Journal of Animal and Feed Sciences, 15, Suppl. 1, 2006, 77–80

Vitamin C affects the antioxidative/oxidative status in rats irradiated with ultraviolet (UV) and infrared (IR) light

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ABSTRACT

Four groups of twenty growing Wistar rats were irradiated with either UV, IR , UV+IR light or were not irradiated (control). Ten rats from each group received a diet supplemented with 0.6% of L-ascorbic acid. The effects of the mega-dose of vitamin C were evaluated by changes in the antioxidative/oxidative status. UV and IR radiation promoted oxidative DNA degradation in rat livers and supplementation with ascorbic acid strengthened the prooxidative effects on DNA oxidation in rats irradiated with UV or IR light. Vitamin C also increased the tiobarbituric acid reactive substances (TBARS) concentration in rats from all groups except UV+IR-irradiated. The combined UV+IR light, corresponding to solar radiation, had no negative effects on redox homeostasis in rats. Furthermore, L-ascorbic acid showed antioxidative properties by increasing the concentration of Total Antioxidative State (TAS) in plasma, hence decreasing the production of reactive oxygen species (ROS) in UV+IR irradiated rats.

KEY WORDS: ascorbic acid, antioxidative/oxidative state, ultraviolet, infrared, rats

INTRODUCTION

The sun is the major source of radiant energy. Solar radiation consists of UV, IR and visible radiation. It is well known that solar UV radiation has been increasing in intensity at the earth's surface as a result of the depletion of stratospheric ozone (He and Häder, 2002). During UV and IR exposure, reactive oxygen species are formed in the body's cells (Nishi et al., 1991; Flanagan et al., 1998). These free

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radicals can produce major interrelated disruptions of cell metabolism, including lipid, protein and DNA oxidation leading, among others, to carcinogenesis. Furthermore, it was demonstrated that infrared radiation may increase the adverse effects of ultraviolet radiation (Kligman, 1982). Consequently, administration of an antioxidant may be a promising strategy to counteract numerous adverse reactions in the animal cell induced by solar light.

Vitamin C is one of the most important water-soluble antioxidants in mammalian tissues. It scavenges ROS and/or prevents their synthesis. (Banhegyi et al., 1997). Vitamin C can, however, react with transition metals such as iron, increasing oxidative damage of lipids, proteins and DNA (Auroma et al., 1999).

We presumed that a high dose of L-ascorbic acid can protect an organism from environmental stress induced by solar radiation. The hypothesis was tested by determining the effects on the redox status of rats supplied with vitamin C and irradiated with UV, IR and UV+IR light.

MATERIAL AND METHODS

Eighty male Wistar rats were kept in individual cages for 41 days under standard conditions: temperature 22^{o}C, humidity 50-70%, light/dark 12/12 h. The rats received a semi-purified mixture containing, %: maize starch 62, casein 14, saccharose 10, cellulose 5, rapeseed oil 5, mineral and vitamin mixture 3.7, L-cystine 0.2 and chloride choline 0.1. The animals were divided into four groups: control, and exposed to UV, IR, or UV+IR light. In each group 10 rats were supplemented with 0.6% of L-ascorbic acid. At the end of the experiment the rats were fasted for 12 h, sedated by intramuscular ketamine at a dose of 50 mg/kg body weight. Blood was sampled from the heart into heparinized tubes, and cooled to 4^{o}C prior to analysis. The animals were euthanized by ketamine overdose and then livers were removed. Determination of L-ascorbic acid in plasma was performed by HPLC. TAS in plasma, activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the red blood cells were analysed by Randox Laboratories Ltd. kits. Liver 8-oxo-2'-deoxyguanosine (8-oxo-dG) was determined using HPLC. Thiobarbituric acid reactive substances (TBARS) were measured by a colorimetric method using 1.2.3.3.-tetraethoxypropane (TEP) as the standard. Statistical analysis was carried out as multifactorial ANOVA and Duncan's multiple range test, using the Statgraphic 4.1 Plus software package.

RESULTS AND DISCUSION

Rats were exposed to three radiation regimes and to supplementation with ascorbic acid. The vitamin C concentration in the plasma of rats was affected by irradiation,

vitamin C supplementation, and interaction of both factors (Table 1). It increased due to UV radiation and supplementing vitamin C enhanced this effect. The marker of lipid peroxidation (TBRAS) increased in control animals receiving vitamin C, as well as in the UV and IR groups, but not in the group irradiated with combined UV+IR, with or without vitamin supplementation. The concentration of 8-oxo-dG in the liver increased in animals irradiated with UV or IR, but not in the UV+IR group and vitamin C augmented this effect. The highly significant effect of UV as well as IR radiation on DNA degradation was enhanced by vitamin C supplementation.

Table 1. Effect of irradiation with UV, IR and UV+IR, and vitamin C supplement on the oxidative status of rats

Irradiation effect	Vit. C	Vit. C µmol/l	TAS[1] mmol/l	SOD[2] U/gHb	GPx[3] U/gHb	TBARS[4] µmol/l	8-oxo-dG5, 8oxodG/ 106dG
Control	-	26.2	0.61	1948	21.0	2.84	4.39
	+	30.4	0.48	2007	20.1	3.44	4.04
UV	-	30.7	0.54	2137	19.3	2.78	6.13
	+	56.3	0.47	2145	19.6	3.46	9.72
IR	-	21.8	0.49	2151	20.9	2.84	8.16
	+	43.5	0.43	2405	21.2	3.60	10.38
UV+IR	-	28.1	0.55	2255	20.7	3.36	4.83
	+	40.7	0.61	2071	14.2	3.03	4.63
ANOVA							
Irradiation	SEM	2.44	0.0330	227.1	1.30	0.0776	0.289
	P	0.0004[a]	0.0321[b]	NS	NS	NS	0.0000[c]
Vit. C	SEM	1.73	0.0192	160.6	92.16	0.0549	0.2307
	P	0.0000	NS	NS	NS	0.0000	0.0001
Irradiation × vit. C	P	0.0135	NS	NS	NS	0.0000	0.0000

[a] UV > control , IR , UV+IR; [b] UV+IR > IR; [c]Cont< UV, IR; UV>UV+IR; IR> UV+IR, UV

[1] total antioxidant status, [2] superoxide dismutase, [3] glutathione peroxidase, [4] tiobarbituric acid reactive substances, 58-oxo-2'-deoxy guanosine

Solar exposure has been linked to formation of free radicals and mobilization of transition metal ions. If the flux of ROS is high and antioxidant regeneration becomes a limiting factor, then oxidative stress will occur, damaging cell molecules (Fuchs, 1998). In our study, a significant increase in 8-oxo-dG levels was detected, confirming the pro-oxidative properties of UV and IR radiation. Both lights used separately did not exhibit significant effects on lipid peroxidation. This was probably because lipid peroxidation is often the final stage of oxidative damage and because the intermediates of lipid peroxidation are not able to reach DNA (He and Häder, 2002). Consequently, the elevated concentration of 8-oxo-dG was caused by an oxidative capacity not related to degradation of cell membranes. The present results indicate that the pro-oxidative action

of UV and IR radiation increases requirements for antioxidants in the body, since the concentration of ascorbic acid in plasma was enhanced. According to Kligman (1982), IR radiation increases the efficiency of UV, however, the present results show that the combined UV+IR radiation had no negative effects on redox homeostasis in rats. The UV+IR radiation corresponds to solar radiation and it is likely that animals are adapted to simultaneous UV and IR radiation, but not to the separate action of UV or IR.

A high level of vitamin C intensified the oxidative degeneration of DNA in the liver caused by UV and IR radiation. In the presence of active Fe^{2+}, vitamin C generates ROS in the Fenton reaction. Moreover, solar radiation, UV in particular, enhances Fe^{2+} mobilization in the skin. Thus, it is likely that supplementing vitamin C to irradiated animals generated substrates for the Fenton reaction and, consequently, increased the synthesis of ROS. On the other hand, it is remarkable that vitamin C had no effect on oxidative degeneration of lipids and DNA when combined UV+IR was applied. Furthermore, ascorbic acid showed antioxidative properties, increasing the TAS concentration in plasma, hence decreasing the production of ROS.

CONCLUSIONS

When used separately, UV and IR radiation generated oxidative stress in the rats. Combined UV+IR radiation (corresponding to solar radiation), had no negative effects. High doses of vitamin C enhanced the negative effects of separate UV and IR, but not combined UV+IR, indicating that vitamin C has relative properties as a antioxidant (or prooxidant).

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