In vitro Drug Sensitivity Testing of Human Testicular Germ Cell Tumours with Cytostatic Drugs and Interferon Alpha-2b

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In spite of the significant advances in the chemotherapy of germ cell neoplasms, some patients do not achieve disease-free status and ultimately die from their diseases. Therefore, it is reasonable to select the best chemotherapeutic agents in these patients by in vitro drug sensitivity assay (IVDSA) in order to apply the most effective agent in case of resistance to primary chemotherapy. Fresh operative cells from 12 testicular germ cell tumours (TGCT) were cultured in vitro. Sensitivity of the tumour cells to interferon-alpha (IFN-alpha), cisplatin, mitomycin C, vinblastine, doxorubicin, etoposide, bleomycin, vincristine (VCR) were tested by a colorimetric assay using MTT. A preexposure viability over 75% was essential for IVDSA. Sensitivity was determined by a more than 50±2 SD% reduction from the control absorbance. All eight drugs in their high concentrations exhibited cell proliferation inhibition in 83.3 of TGCT. But in low concentrations efficacy of IF and VCR were found to be lower than the others (33.3% and 58.3%, respectively). The results indicated that although TGCT are highly sensitive to various agents IVDSA may help to identify the effective agents which might be necessary for second line chemotherapy in a small percentage of patients.

Introduction

Although 70 to 80% of patients presenting with metastatic testicular cancer are cured of their disease through the use of modern cisplatin based chemotherapy regimens with or without surgery, those patients who progress or relapse present a difficult clinical problem [1, 2]. Up to 30% of patients with testicular cancer have become candidates for salvage chemotherapy and it is possible to cure approximately one-third of these patients, but the majority are destined to die of testicular cancer [3, 4]. The unpredictable response of some testicular germ cell tumours (TGCT) to chemotherapy forced us to use in vitro assays that determine drug sensitivity and resistance prior to chemotherapy. In the present study, untreated fresh human TGCT cells from 12 patients were studied in vitro, exposing them to interferon-alpha (IFN) and cytotoxic agents. For this purpose cisplatinum (CDDP), mitomycin C (MMC), vinblastine (VBL), doxorubicin (DOX), etoposide (ETOP), bleomycin (BLM), vincristine (VCR) were used. Cell viability was assessed by a colorimetric assay using dimethylthiazol diphenyltetrazolium bromide (MTT).
Materials and methods

The study consisted of 12 men with primary TGCT. Characteristics of the patients are shown in Table 1. Tumour tissues of these patients were obtained by radical orchiectomy. Resected tumour tissue was divided into two similar components which were used for pathological examination and in vitro drug sensitivity assay (IVDSA). Care was taken to obtain a sample which lacked haemorrhage or necrosis.

Table 1

Patient characteristics

<table>
<thead>
<tr>
<th>Total number</th>
<th>12</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>34.8</td>
</tr>
<tr>
<td>Median</td>
<td>34.8</td>
</tr>
<tr>
<td>Range</td>
<td>24–54</td>
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</tbody>
</table>

* According to Royal Marsden Hospital Classification [5].

After mechanical dissection and enzymatic digestion using trypsin, tissue fragments were centrifuged and washed twice in RPMI-1640 medium. Subsequently nucleated cells were suspended in medium RPMI-1640 supplemented with 15% foetal calf serum and 1% penicillin-streptomycin. Tumour cell clusters were incubated in 24-well multiplate for 3 days at 37 °C in a humidified atmosphere at 5% CO₂/95% air. Incubation was carried out in triplicate in the presence and absence (control) of various concentrations of chemotherapeutic agents (Table 2). Only IFN was studied in two different concentrations. For each assay tumour cell viability was tested after enzymatic digestion. A viability over 75% was essential for IVDSA.

Drug sensitivity assay

Drugs were initially diluted in 0.9% NaCl and these stock solutions were stored at −20 °C. The following dilutions were done in culture medium fresh before each assay to obtain the concentrations shown in Table 2.

This range of concentrations and drug exposure was obtained from in vitro studies published earlier [6, 7]. The doses used by Leone et al. [6] for VCR were 20, 40 and 80 µg/ml which are hardly achievable in vivo during a standard 1.4 mg/sq.m dose application. Therefore we decreased final VCR concentration to 1, 5 and 10 µg/ml.