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Modulation of gamma-ray-induced apoptosis in human peripheral blood leukocytes by famotidine and vitamin C

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Abstract

To study the radioprotective effects of vitamin C and famotidine against radiation-induced apoptosis in human peripheral blood leukocytes, peripheral blood was obtained from six healthy volunteers including three males and three females. Twelve microlitres of blood sample diluted in 1 ml complete RPMI-1640 medium was irradiated with various doses of gamma-rays (4, 8 and 12 Gy) in the presence or absence of various doses of vitamin C and famotidine. After 48 and 72 h incubation in a 37 °C CO_2 incubator, neutral comet assay was performed for all samples. At least 1000 cells were analyzed for each sample for presence of apoptosis. Data were statistically evaluated using Mann–Whitney non-parametric and ANOVA tests. Results show a significant increase in apoptosis induction following γ-irradiation with a dose dependent manner compared to controls ($p < 0.001$). Presence of famotidine at 200 μg/ml produced a significant protective effect against radiation-induced apoptosis for various doses of radiation. Similar effects were observed for vitamin C at much lower doses (10 μg/ml). Dose reduction factor (DRF) calculated for famotidine treatment was about 1.5, and above 2 for vitamin C treatment. These results suggest that both vitamin C and famotidine suppresses radiation-induced apoptosis when used with various doses of gamma-irradiation (4–12 Gy) probably via •OH radical scavenging and an intracellular antioxidation mechanism.

Keywords: Apoptosis; Human leukocytes; Gamma-rays; Vitamin C; Famotidine; Comet assay

1. Introduction

Exposure of mammalian cells to ionizing radiation activates genetic cascades of signaling events leading to cellular damage primarily through a spectrum of lesions induced in DNA. Recognition of these damages leads to the activation of specific proteins that in turn activate repair, cell cycle checkpoints, and apoptosis [1].

Apoptosis is a common mode of cell death in a variety of normal tissues following cellular insults including ionizing radiation [2–4]. Although apoptosis is not the major mode of cell death in irradiated cells in culture [5], however radiation-induced apoptosis has been documented in normal tissues such as small intestine, salivary glands and hematolymphoid tissues and some cells such as lymphocytes, male germ cells, thymocytes and epithelial cell lines [6–8]. Apoptosis is a well-characterized cell death process and follows a sequence of genetically programmed events. Fragmentation of chromosomal DNA is the biological hallmark of apoptosis [9,10]. It is well known that ionizing radiation induces variety of DNA damages including single and double strand breaks. As a result of double strand break (DSB) formation, chromosomal aberrations and micronuclei are formed. In

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addition, mitotic homologous recombination, mutations and oncogenic transformations may result from DSB [11]. It was shown that induction of apoptosis is not independent of DNA damage [12] and is likely to be activated by DSB which can be considered as a critical ultimate lesion in the apoptotic process [13]. Radiation-induced DNA damage is mainly due to water radiolysis in the vicinity of the DNA molecule and production of free radical and reactive oxygen species (ROS) [2,14].

These oxygen radicals are considered as mediators of apoptosis. The $^{\bullet}OH$ is generally considered the most damaging of the oxygen-based free radicals and it is believed to account for an estimated 50% of the total damage induced by free radical mechanisms [15]. Thus, any molecule that would efficiently scavenge the $^{\bullet}OH$ would be considered as important antioxidant.

Previous approaches toward therapeutic intervention against ROS damage have included administration of radical scavenger compounds, use of novel drugs that increase cellular production of constitutive antioxidants, or pharmacologic agents that modify the intracellular transport of antioxidants. In order to protect normal tissues from potential radiation damage, radioprotective agents are developed to reduce damaging effects of ionizing radiation, including lethality [16]. Extensive radiobiological research yielded numerous agents which, when given before radiation exposure, protected animals (primarily rodents) against radiation injuries. Unfortunately, most of these compounds at radioprotective doses were found to be toxic to humans [17].

Famotidine, a histamine H_2-receptor antagonist usually used for peptic ulcer treatment, has been shown previously to reduce radiation-induced DNA damages expressed as chromosomal aberration and micronuclei both *in vivo* and *in vitro* [18–20] and in leukocytes as assayed using the comet assay [21]. Famotidine has also been demonstrated that is highly powerful hydroxyl radical scavenger [22].

Also there are evidences that demonstrate the radioprotective ability of dietary antioxidants such as vitamins C, E, and beta-carotene [23–26]. Among these, vitamin C (ascorbic acid), the major water soluble antioxidant in blood, tissue and intracellular fluid is a very potent free-radical scavenger and has attracted tremendous attention. It has been shown that vitamin C and E reduced radiation-induced mutations and chromosomal damage in mammalian cells and radiation-induced lethality [23–26]. Also, vitamin C added at low concentration (1 μg/ml) before exposure of the cells to radiation, prevented induction of micronuclei [27]. In addition, administration of ascorbic acid has protected mice against radiation-induced sickness, mortality and improves healing of wounds after exposure to whole-body γ-radiation [28].

Based on the observations that demonstrate famotidine and vitamin C can reduce DNA damages, these compounds were used in the present study to investigate their protective effects against radiation-induced apoptosis.

Different methods of measuring apoptosis have been developed. Some are based on morphology, others on biochemical such as DNA laddering, TUNEL assay (transferase-mediated dUTP-biotin nick end labeling of DNA fragments), in situ end labeling (ISEL), comet assay and flow cytometric methods. However, there is a view that it is hard to diagnose apoptotic cells solely based on the phenotypes of cells [29]. The value of comet assay in measuring apoptosis in comparison with electron microscopy, flow cytometry using Annexin V and propidium iodide is shown by other investigators [30,31]. These studies confirm the consistency of the comet assay for detection of apoptosis in single cells and provide evidence for its applicability as an additional method to detect apoptosis. The comet assay has been used to measure apoptosis in several other studies describing apoptotic cells as structures with diffuse fan-like tails and small heads, whereas normal cells form larger, more defined heads with minimal DNA diffusion [10,30–32].

In this study, by the use of the neutral comet assay, the effect of famotidine and vitamin C was examined and compared on radiation-induced apoptosis in human peripheral blood leukocytes.

2. Materials and methods

2.1. Blood sampling and drug treatment

Peripheral blood samples were obtained by venopuncture from six healthy volunteers including three men (mean age 24 ± 5 years) and three women (mean age 24 ± 4 years), for famotidine treatment and various samples from two female donors (mean age 26.5 ± 0.1) for vitamin C treatment. These volunteers were all non-smokers without infectious disease, antibiotic consumption and X-rays during the last month prior to sampling. Fresh blood samples (12 μl) was transferred into a microtube (Eppendorf) containing 1 ml RPMI-1640 medium (Sigma) supplemented with antibiotics (Penicillin 100 IU/ml, Streptomycin 100 μg/ml), 10% L-Glutamine (2 mM) (Sigma) and 15% fetal bovine serum (FBS, Gibco, BRL).

The samples were prepared either in the complete RPMI-1640 medium or the medium containing famotidine or various doses of vitamin C. Famotidine powder as well as vitamin C (provided by Chemodaru Pharmaceutical, Tehran, Iran) separately, were dissolved in RPMI medium to achieve the appropriate concentrations (50, 100 and 200 μg/ml famotidine; and 10, 50 and 100 μg/ml vitamin C). Prepared samples in the