 + y)]
\]

with initial value \( y(0) = 701 \).

We consider this to be the best model currently available, and have solved it to revise our estimate of the radiosensitivity of the human oocyte based upon additional data from young women who developed ovarian failure following treatment with TBI.

### Patients

We studied two cohorts of women with ovarian failure secondary to radiotherapy treatment for childhood cancer. The first cohort comprised of eight post-pubertal women, median age 17.1 years (range 15.4–21.5), recruited from paediatric oncology late effects clinics throughout Scotland (Bath et al., 1999) (Table I). The patients had been treated with TBI, 14.4 Gy in eight fractions over 3 days, during first or second remission for leukaemia at age 11.5 years (range 4.9–15.1). No shielding to the ovary was applied. All patients received chemotherapy with standard Medical Research Council (MRC) trials for acute lymphoblastic leukaemia or acute myeloid leukaemia.
The second cohort comprised 19 patients from our previous study describing the radiosensitivity of the human oocyte (Wallace et al., 1989a) (Table II). The 19 patients had been treated during childhood for an intra-abdominal tumour with whole abdominal radiotherapy (total dose: 30 Gy, 16–26 fractions), surgery and chemotherapy between 1966 and 1975 at the Christie Hospital, UK (Wallace et al., 1989b). The median age at treatment was 4 years (range 1.3–13.1) and between 1966 and 1975 at the Christie Hospital, UK (Wallace et al., 1989a) (Table II). The 19 patients had been treated during childhood:

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at irradiation (years)</th>
<th>Age ovarian failure diagnosed (years)</th>
<th>Surviving fraction of oocytes (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>14.1</td>
<td>0.42</td>
</tr>
<tr>
<td>2</td>
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<td>13.1</td>
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<td>4</td>
<td>5</td>
<td>12.8</td>
<td>1.77</td>
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<tr>
<td>5</td>
<td>13</td>
<td>15.7</td>
<td>0.72</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>12</td>
<td>0.77</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>11.9</td>
<td>1.99</td>
</tr>
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<td>2</td>
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<tr>
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<td>4</td>
<td>10.5</td>
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<td>1.66</td>
</tr>
<tr>
<td>19</td>
<td>7</td>
<td>NF</td>
<td>NA</td>
</tr>
</tbody>
</table>

NF = normal ovarian function; NA = not applicable.

Results

Using the numerical solution procedure, sol, derived as above, we obtained a procedure which takes an age at irradiation and age of diagnosis of ovarian failure, and returns (as a percentage) a surviving fraction of oocytes. Suppose child A is treated at age \( r \) and has ovarian failure diagnosed at age \( s \). Then, assuming that (i) decay occurs after irradiation at the rate given by the Faddy–Gosden model, and (ii) age of ovarian failure is close to the age of diagnosis of ovarian failure, we can estimate that the number of follicles remaining after irradiation is \( \text{sol}(51 - s + r) \). The surviving fraction as a percentage is then 100 times this number divided by \( \text{sol}(r) \). The mean surviving fraction for the six cases in the first cohort is 0.66% (see Table I). If we assume a logarithmic increase in the number of follicles removed with increasing dose, then we can obtain a function, \( g(z) \), which decreases in value from 100% at zero dosage to the mean value at dosage \( z = 14.4 \) Gy given to the six cases. We have \( g(z) = 10^{mzc} \), where \( c = \log_{10}100 = 2 \), and \( m = [\log_{10}(0.66) - c]/14.4 \).

Solving \( g(z) = 50 \) gives an \( \text{LD}_{50} \) of 1.99 Gy

Figure 2 demonstrates the estimation of \( \text{LD}_{50} \) for the human oocyte. The dose required to completely destroy the follicle pool, \( D_0 \) (\( \text{LD}_{100} \)), is an infinite number of Gy, since we are assuming a logarithmic model.
We previously determined the radiosensitivity of the human oocyte based on an analysis of the 19 patients treated with whole abdominal irradiation. The calculation of an estimated surviving fraction for case 5 in Table I is shown: ovarian failure occurred at age 13 years in case 5. By applying the Faddy–Gosden model, we can determine the size of the surviving fraction following radiotherapy at age 10.5 years to be 0.56%.

We previously determined the radiosensitivity of the human oocyte based on an analysis of the 19 patients treated with whole abdominal irradiation. The surviving fraction of oocytes following irradiation was calculated for each of the 18 patients who developed ovarian failure. By plotting the surviving fraction logarithmically against the dose of radiation received, the LD$_{50}$ was estimated to be <4 Gy. With new evidence (Faddy et al., 1992), it is clear that primordial follicle decay does not follow a simple logarithmic decline, but rather represents an instantaneous rate of temporal change based upon the remaining population pool. Using the new mathematical model of follicle decline, we determined the surviving oocyte fraction in our second population of 18 patients with ovarian failure and revised our estimate of the LD$_{50}$ for this group. Replacing $z$ with a dose of 30 Gy for 14.4 Gy for the second cohort, our original data set of 18 cases (Table II) gives an LD$_{50}$ of 5.15 Gy.

**Discussion**

We estimate the LD$_{50}$ of the human oocyte to be <2 Gy, calculated by applying the solution of the Faddy–Gosden mathematical model of ovarian follicle decay to six patients with ovarian failure secondary to TBI as part of the treatment for childhood cancer. The human ovary is endowed with a fixed number of primordial follicles at birth, which undergo attrition and recruitment towards ovulation, culminating in menopause. Ovarian failure and a premature menopause will ensue from any cytotoxic insult, which either depletes the oocyte pool or hastens its decline. Radiotherapy is well recognized to cause destruction of the ovarian pool, although the extent of the damage is difficult to determine. Ovarian failure following radiotherapy during childhood may manifest in adolescence as failure of spontaneous puberty or pubertal arrest, or in adulthood as infertility and premature menopause.

The aetiology of ovarian failure in both our cohorts of patients is likely to be irradiation induced. The patients in the first cohort who had received TBI were treated with standard MRC chemotherapy protocols that include alkylating agents and cytotoxic. Ovarian function appeared to be preserved after standard treatment for acute lymphoblastic leukaemia (Wallace et al., 1993). We have described decreased LH secretion and short luteal phases in young women whose treatment included low dose cranial irradiation (Bath et al., 2001), but premature ovarian failure is not described, although these women may go on to have an early menopause.

With no biochemical markers available to predict those patients for whom premature ovarian failure is likely, information to determine the extent of radiotherapy-induced damage and prediction of the likely fertile window will be helpful for reproductive counselling. Oocyte radiosensitivity differs tremendously between species, with the mouse oocyte (LD$_{50}$ 0.15 Gy) being about 350 times more radiosensitive than that of the monkey (LD$_{50}$ 50 Gy). The radiosensitivity of the human oocyte has been reported in a number of studies, although the majority of these have focused on radiotherapy treatment in adult patients. Bianchi estimated the LD$_{50}$ for human oocytes to be 6–18 Gy (Bianchi, 1983). In these patients, radiotherapy was administered for the treatment of benign gynaecological disorders to induce an artificial menopause. Permanent ovarian failure was induced in a group of 72 patients, most of whom were >40 years old at time of treatment, following administration of 625 roentgens (~6 Gy).
In a study of 2000 females treated with radiotherapy for menorrhagia, permanent ovarian failure was induced in 97% of patients following irradiation with 5–10.5 Gy. In this study, our revised estimate of the LD50 for the human oocyte is significantly less than previously reported.

We have based our calculations on the assumption that the rate of decline of the surviving fraction of oocytes is not greater than that for a non-irradiated ovary. This is based upon the observation that oocytes die in interphase within a few hours of irradiation, becoming pyknotic and then removed by phagocytosis within a few days (Lindop, 1969). Furthermore, in irradiated fetal rat ovaries, severely damaged germ cells degenerate rapidly and are eliminated from the ovary within a few days of exposure. It is reported that the subsequent rate of oocyte depletion is lower in irradiated animals than in controls (Beaumont, 1964). If this were true, then the surviving fraction for each patient would be lower than we have estimated, indicative of increased radiosensitivity of the human oocyte and, consequently, a lower LD50.

Radiotherapy treatment before puberty may result in significant ovarian follicle depletion. However, the earliest clinical manifestation of ovarian follicle exhaustion is failure of pubertal development in association with elevated gonadotrophins. Delay in the diagnosis of ovarian failure would result in an overestimation of the LD50. Estimation of the LD50 (<2 Gy) for the cohort of patients treated with 14.4 Gy TBI was lower than our previous estimate of that (4 Gy) for our original cohort of 19 patients who received 30 Gy abdominal irradiation. The LD50 of 4 Gy is likely to be an overestimation, largely attributable to the long lag period between treatment and the earliest detection of clinical ovarian failure. It is now clear that the oocyte pool of these females would have been exhausted at an earlier age than that at which clinical manifestations could be detected. Using the solution to the Faddy–Gosden model has enabled us to recalculate the LD50 for the original cohort and, to our surprise, the estimate (5.15 Gy) is higher than we previously reported. The reason for the overestimate remains the higher radiation dose received by the first cohort, who were treated at a younger age, with a resulting longer lag period between treatment and the detection of ovarian failure. We therefore maintain that the most accurate estimate (LD50 <2 Gy) is calculated from applying the Faddy–Gosden solution to the second cohort, who were treated at an older age [median 11.5 (range 4.9–15.1) versus 4 (1–13) years] and with a lower total radiation dose.

A further consideration when applying our construct clinically is the uncertainty of the impact of fractionated doses of radiotherapy. Our estimation of the LD50 may be considered as an upper limit, because it does not take into consideration the fractionated schedule of radiotherapy.

Solving the Faddy–Gosden mathematical model for ovarian follicle decline using a seventh-eighth order continuous Runge–Kutta numerical method, and applying the solution to new clinical data on age at development of ovarian failure after TBI (14.4 Gy), has enabled a more accurate estimate of the radiosensitivity of the human oocyte. Calculation of the dose of radiation received by each ovary, combined with a more accurate estimate of the radiosensitivity of the human oocyte, will facilitate our ability to provide more scientific fertility counselling to young women at risk of a premature menopause following the successful treatment of cancer.

References


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