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Effects of radiation and vitamin C treatment on metronidazole genotoxicity in mice

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ABSTRACT

The impact of exposure to low dose radiation (LDR) on human health is not clear. Besides, cross adaptation or sensitization with pharmaceutical agents may modify the risk of LDR. In the present study, we analyzed the interaction of radiation and metronidazole (MTZ) in inducing chromosome aberration (CA) and micronucleus (MN) in the bone marrow cells of Balb/C mice in vivo. Further, we evaluated the efficacy of vitamin C to reduce MTZ induced genotoxicity. We found that 10, 20 and 40 mg/kg of MTZ induced dose dependent increase in the frequency of CA ($r = 0.9923$, $P < 0.01$) as well as MN ($r = 0.9823$, $P < 0.05$) in polychromatic erythrocytes. However, MTZ did not affect the ratio of polychromatic erythrocytes to normochromatic erythrocytes indicating lack of cytotoxicity. Supplementation with vitamin C prior to MTZ treatment significantly reduced the frequency of CA ($P < 0.001$) as well as MN ($P < 0.001$). Radiation (0.5 Gy) exposure prior to MTZ treatment produced a less than additive (for CA) to additive (for MN) effects. However, radiation exposure following MTZ treatment produced additive (for CA) and synergistic (for MN) effects. Further, vitamin C pre-treatment also reduced the genotoxicity indices following the combined treatment of MTZ and radiation. Our findings suggest that MTZ may sensitize bone marrow cells to radiation exposure and enhances genotoxicity. We recommend more studies on the interaction of LDR and marketed pharmaceuticals to minimize possible harmful outcomes through appropriate precautionary measures.

1. Introduction

Modern medical diagnostic technology has revolutionized the health care system. However, this has also resulted in the exposure of a significantly large population (both stakeholders and professionals) to low dose radiation (LDR) emanating from radiodiagnostic procedures. The major debate around LDR is on possible health risks to the exposed population. Concerns about the long-term risks associated with LDR have widened [1–3]. Most of the radiation protection agencies adopt the linear non-threshold (LNT) model. The LNT hypothesis assumes that any radiation dose, no matter how low, has the potential to increase the risk of cancer and heritable mutations [4]. However, the LNT hypothesis has been challenged both by those who believe that LDR is more damaging than the hypothesis predicts as well as those who believe that it is less harmful, and possibly even induce adaptive response (reviewed in [5]).

Olivieri et al. [6] initially advocated the concept of adaptive response (often called hormesis). In recent times, several reports have appeared in the literature on LDR induced adaptive response in exposed individuals to higher dose of radiation [7–9] as well as chemical agents (reviewed in [10]). On the contrary, increased cancer risk [11,12], chromosomal alterations [13,14], mutations and genome instability [15] have been reported following exposure to LDR.

The impact of exposure to LDR on human health remains inconclusive. Uncertainties remain regarding many aspects of LDR induced adaptive response and the underlying mechanism. As it has been rightly said, 'life is a risky business'. In our day-to-day life activities, we are exposed to numerous natural and manufactured chemicals. Cross adaptation or sensitization may play important role in modifying the risk of LDR. Therefore, it is imperative to have more information on such phenomena [16]. In a previous study on the interaction of radiation and chloroquine, we [17] found that exposure to 0.5 Gy either prior to or following chloroquine treatment produced no synergistic effect in genotoxic evaluations in somatic cells, but synergism was found in the germ cells assessed using sperm head morphology.

In the present study, we analyzed the interaction between radiation and metronidazole (MTZ) to induce genotoxicity, using

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chromosome aberration (CA) and micronucleus (MN) as study parameters in the bone marrow cell of Balb/C mice in vivo. Further, we evaluated the efficacy of vitamin C to reduce MTZ and/or combined treatment of MTZ and radiation induced genotoxicity. MTZ is one of the most used antibacterial and antiprotozoan drugs and figures in the essential drug list of the World Health Organization [18]. According to International Agency for Research on Cancer [19], MTZ has proven carcinogenic properties in animals but suspected carcinogenic effects in man. The present study is relevant in the context that patients or professionals exposed to radiation may also be under MTZ medication or vice versa.

2. Materials and methods

2.1. Test animals

The Institutional Animal Ethics Committee of Assam University, Silchar, India has approved the present study. We used inbred Balb/C mice for the experiments. The animals were maintained in closely inbred colony under conventional laboratory conditions at a room temperature of 25 ± 3 °C and in 12 h dark and light cycles. Commercial food pellets (Amrut Laboratory Animal Feeds, New Delhi) and water were provided ad libitum. We used both male and female mice in the age group between 6 and 8 weeks, weighing 20–25 g for the experiments. The animals were randomly assigned to different treatment groups of three animals per group, and it was ensured that no group had either exclusively males or females. The cages were randomly assigned to different treatment groups without gender bias, and the experiments were repeated twice.

2.2. Test chemicals

Metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole], CAS Registry No. 443-48-1, chemical formula $C_6H_9N_3O_3$ (Fig. 1), molecular weight of 171.16, was obtained from J.B. Chemicals and Pharmaceuticals Ltd, India. L-Ascorbic acid (Catalogue No. A92902; >99% pure) and colchicines (Catalogue No. C9754; ≥95% pure) were purchased from Sigma Chemicals (St Louis, MO, USA), mitomycin C was purchased from Biochem Pharmaceuticals Industries Ltd., Daman, under license from Kyowa Hakko Kogyo Co. Ltd., Japan (Batch No. LB7122002). Giemsa's stain was procured from HiMedia Laboratories, Mumbai, India. All other chemicals used were of analytical grade. Cobalt-60 γ radiation was given at the Radiology Department of Silchar Medical College, Assam. The buffer for stains and reagent solutions were always prepared in glass-distilled water. The chemicals to be administered to the mice were prepared in physiological saline except for colchicines, which was prepared in double distilled water. The concentration was adjusted so as to administer at a final volume of 1 μl/g of body weight.

2.3. Dose and treatment

The highest sub-acute dose (40 mg/kg) used in the present study was lower than the human equivalent dose [20]. Three sub-acute doses (10, 20 and 40 mg/kg bw) and one chronic dose (3.4 mg/kg bw × 3 days) administered through the intra peritoneal (i.p.) route. The bone marrow cells were fixed at 24 h after the treatments. Mitomycin C (2 mg/kg bw) was used as positive control. The control animals received an equal volume of normal saline. In vitamin C intervention study, the dose of vitamin C used was 500 mg/kg bw per day. Vitamin C was given orally by gavage for 5 consecutive days prior to treatment with MTZ.

Radiation exposure was given using a Phoenix make external beam radiotherapy unit (Theratronics, Canada). The radioisotope used was cobalt-60. We used a dose rate 115 cGy/min at the time of irradiation and energy 1.25 MeV. Radiation was given through whole body exposure either 30 min prior to or 30 min following MTZ treatment. The radiation exposure facility is located in a driving distance of approximately 25 min. On account of the time interval between the treatments and radiation exposure and vice versa as well as any possible transport related stress factor, all the animals including the controls were transported to the radiation exposure facility 1 day prior to the exposure. Following an appropriate time-lapse post radiation exposure, all the animals were brought back to the laboratory.

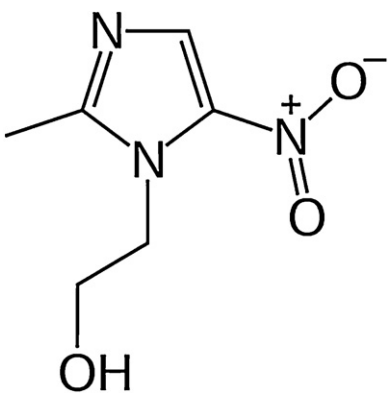

Fig. 1. Chemical structure of metronidazole.

2.4. Chromosome aberration assay

Experimental animals were sacrificed 24 h after the last treatment. Colchicine (2 mg/kg bw) was injected (i.p.) 1.5 h prior to sacrifice. Bone marrow cells were collected from both the femora by flushing in KCl (0.56% at 37 °C), incubated at 37 °C for 18 min. The material was then centrifuged at 1000 rpm for 5 min, fixed in acetomethanol (acetic acid:methanol, 1:3). Centrifugation and fixation (4 °C) were repeated twice at an interval of 30 min. The cell pellet, suspended in a small volume of the fixative, was dropped on to chilled slides, flame-dried and stained in the following day in 5% buffered Giemsa (pH 7.0). One hundred good metaphase spreads were examined per animal and CA were classified as indicated in Table 1. Gaps have not been considered for statistical analysis because of their controversial genetic significance.

2.5. Micronucleus assay

After 24 h of treatment, the animals were sacrificed by cervical dislocation. Bone marrow smears and staining was done following the method of Schmid [21] with minor modifications. In brief, bone marrow cells were collected in 0.9% NaCl (37 °C) instead of fetal calf serum. Staining was done in 5% buffered Giemsa (pH 7.0). The slides were mounted with cover slip using DPX mountant (a resin based mounting medium consisting of distyrene, a plasticizer and xylene). Polychromatic erythrocytes (PCEs) stain light blue to gray and normochromatic erythrocytes (NCEs) stain light orange to straw yellow. At least 1000 PCEs per animal were analyzed for the presence of MN. The corresponding number of NCEs was recorded for determination of PCE/NCE ratio.

2.6. Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze CA and MN data at different dose levels. Prior to ANOVA, an *F*-test was performed. The data were square root transformed wherever these did not meet the assumptions of normality. Post hoc analysis was performed for multiple comparisons among the treatment groups. Gender effects were analyzed using pair sample *t*-test. Dose–response analysis was performed using linear regression analysis. We determined the predicted frequency of CA and MN using Abbot formula for the combined treatment of radiation and metronidazole for comparison with the observed effects. We used the expression $C_{exp} = A + B - (AB/100)$, where C_{exp} is the predicted frequency of the combined treatment of radiation and MTZ, and A and B are the individual frequencies of radiation and MTZ respectively. The analyses were performed using SPSS® 18.0 statistical software. Variances were considered to be significant at P value less than 0.05.

3. Results

The animals tolerated the highest dose without any visible symptom of toxicity. Qualitative assessment of metaphase plates from the bone marrow cells revealed various types of CA, which consisted of chromatid and isochromatid types of gaps and breaks, exchanges and sister chromatid union following MTZ treatment (Table 1). Sister chromatid unions result in the formation of ring-shaped chromosomes formed due to special type of chromosome breaks producing sticky ends which join together. The gaps and breaks were more frequent than other types of aberrations. A preliminary assessment of the distribution of breaks and gaps revealed that the distal regions of the long chromosomes were more vulnerable to MTZ.

Quantitative analysis of CA showed dose-dependent increase in the frequency of aberrations following MTZ treatment (Table 1). Except for the lowest dose (10 mg/kg), 20 and 40 mg/kg of MTZ induced significant ($P < 0.02$ and $P < 0.001$ respectively) increase in the frequency of CA as compared to the negative control. The fractionated dose (13.4 mg/kg × 3 days) also induced higher frequency of CA ($P < 0.01$). Pair wise comparison among MTZ treated groups showed significant difference between the groups 10 vs. 40 mg/kg

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