Cyclophosphamide (CPA) is a nitrogen alkylating agent used for various types of cancer & some autoimmune diseases. It is a prodrug that is converted to its active metabolites in liver. At higher doses, it interrupts cell cycle by forming DNA cross linking, DNA lesions. It mediates G0/G1 and S phase arrest. At chromosomal level, this anti-neoplastic drug causes chromosomal aberration (CA) and increase in frequency of sister chromatid exchange (SCE). It also causes apoptosis and cell toxicity. Due to its wider clinical use, study of its genotoxicity has significant practical aspect. Recent studies strongly evidence that the compounds showing antioxidant activity have protective effect against chemical induced toxicity of CPA. The present review focuses on past and present studies regarding the genotoxic effects of cyclophosphamide & remedies for its cytotoxic action to be a better therapeutic agent.

Key words: CPA, CA, SCE, Genotoxicity

INTRODUCTION

Cyclophosphamide (cytotoxan) is an alkylating anti-tumor drug. Its chemical formula is C16H10Cl2N2O2P. Its st. is shown in Fig-1, was identified by X-ray diffraction. CPA is a member of oxazaphorine group. The other members of this group are Ifosfamide & Trofosfamide. Another synthetic analog of this group is Mafoside which is a stable sulfonic acid salt of 4-CPA. Cyclophosphamide shows anti-tumor activities against a broad range of cancers including malignant lymphomas, myeloma, leukemia, neuro-blastoma, adeno-carcinoma, retino-blastoma, and breast-carcinoma. CPA given at low doses act as either an anti-angiogenic or an immuno-stimulatory agent in combination with other immunotherapies in the treatment of cancer. CPA is also used for the mobilization of hematopoietic progenitor cells from the bone marrow into peripheral blood.

It is also a well known immunosuppressive agent used for graft rejection in case of renal, hepatic, and cardiac transplantation. Its therapeutic use as an anticancerous drug is limited due to its side effects.

DNA DAMAGE BY CPA

DNA is the primary target in terms of the teratogenic, mutagenic, and antineoplastic effects of CPA. Effects of CPA on DNA have been reported widely in mammalian cells, both of somatic and germ cell origin. CPA is supposed to exert its cytotoxicity via the cross-linking of cellular DNA, and studies demonstrated that following drug exposure there is occurrence of interstrand and DNA-protein cross-links, but no single strand breaks. CPA inhibits embryonic DNA synthesis and does so prior to its effect on RNA or protein synthesis. Although CPA is known to produce DNA cross-links, other DNA lesions are produced as well. CPA mediates G0/G1 and S phase arrest, accumulation of cells in G0/G1, in comparison to the control, whereas higher concentrations causes dose-dependent G0/G1, S phase and G2/M phase inhibition.

The cytotoxic action of CPA results mainly due to phosphoramide mustard-induced DNA cross-linking. Phosphoramide mustard is a bifunctional alkylating agent that binds to the N-7 position of guanine, to the phosphate backbone of DNA. Exposure of cells to phosphoramide mustard results in the induction of a mixture of interstrand, DNA-protein cross-links & enlargement of cells. Phosphoramide mustard significantly increases the amount of cross-linked DNA after incubation with intact LM4 cells or nuclei isolated from these cells. Acrolein, on the other hand is reported to bind to proteins, to form DNA adducts, to create abasic sites, and to induce DNA single-strand breaks (SSB).

The concurrent results of biological and biochemical experiment in S.cerevisiae indicate that non-activated CPA has a weak but detectable monofunctional alkylating potency, leading to DNA strand breaks where as activated CPA has the ability to induce both DNA strand breaks and interstrand cross-links.

A number of studies have confirmed that CPA is a DNA-damaging agent during spermatogenesis. Exposure of rat spermatocytes to CPA results in synaptic fragmentation, fragmentation of the synaptonemal complex, and altered centromeric DNA sequences. Exposure of CPA for 6wk induces a significant increase in...
both DNA single strand breaks and cross-links in spermatozoal nuclei; the cross-links were primarily attributed to DNA-DNA linkages. As there is no DNA repair during spermatogenesis, damage to the genome at this stage results in the production of dysfunctional germ cells.[31] It also causes nucleosomal DNA fragmentation in lymphocytes at higher concentration.[32] DNA Strand breaks and DNA cross-links were detected in peripheral mononuclear blood cells of 15 ovarian carcinoma patients who received CPA therapy.[33]

CHROMOSOMAL DAMAGE BY CPA

In somatic cells CPA has been shown to produce gene mutation, chromosomal aberration, micronuclei and sister chromatid exchanges in a variety of cultured cells. Chromosome analysis of mice was performed in unfertilized metaphase II oocytes after induced ovulation against the alkylating agent CPA. The investigation demonstrated the dose-dependent frequency of the induced types of chromosomal abnormalities. In another experiment the epipodymal spermatozoa from male rats were treated with CPA and the chromosomal abnormalities were studied by using fluorescent in-situ hybridization (FISH) assay. Results showed that CPA disrupts meiotic events before pachynema stage. The various abnormalities seen were disomy, nullisomy and diploidy spermatozoa with double size than normal cells.[34]

Patients receiving CPA chemotherapy were studied for acute cytogenic effect on the peripheral blood lymphocytes by chromosomal aberration and micronuclei assay. Results showed highest level of chromosomal damage as well as highest incidence of micronuclei.[35] To determine the frequencies of chromosomal aberrations (CA) and sister chromatid exchanges (SCE) in peripheral blood lymphocytes, cultures of women breast cancer cells treated by chemotherapy along with cyclophosphamide were investigated. The number of patients varies from 1 to 3 for SCE and 2 to 5 for CA.[36]

Chromosomal aberration also seen in lymphocytes of medical staffs continuously exposed to cytostatic agents such as CPA. The chromosome and chromatid breaks increases due to long term occupational exposure to these drugs.[37] In another study when a male rat is exposed to CPA there occurs synaptoneural chromosomal damage and this damage is more as compared to meiotic metaphase chromosomal damage.[38] The chromosomal aberration was seen as translocations in bone marrow cells of rat exposed to CPA along with oxidative stress.[39] In another study CPA was given to pregnant rats on 12th day of gestation. The pups were collected and liver dissected for cytogenetic analysis. The dispersed metaphase plates showed a variety of abnormal chromosomal features such as gaps, chromatid & chromosomal breaks,acentric fragments,centromeric breakage,chromatin bodies and aneuploidy.[40]

The effect of CPA on chromosome also examined by using animal model. The fifth instar nymphs of grasshopper were treated with various conc. of CPA. The effect was analysed at 42h time point which shows that the aberrations observed were mainly chromatid and chromosome breaks. It also showed that CPA induced a dose and time dependent increase in number of CA per cell.[41]

MUTAGENICITY AND APOTOPSIS DUE TO CPA

The mutagenic effect of CPA is mainly due to its active metabolite phosphoramide mustard, which forms mono & bi-functional guanine adducts. Nitrogen mustards are genotoxic in virtually every assay, and produce a wide array of mutations, including base substitutions at both G.C and A.T base pairs, frameshifts, centromeric breakage, chromatin bodies and aneuploidy.[42]

It was shown that CPA induces apoptosis in mature human lymphocytes at concentrations that are achieved in vivo. Apoptotic cell death of lymphocytes and adenine adduct.[43] After biotransformation of isolated rodent liver homogenates (S9 fraction) through CPA and its analogs exhibit mutagenic activity in E.coli gal-+ and arg-+ and his-+ in Salmonella typhimurium TA1535. Both mutations are back mutation in nature.

PREVENTION OF CPA INDUCED CHROMOSOME & DNA DAMAGE

Antioxidants, polysaccharides, flavonoids were found to inhibit DNA & chromosomal damage induced by CPA. To study the effect of lemon juice on CPA induced cytotoxicity, mice were treated orally with CPA prior to and after treatment with orange juice. DNA damage was evaluated by the comet assay in peripheral white blood cells. Under these experimental conditions, orange juice reduced the extent of DNA damage caused by this mutagen.[44] Pretreatment with 50-250 mg/kg body wt of mustard leaf extract (MLE) for seven days significantly increases the frequency of chromosomal damage by CPA. The protective effect against chromosomal damage was associated with modulation of lipid peroxidation as well as an increase in GSH and the GSH-dependent enzyme glutathione S-transferase.[45] These findings indicate that intake of the green leafy cruciferous vegetable mustard leaf can lead to protection against in-vivo genotoxicity and oxidative stress.

In another study the anticancer drug CPA was administered intra-peritonially to induce mutagenic effect in Swiss albino mice. The pretreatment with tutsin, a naturally occurring tetrapetide abolished effects such as CA, formation of micronucleated polychromatoid erythrocytes (MNPC’s), as well as oxidative stress and decrease in lipid peroxidation in liver of the animals in dose-dependent manner and also increased mitotic index in the experimental animals.[46]

To study the effect of carotenoids on CPA induced genotoxicity β-Carotene at different conc. was administered by gavage for 5 consecutive days. 4 h after the last treatment with β-carotene, the mice were injected intraperitoneally with CPA, and the bone marrow cells were evaluated for the frequency of chromosome aberrations. The results showed that β-carotene was effective in decreasing the frequency of chromosome aberrations induced by CPA in the bone marrow cell culture of mice treated at a concentration up to a level after which this effect was not observed.[47] In another experiment rats were treated with alpha-lipoic acid (LA) and CPA, the frequency of CA and single strand breaks were significantly decreased in comparison to those given CPA alone. CPA administration distinctly triggered the apoptosis and necrotic cell death, and the pretreatment affected cell death by decreasing the number of apoptotic and necrotic cells.[48]

To study the effect of flavonoids on CPA teratogenicity, folic acid (FA) and soybean isoflavone (SIF) was given in combined supplementation & the results showed that flavonoids decreased CPA induced damage, such as damaged nuclear DNA, early apoptotic morphological changes.[49] Mouse bone marrow cells were orally pretreated with saffron, garlic and citrus either alone or in combination for five consecutive days, 2h prior to the administration of CPA. Maximum reduction in the frequencies of micronucleated polychromatoid erythrocytes (MNPC) was induced by CPA and it was observed when all the three compounds were administered together.[50]

To study the effect of organo-sulphur compounds three different doses of garlic extract were tested for their modulatory capacity on the mutagenicity exerted by CPA. The results indicate a significant decrease in the frequency of CA and SCE suggesting that the garlic extract modulates the CPA induced genotoxicity in a dose dependent manner.[51] In another research CPA was administered intraperitoneally (50 mg/kg) and diphenylmethyl selenocyanate given orally (3 mg/kg) in a pretreatment and concomitant treatment. Results showed that diphenyl-methyl selenocyanate significantly increased GSH, glutathione peroxidase, and catalase levels whereas decreased the lipid peroxidation levels in both liver and lung tissues of the animals given CPA.[52] Mice were orally (gavages) pretreated with solutions of hesperidin at four different doses for five consecutive days. Then they were injected intraperitoneally on the fifth day with CPA. It showed that hesperidin, with anti-oxidative activity, reduced the oxidative stress and genotoxicity induced by CPA in mouse bone marrow cells.[53]

CONCLUSION

Despite of CPA's antitumor activity against a broad range of tumors, its clinical activity is limited due to its pharmacokinetic variability, resistance & severe host toxicity. So synthesis & development of novel oxazaphosphorine analog with favourable pharmacokinetic-dynamics properties is needed. As CPA requires metabolic activation by CYP2B & other polymorphic enzymes. Hence it was suggested that the inducing agent liver P-450 targeting at these enzymes could be used to enhance its therapeutic use. In order to study its effect on other cellular functions, it is necessary to develop derivatives of CPA which can be stable in crystalline form & hydrolysed in-vitro without metabolic activation.

As many anti-oxidants, flavonoids, polysaccharides, cytochromes, peptides have modulatory effect on the genotoxicity induced due to CPA. So the components of these compounds which are exactly necessary for exerting the anti-oxidative effect should be purified. This will lead to development of drugs which can be given in combination with CPA to reduce its side effects. Although positive results have been reported for CPA both as a single agent as well as in combination therapy. The results reviewed interpreters that in order to effectively use this drug for clinical purpose more studies need to be done regarding its genotoxic effect & factors that regulating them especially in human cells. Despite great advances in understanding its effects on DNA & chromosome, some important questions regarding its genotoxic effects remain to be answered.

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