Chromosomal breakage analysis in dyskeratosis congenita peripheral blood lymphocytes

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Summary. Dyskeratosis congenita (DC) is a rare inherited disorder characterized by reticulate skin pigmentation, nail dystrophy and mucosal leucoplakia. Bone marrow failure occurs in the majority of cases and there is a predisposition to malignancy. Following conflicting reports of increased spontaneous and induced chromosomal breakage in DC lymphocytes, we examined chromosomal breakage with and without clastogen treatment in 10 DC patients from six different families. Peripheral blood cultures were stimulated with phytohaemagglutinin and treated with three clastogenic agents and γ-irradiation. There was no significant difference in the chromosomal breakage in DC lymphocytes with or without exposure to bleomycin, DEB, MMC or γ-irradiation. DC can therefore be distinguished from Fanconi’s anaemia in which lymphocytes show increased spontaneous and clastogen-induced chromosomal breakage.

Keywords: bone marrow failure syndrome, chromosomal breakage, clastogens, dyskeratosis congenita, lymphocytes.

Dyskeratosis congenita (also known as Zinsser-Engmann-Cole syndrome) is a rare inherited disease, with a prevalence of <1 per million. Although it is a heterogenous disorder, a typical patient will usually present by the age of 10 years with the triad of skin pigmentation, nail dystrophy and leucoplakia. The nail dystrophy can progress to complete nail loss. In addition, a given patient may have a wide range of other abnormalities (Sirinavin & Trowbridge, 1975; Drachtman & Alter, 1992). Bone marrow failure occurs in the majority of patients and is the principal cause of early mortality (Dokal, 1996). In addition, some patients die of malignancy or pulmonary complications. Early diagnosis is therefore desirable.

Dyskeratosis congenita (DC) has many features in common with Fanconi’s anaemia (FA) in which there is a hypersensitivity to clastogenic agents such as mitomycin C (MMC), which is a DNA alkylator. Some authors have reported excessive chromosome breakage in DC, either spontaneous (Morrison, 1974) or induced (Pai et al, 1989; DeBauche et al, 1990), whereas others disputed these findings (Sirinavin & Trowbridge, 1975; Drachtman & Alter, 1992), with the result that confusion exists over the susceptibility of DC cells to clastogenic agents. We therefore chose a range of agents, based on previous reports suggesting susceptibility, to determine which agents would produce an increased level of breakage in DC lymphocytes over that seen in normal controls. The ultimate aim was to define a reproducible method which could be used as a reliable diagnostic tool for this disease.

MATERIALS AND METHODS
It was crucial to ensure that only patients with a convincing diagnosis of DC were included in the study. Therefore patients with the classic triad of skin pigmentation, nail dystrophy and leucoplakia were selected from the Dyskeratosis Congenita Registry (DCR) established at the Hammersmith Hospital in 1995.

Heparinized peripheral blood (PB) was collected from each patient and a normal control at the same time. 10 DC patients aged 8–35 years were included in this study. Each patient (or guardian) gave consent for these studies. A total of six cultures containing fresh whole blood in 5 ml of complete medium were set up from each patient and each control, with culture types as follows. One culture was left untreated to assess spontaneous chromosomal breakage. The clastogenic agents diepoxidebutane (DEB) and MMC were added separately to a further two cultures, since cells from FA patients, with which DC has sometimes been confused clinically, show hypersensitivity to these agents; MMC (0–1 μg/ml) was added to one set of cultures at the time...
patients over a wide range (8–35 years). There is a possibility of differences in the age of patients, since our study included et al 1989; DeBauche 1989; DeBauche et al, 1990) are unlikely to reflect differences in the age of patients, since our study included patients over a wide range (8–35 years). There is a possibility that the discrepancies between our study and that of the previous ‘positive’ reports reflect genetic heterogeneity. It is of interest that in the bleomycin-treated cultures, in one patient the number of breaks per cell was relatively high (2·73/cell) compared to the range of 0·22–1·04 for the other seven patients. This suggests that a subset of DC patients may have increased chromosomal breakage following exposure to bleomycin. This may have been the basis of those reports (Pai et al, 1989) which alluded to a bleomycin effect on DC lymphocytes. However, the breakage levels in the controls was also high, with a wide range, and the difference was not statistically significant (Mann-Whitney test).

It is noteworthy that fibroblast studies (Dokal et al, 1992) have revealed the presence of chromosomal rearrangements in the absence of clastogens, and some studies on myeloid cells (Dokal et al, 1992; Demiroglu et al, 1997) have also shown chromosomal rearrangements. The primary defect in DC therefore appears to have a different effect on lymphocytes and fibroblasts with regard to the predisposition to chromosome instability, and it is possible that other cell lineages may respond differently to the T lymphocytes studied here.

The lack of chromosomal breakage in DC lymphocytes with and without clastogens now enables this condition to be distinguished from FA. It is noteworthy that cell-cycle studies using Epstein-Barr-virus-transformed lymphoblastoid lines showed similar profiles in DC and controls, whereas those from FA patients showed significant accumulation of cells in G2 (Racchi et al, 1998).

Cytogenetic analysis based on lymphocytes has as yet been unrewarding in terms of developing a reliable diagnostic test for DC. Fortunately, because of the genetic resource of the DC registry it has been possible to develop a carrier test for the X-linked form of DC; carriers of DC show skewed X-chromosome inactivation patterns (XCIPs) (Vulliamy et al, 1997). Furthermore, it has been possible to achieve fine mapping of the X-linked gene (DKC1) in Xq28 (Knight et al, 1996, and unpublished observations) and this has led to the identification of this gene (Heiss et al, 1998). The combined use of XCIPs together with mutational analysis of the DKC1 should now provide a robust system of carrier detection and early diagnosis of the disease in X-linked families.

Table I. Chromosomal breakage scores for DC patients and controls. There was no significant difference between DC and control lymphocytes with and without exposure to clastogens.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Patients</th>
<th></th>
<th>Controls</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Breaks</td>
<td>Range</td>
<td>n</td>
<td>Breaks/cell</td>
</tr>
<tr>
<td>Untreated</td>
<td>9</td>
<td>0·01</td>
<td>0·0–0·04</td>
<td>9</td>
<td>0·004</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>7</td>
<td>0·35</td>
<td>0·06–1·23</td>
<td>7</td>
<td>0·215</td>
</tr>
<tr>
<td>Diepoxybutane</td>
<td>5</td>
<td>0·019</td>
<td>0·0–0·06</td>
<td>6</td>
<td>0·018</td>
</tr>
<tr>
<td>50 cGy</td>
<td>6</td>
<td>0·113</td>
<td>0·0–0·16</td>
<td>6</td>
<td>0·137</td>
</tr>
<tr>
<td>100 cGy</td>
<td>6</td>
<td>0·207</td>
<td>0·02–0·52</td>
<td>6</td>
<td>0·16</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>8</td>
<td>0·913</td>
<td>0·22–2·73</td>
<td>9</td>
<td>0·612</td>
</tr>
</tbody>
</table>

of incubation, and DEB (0·1 μg/ml) to another set after a period of 24 h in culture.

Gamma-irradiation has been suggested to induce genetic damage in DC fibroblasts (DeBauche et al, 1990). Therefore two cultures from each patient sample were treated with 50 cGy and 100 cGy of γ-irradiation at 68 h, i.e. 4 h prior to harvest, which was targeted to have an effect in the G2 phase of the cell cycle. Finally, bleomycin had been suggested in the literature (Pai et al, 1989) to have a breakage effect on DC lymphocytes, and so this was added (25 μg/ml) to our final culture (sixth culture) 4 h before harvesting.

Phytohaemagglutinin (PHA) was added to all cultures at the time of incubation to stimulate T-lymphocyte cell division, and, after a total of 72 h at 37°C, cells were arrested in metaphase with Colcemid, and harvested. Slides were stained with Leishman’s stain only, without trypsin banding. We aimed to screen 50 complete metaphases from each culture, from the patient and control. All slides were coded and scored blind.

RESULTS

We found no significant elevation in the level of chromosome breakage in lymphocytes from patients over that seen in the controls for the untreated, bleomycin, DEB, MMC and irradiation treated cultures (Table I).

DISCUSSION

Peripheral blood lymphocytes from DC patients showed no significant difference in the level of chromosome breakage compared with normal controls without or after treatment with a variety of clastogens (bleomycin, DEB and MMC) and γ-irradiation. We therefore conclude that this form of breakage screening, on T lymphocytes from PB, is unsuitable as a basis for developing a diagnostic test. Our study supports the findings of previous case reports (Sirinavin & Trowbridge, 1975; Drachtman & Alter, 1992) which had shown normal chromosomal breakage analysis. Previous case reports of increased chromosomal breakage (Morrison, 1974; Pai et al, 1989; DeBauche et al, 1990) are unlikely to reflect differences in the age of patients, since our study included patients over a wide range (8–35 years).
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REFERENCES


