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Persistence of cisplatin-induced DNA interstrand crosslinking in peripheral blood mononuclear cells from elderly and young individuals

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Abstract DNA interstrand crosslinks (ISCL) produced by the incubation of human peripheral blood mononuclear cells (PBMs) with cisplatin (CDDP) were measured in two populations of volunteers, one aged less than 25 years and the other, greater than 72 years. The technique of fluorometric alkaline elution was used to measure DNA interstrand crosslinking with time after a 1-h exposure to drug. The samples from the young group showed a more consistent pattern of crosslink formation and removal, with extensive, or in some cases complete, repair at 48 h. Those from the elderly group showed considerable inter-individual variation and significantly higher mean levels of crosslinking at 24 and 48 h. No samples showed complete repair at 48 h in this population. These results indicate an impaired DNA repair capacity in the cells from the elderly group. This may be a factor in the poor tolerance of chemotherapy in the ageing population.

Key words Cisplatin · DNA crosslinking · DNA repair · Ageing

Introduction

It is a commonplace of cancer chemotherapy that toxicity increases with age yet most tumours occur in the elderly, with about 50% of all solid tumors presenting in patients over 70 years [1]. Many clinical trials exclude patients over a certain age, and most studies do not report response rates and side effects related to age. There has been little systematic investigation of the reasons for increased toxicity, despite this problem being the basis for decisions about treatment with chemotherapy in the elderly.

Cisplatin is used widely for the treatment of solid tumours. It is the first-line single-agent treatment for carcinoma of the ovary [2] and is widely used in treatment of testicular [3], non-small-cell lung and bladder cancers. The main early toxicity is vomiting, and later toxicity can be severe, causing neuropathies and renal failure [4, 5]. The major cytotoxic target of cisplatin appears to be DNA. The type of DNA lesion responsible for the cytotoxicity and antitumour activity, however, is not clearly established. The cytotoxicity of cisplatin in cultured cells has been found to be directly related to total platinum binding [6, 7] interstrand crosslinking [8, 9], and intrastrand crosslinking at d(GpG) and d(ApG) sites [10]. The relative percentages of these lesions have been studied in isolated DNA [11], DNA isolated from drug-treated murine cells [12] and in leukocyte DNA from cancer patients receiving cisplatin-based chemotherapy [13, 14]. In each case the relative percentages were similar, with intrastrand adducts accounting for approximately 90% of lesions, interstrand crosslinks less than 1%, and the remainder the result of a variety of lesions, including DNA–protein crosslinks and monoadducts. Increased DNA repair capacity plays a major role in resistance to cisplatin in several mammalian cell lines, including human ovarian and bladder cells [12, 15, 16].

The present study investigates the onset and disappearance of crosslinks in the peripheral blood mononuclear cells from either young (< 25 years) or elderly (> 72 years) volunteers exposed in vitro to doses and durations of cisplatin achievable clinically.

Materials and methods

Preparation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMs) were isolated from two populations of individuals. The elderly group (mean age 75 years, age range 72–81 years) was made up of patients admitted to the orthopaedic wards for surgical operations, who were otherwise fit...
and not taking any significant medication. Blood was taken pre-operatively and with informed consent. The younger group (mean age 24, age range 21–25 years) were laboratory staff, nurses or medical students, who were fit and not taking any medication. A sample of 30 ml was taken from each person, and PBMs were isolated using the ficoll-hypaque method, which included washing four times with MEM containing 10% fetal calf serum (FCS) before incubation in the same medium. Each experimental point was performed in triplicate.

Drug treatment in vitro

Cisplatin (Sigma Chemical Company) was dissolved in serum-free MEM by heating to 37°C for 30 min immediately prior to use. Where appropriate the PBMs were exposed immediately to drug for 1 h at 37°C. Cells were then washed twice in drug-free medium, resuspended in fresh medium, and reincubated for the requisite post-incubation time. Cell viability was assessed using trypan blue exclusion and cells counted on a haemocytometer at each time point. Viability always exceeded 90%.

Measurement of DNA interstrand crosslinks

A modification of the alkaline elution technique of Kohn [17] was used, in which the DNA is quantitated fluorometrically [18]. After appropriate post-incubation times, PBMs were chilled on ice and irradiated to introduce random DNA single-strand breaks (6 Gy, dose rate 4.5 Gy/min). They were then deposited onto polycarbonate filters (Nucleopore 25 mm diameter, 0.2 μm pore size) pre-chilled with cold PBS at 1.5–2.5 x 10^6 cells per funnel. The funnel outlet was plugged and cells were lysed with 2 ml sarkosyl lysis solution (0.2% sarkosyl, 0.4 M EDTA-Na2, 2 M sodium chloride, pH 10) containing 0.5 mg/ml proteinase K (Sigma) for 20 min. The funnels were then unplugged, allowing the lysis solution to drip through. The DNA was washed with 10 ml of 0.2 M EDTA-Na2, pH 10. After washing, the funnels were then reconnected to the pumps and the DNA was eluted at a constant rate of 2 ml/h with elution buffer (tetraethylammonium hydroxide, 0.02 M EDTA-Na2, pH 12.2). Five 6-ml fractions were collected over a 15-h period.

After this, 3 ml of each of the fractions was adjusted to pH 6.8–7.1 by the addition of 1 M potassium phosphate. The filters were removed from the funnels and incubated at 55°C for 1 h in elution buffer and then vortexed rapidly for 2 min. To each 3 ml sample, 0.8 ml 2.25 μM Hoechst 33258 was added, and after mixing the samples were allowed to stand for 30 min at room temperature. The fluorescence was then read on a fluorimeter (Perkin Elmer Model LS-2B) at an excitation of 350 and emission 475 nm. A blank elution curve was constructed using calf thymus DNA in the same buffer. The fraction of DNA remaining on the filter was calculated at each time point and elution curves constructed from control and drug-treated cells. The crosslink index was calculated at 12 h of elution according to the formula:

Crosslink index (in rad equivalents) = \[ \frac{\sqrt{(1 - R_o)\cdot(1 - R)} - 1}{R_o} \times 600 \]

where \( R_o \) and \( R \) are the relative retentions for the control and drug-treated irradiated cells, respectively. In each experiment control lanes containing DNA from unirradiated and irradiated human K562 cells were run to ensure consistency.

Results

Cisplatin-induced DNA ISCL were measured in PBMs from young and elderly volunteers following a 1-h exposure to the drug. Initial experiments were performed at a dose of 50 μM in 8 young and 7 elderly patient samples. Crosslinking was measured at 6, 24 and 48 h after incubation. In the samples from young patients the range of the cross-link indices observed at 6 h was 0–71. At 24 h fewer crosslinks were detected than at 6 h in all samples except one, and in half of the samples no crosslinks were detectable. By 48 h no crosslinks were detected in any sample. In the PBMs from the 7 volunteers over 72 years the crosslink indices ranged from zero to 55 at the 6-h time point. At 24 h 4 had an increased number of crosslinks and only 2 had undetectable levels. By 48 h post incubation only 3 had no detectable crosslinking.

Despite considerable inter-individual variation the data from this small sample number suggested distinct differences between the two populations and suggested that there might be differences in the cellular processing of DNA interstrand crosslinks between the two groups. This prompted a larger study with the higher concentration of 70 μM cisplatin, in order to maximise any differences between the two age groups. In this study, 12 volunteers were studied in each age group and crosslink levels were again measured at 6, 24, and 48 h following a 1-h treatment. The data from the young volunteers are shown in Fig. 1. At 6 h the range of crosslink indices was 14–73. In 6 samples more crosslinking was observed at 6 h than at 24 h, whereas in the other 6 increased levels were observed at the 24-h time point. In all samples, however, the number of crosslinks was smaller at 24 h than at 24 h. In 2 samples there was complete repair by 48 h, and in 11 of the 12 samples the crosslink index was less than 20 following this dose of cisplatin.

The results in the 12 samples from the elderly volunteers (Fig. 2) show considerable inter-individual variation, which is more marked than in the young group.

![Fig. 1 DNA interstrand crosslink profiles in PBMs obtained from young volunteers and incubated with 70 μM cisplatin for 1 h](image-url)