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CYTO GENTIC ANALYSIS OF VITAMIN B12 INDUCED BY CYCLOPHOSPHAMIDE MOUSE BONE MARROW CELLS

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Abstract:

In the present investigations, the preventive effect of Vitamin B12 was evaluated against cyclophosphamide induced cytogenetic analysis in the mouse bone marrow cells. The single ip administration of Vitamin B12 at the dose of 125, 250 and 375 mg/kg body weight, 24 hours prior the administration of cyclophosphamide (at the dose of 50 mg/kg) have significantly prevented the cytogenetic analysis formations in dose dependent manner in bone marrow cells of mice as compared to cyclophosphamide group. However, Vitamin B12alone has not induced cytogenetic analysis formations in bone marrow cells as compared to control group. Therefore seems to have a preventive potential against CP-induced formation in Swiss mouse bone marrow cells.

Keywords: Vitamin B12, Cyclophosphamide, Cytogenetic Analysis

Number of Tables : 1 Number of References: 15

INTRODUCTION

More than 50 cytotoxic drugs are commercially available for treating cancer patients. Clinical and laboratory studies have proved many of them to be mutagens, carcinogens, or teratogens in humans and animals, while patients that received therapeutic dosages of these drugs have exhibited a long list of acute and chronic adverse effects, including second cancers (Baker ES, Connor TH, 1996).

Most antineoplastic drugs are soluble in body fluids and can thus be delivered systemically. Although therapeutic efforts are directed toward cancer tissue, the DNA of non-cancer cells is also subjected to damage during chemotherapy. There is an increasing interest in the assessment of biological markers that detect the damage produced after cancer chemotherapy. As the target cells in determination of the correlation between exposure of cancer patients to antineoplastic drugs and / or radiation and alterations of DNA repair efficiency, peripheral blood lymphocytes are frequently used (Rigaud O, Guedeney G etal., 1990). After intravenous administration of various antineoplastic drugs in the peripheral blood leukocytes of all cancer patients studied showed a significantly increased level of DNA damage compared to the pre-treatment values. Administration of antineoplastic drugs in standard protocols is accompanied by significant DNA damage in peripheral blood leukocytes (Baker ES, Connor TH, 1996).

A number of antineoplastic drugs are used to combat with different types of cancer which have also shown to be mutagenic in various test systems. Various antineoplastic drugs such as cisplatin, cyclophosphamide, Tamoxifrn Genecitabine and Paclitaxel etc have shown to be clastogenic effects in various test systems (Garrone et al., 1993; Takeda et al., 2001; Padmalatha Rai and Vijaylakshmi, 2001; Boffetta et al., 2007; Padmanabham et al., 2008).

In early studies (Arinaga S, Akiyoshi T etal., 1985) of the lymphocyte activation induced by allogeneic cells in a mixed lymphocyte culture, it was found that peripheral blood lymphocytes obtained from cancer patients 7 days after treatment with doxorubicin showed an about twice as high cytotoxicity with respect to the pretreatment control values.

The chromosome aberration assay is a powerful classical cytogenetic tool for genotoxicity testing and can be used as a validation test for Comet assay results (Hartmann et al., 2003).

Genotoxicity studies have frequently been conducted on mammalian systems to evaluate the mutagenic potential associated with acute or chronic exposure to chemical agents. Recently, particular attention has been devoted to the Comet assay in order to identify substances with genotoxic activity. This test allows the detection of DNA damage such as single and double-strand breaks and alkali labile lesions in individual cells after acute and/or chronic exposure to a genotoxic agent (Tice et al., 2000).

Doxorubicin induced a significant increase ($p < 0.05$) in DNA damage score and the frequency of chromosome abnormalities, these results being consistent with those reported by other authors (Anderson et al., 1998). The anticancer drug doxorubicin (DOX) is toxic to target cells, but also causes endothelial dysfunction and edema, secondary to oxidative stress in the vascular wall. Thus, the mechanism of action of this drug may involve chemotoxicity to both cancer cells and to the endothelium (Matthew B, Wolf and John W, Baynes, 2006).

The effect of various concentrations of Aegle marmelos (AME) on the doxorubicin (DOX)-induced genotoxic effects in mice bone marrow was studied. Treatment of mice with different concentrations of DOX resulted in a dose-dependent elevation in the frequency of micronucleated polychromatic (MPCE) as well as normochromatic (MNCE) erythrocytes in mouse bone marrow. The frequencies of MPCE and MNCE increased with scoring time, and the greatest elevation for MPCE was observed at 48 hours post-DOX treatment, whereas a maximum increase in MNCE was observed at 72 hours post-DOX treatment. This increase in MPCE and MNCE was accompanied by a decline in the polychromatic erythrocytes–normochromatic erythrocytes (PCE/NCE) ratio, which showed a DOX-dose-dependent decline. Treatment of mice with 200, 250, 300, 350, and 400 mg/kg body weight of AME, orally once daily for 5 consecutive days before DOX treatment, significantly reduced the frequency of DOX-induced micronuclei accompanied by a significant elevation in the PCE/NCE ratio at all scoring times. The greatest protection against DOX-induced genotoxicity was observed at 350 mg/kg AME. The protection against DOX-induced genotoxicity by AME may be due to inhibition of free radicals and increased antioxidant status (Ponemone Venkatesh, Bellary Shantala etal., 2007).

MATERIALS & METHODS

In the present investigations, the preventive effect of Vitamin B12 was evaluated against cyclophosphamide induced cytogenetic analysis in the mouse bone marrow cells. The single ip administration of Vitamin B12 at the dose of 125, 250 and 375 mg/kg body weight, 24 hours prior the administration of cyclophosphamide (at the dose of 50 mg/kg).. Animals were dissected out for femur bones and flushed out bone marrow into a Petridish containing 0.75m KCl (hypotonic) solution to get a homogenous suspension. The cell suspension was collected in clean centrifuge tubes and incubated at 370c for 45minutes. After incubation the tubes were centrifuged for 10minutes at 1000rpm. The supernatant was discarded and to the pellet 5ml of freshly prepared pre-chilled fixative (3:1 methanol and acetic acid) was added and allowed to stay at room temperature for 10minutes.

This step was repeated 4 to 5 times. Finally the cells were fixed in fresh fixative. Air-dried slides were prepared by dropping one or two drops of the final suspension on the grease-free, pre-chilled slides with the Pasteur pipette. The slides were dried

immediately by air-drying method, coded and stained in 2% Geimsa (2mL of Geimsa + 2mL of Sorenson's buffer + 46 mL of distilled water) for 10 minutes.

RESULT & DISCUSSION

The mutagenic potential of any test compound is evaluated by scoring and analyzing the frequency of structural aberrations and numerical aberrations (Datta et al., 1970). Although chromosomal damage in somatic cells is not transmitted to the offspring (Evans, 1976), there is every possibility of transmission of these aberrations to further filial generation as stable abnormalities (Hirschhorn And Collins, 1969).

Among the structural abnormalities, gaps are termed as achromatic lesions and remain unstained by the feulgen technique (Reiger et al., 1976) reported gaps in metaphase chromosomes to be the result of insufficient folding of chromosome fibres.

Cyclophosphamide	CE±SE
Vitamin B12 (125mg/kg) + CP (50 mg/kg)	3.34± 0.926
Vitamin B12 (250mg/kg) + CP (50 mg/kg)	1.40 ± 0.603
Vitamin B12 (375mg/kg) + CP (50 mg/kg)	1.23 ± 0.495
Vitamin B12 (125 mg/kg) Alone	0.45 ± 0.22

Table 1: *Shows the effect of Vitamin B12 in bone marrow cell*

* Denotes Statistical Significance at P<0.05 in't' test. When compared with respective positive control group. Each group consists of six animals.

Cyclophosphamide plus etoposide was associated with a (EBMT complete plus partial plus minimal) response rate of 55.6% in our study. However, the regimen was toxic, with 75% of patients requiring hospitalization, 70.5% of patients developing neutropenic fever, and 67% of patients requiring platelet transfusions for severe thrombocytopenia. It is interesting to note that the overwhelming majority of bacteremic episodes in our patients were due to Gram-positive normal flora, which we suspect may have gained access to the blood through central catheters or damaged mouth or bowel mucosa. Dimopoulos et al17 were the first to report using a combination of cyclophosphamide and etoposide for the treatment of MM, although the doses and schedule used (600 mg/m2 cyclophosphamide plus 180 mg/m2 etoposide daily 5 days) were different from ours. In a group of 52 patients with advanced or VAD- refractory myeloma, this regimen resulted in a response (75% M protein reduction, disappearance of Bence Jones protein and decrease of bone marrow

plasmacytosis to <5% for 2 months) of 42%. Neutropenic fever occurred in 49/52 (94%) of patients, and two died of that complication. Severe thrombocytopenia (<20 109/l) occurred in 65% of patients. These results are similar to ours. All of the 77 patients in our study were able to undergo ASCT, receiving a median of 7.03 (range 1.1–25.6) or 7.92 (1.96–38.24) million CD34+ progenitor cells/kg in the cyclophosphamide or cyclophosphamide plus etoposide group, respectively. As expected, this number of cells resulted in prompt neutrophil and platelet count recovery following transplant in almost all patients. Thus, we could not identify any factors that predicted the tempo of engraftment.

Our study suffers from several limitations. Patients were not randomly assigned to cyclophosphamide or cyclophosphamide plus etoposide, so that comparisons between the two groups have limited applicability. In addition, because these patients proceeded to receive ASCT at an average of 6 weeks after mobilization chemotherapy, it was not possible to observe the duration or maximal effect of chemotherapy achieved by the mobilization regimen. The method of progenitor cell collection (CD34+ cell tracking followed by large volume leukapheresis) was highly efficient, thus blunting the effect of some pre-treatment variables on mobilization yield. Nevertheless, this study confirms previous reports of cyclophosphamide plus growth factor as a useful regimen for the mobilization of progenitor cells for autologous transplantation, and showing that the regimen is also associated with a remarkable disease response. The addition of etoposide provides at least as many progenitor cells and perhaps a better antimyeloma effect; unfortunately, however, it was clearly more toxic. Future studies should address the contribution of pre-ASCT disease bulk reduction on event-free and survival rates, and should aim at reducing the severe toxicities associated with this approach to mobilization.

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