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Effect of Radiation on Glyoxalase System Activities in Human Red Blood Cell

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Abstract: Human red blood cells were irradiated with different doses (0-25 Gy) of radiation at a dose rate of 0.5 Gy s⁻¹. The specific activity of glyoxalase I (GLO I) and glyoxalase II (GLO II) were determined. The results indicated that the glyoxalase system is dose-rate radiosensitive. Reduced glutathion(GSH), caffeine (CAF) and chlorpromazine (CPZ) were proved as a modifiers of radiation effects on glyoxalase system. Since the glyoxalase system involves in antioxidative defence mechanism and could play an important role in the regulation of cell division and differentiation, radiation effects on this system may have some biochemical consequences.

Key words: Radiation, glyoxalase system, specific activity, modifier

INTRODUCTION

The glyoxalase system is an enzymatic pathway found in the cytosol of all cells. It is vital for life support from the earliest stages of embryogenesis, through maturation and development, until ageing and death. It has been implicated in control of cell growth, detoxification of α -oxoaldehydes and bypass for the triosephosphate^[1]. The glyoxalase system catalyses the conversion of methylglyoxal to D-lactate via the intermediate S-D-lactoylglutathione. It is comprised of two enzymes, glyoxalase I and glyoxalase II and a catalytic amount of reduced glutathione (GSH)^[2]. Glyoxalase I (EC 4.4.1.5) (GLOI) catalyses the formation of S-D-lactoylglutathione from the hemithioacetal formed non-enzymatically from methylglyoxal and reduced glutathione^[3,4]. Glyoxalase II (EC 3.1.2.6)(GLOII) catalyses the hydrolysis of S-D-lactoylglutathione to D-lactate, regenerating the reduced glutathione consumed in the glyoxalase I catalysed reaction^[5,6].

It is well established that radiosensitivity is directly proportional to the rate of cell division and inversely proportional to the degree of differentiation^[7]. The glyoxalase system is modulated during cell division and differentiation^[1,8] and might also play an important role in cellular radiosensitivity. Moreover glutathione is a cofactor in the glyoxalase system and an important factor in determining inherent cellular radiosensitivity^[9,10]. Many investigators^[7,10-12], have found that depletion of intracellular GSH increased the radiosensitivity of cell. GSH depletion leads to less detoxification of radiation-

produced peroxy radicals or hydroperoxides^[12,13]. Thus it appears that there might be some relationships between cellular damage by radiation and the glyoxalase system.

This enzyme system is considered to be vital for biological functions especially its involvement in antioxidative defence mechanism, therefore, present study was attempted to study the effect of radiation on the glyoxalase system in human red blood cell.

MATERIALS AND METHODS

Samples: Human blood was collected from 44 normal mature people (25 males and 19 females).

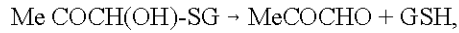
Chemicals: All chemical used were purchased from Sigma chemical Co. and all were of analytical grade. Methylglyoxal was prepared according to the method used by McLellan^[14]. S-hexylglutathione was prepared according to the method of Vince^[15] and recrystallized twice from water/ethanol according to^[16].

Irradiation of RBC: Irradiation of RBC was carried out in air at room temperature at a dose- rate of 0.5 Gy s⁻¹ in the laboratories of the ministry of energy /Amman.

Purification of glyoxalase I and II: After irradiation, glyoxalase I and glyoxalase II were isolated and purified^[14,16].

Assay of the activity of glyoxalase I: The activity of GLO I was assayed at 37°C by measuring the initial rate of

formation of S-D-lactoylglutathione at 240 nm^[9]. The assay mixture contained, in a final volume of 1 mL, 100 mM sodium phosphate buffer (PH 7.2), 3.5 mM methylglyoxal, 1.7 mM GSH and 16.0 mM MgSO₄ 7H₂O. The mixture was incubated for 10 min followed by addition of the supernatant. Initial concentration of hemithioacetal was 2 mM, calculated from equilibrium :



where, $K_{\text{diss}} = 3 \text{ mM}$ and using a molar absorption coefficient of $3.4 \text{ mM}^{-1} \text{ cm}^{-1}$. The enzyme activity is given as units/mg protein. One unit of the enzyme is defined as the amount of enzyme catalysing the formation of 1 umol of S-D-lactoylglutathione/min under the assay condition.

Assay of the activity of glyoxalase II: The activity of glyoxalase II was assayed by measuring the initial rate of hydrolysis of S-D-lactoylglutathione to reduce glutathione and D-lactic acid^[17]. The initial concentration of S-D-lactoylglutathione was 0.3 mM in 50 mM Tris/HCl, pH 7.4 at 37°C. An aliquot of cell lysate was added to S-D-lactoylglutathione solution in 50 mM Tris/HCl, pH 7.4 and the rate of hydrolysis was followed by measuring the absorbance at 240 nm. The activity of glyoxalase II was calculated in U, where, 1 U glyoxalase II activity catalyses the hydrolysis of 1 mmol S-D-lactoylglutathione/min under assay conditions.

Protein determination: Protein concentration was determined by the method of Bradford^[18] using bovine serum albumin as standard.

RESULTS AND DISCUSSION

The effects of various doses of radiation (0-25 Gy) on enzyme glyoxalase I and II activities in human red blood cells are shown in Table 1. The effect of radiation on the activity of GLO I was found to be biphasic. The activity increased with dose up to 6 Gy and declined progressively at 8 Gy and beyond in dose-dependent manner. These changes in GLO I activity may be associated with factors such as unscheduled DNA

synthesis^[7] and activation of the immune response which may be required for the repair and later for regeneration^[9,1].

In case of GLO II, also the activity was increased at radiation up to 6 Gy and then inhibited at 8 Gy and beyond as in Table 1. Although the dose- response relationship was not the same as observed for GLO I activity. Lowering of GLO II activity and an increase in GLO I activity may lead to accumulation of S-D-lactoylglutathione, which is known to be involved in several biological responses^[1]. At high doses decrease in enzyme activities, may be indicative of irreversible damage to the cell or to the enzymes themselves^[9].

Our results have clearly shown that GLO I and GLO II respond differently towards ionizing radiation. Free radicals generated in the radiolysis of water may react with enzymes (the indirect effect) and bring about changes in their structure and function^[9]. Accessibility of free radicals towards different sites of GLO I and GLO II may not be same, which may result in the differential radiosensitivity^[7,20].

A large number of chemicals modify the effect of radiation if present at the time of irradiation^[12,21]. Therefore an attempt has been made to see whether the radiation-induced effect on the glyoxalase system could also be modulated in the presence of chemical modifiers : GSH, caffeine and chlorpromazine (CPZ). As the radioresponse of both GLO I and GLO II were maximum at 6 Gy, the

Table 1: Effect of various doses of γ -rays on GLO I and GLO II in human red blood cells

Percentage specific activity		
Dose (Gy)	GLO I	GLO II
0	100±4.1(0.144±0.03)	100±3.3(0.09±0.002)
0.5	128±3.3(0.03±0.004)	92±3.0(0.05±0.002)
1	134±5.6(0.21±0.012)	94±3.8(0.060±0.006)
4	138±6.4(0.26±0.018)	105±4.2(0.080±0.004)
6	147±8.2(0.32±0.026)	109±5.1(0.220±0.004)
8	122±4.4(0.21±0.006)	102±5.1(0.052±0.003)
10	92±5.1(0.18±0.001)	90±2.1(0.050±0.002)
15	84±2.4(0.122±0.003)	88±1.8(0.055±0.002)
25	71±2.1(0.102±0.006)	72±1.6(0.044±0.001)

Each value represents an average of at least three experiments+standard deviation . The values in parentheses represent the absolute value of the enzyme activity in units/mg protein

Table 2: Effect of chemical modifiers of radiosensitivity on GLO I and GLO II in human red blood cells

Percentage specific activity				
GLO I	GLO I		GLO II	
	Unirradiated	Irradiated	Unirradiated	Irradiated
None	100(1.032±0.031)	147±8.2(1.444±0.040)	100(0.055±0.002)	109±5.1(0.530±0.002)
GSH	94±6.6(0.144±0.006)	158±8.5(0.252±0.008)	97±6.2(0.066±0.005)	104±7.1(0.047±0.003)
Caffeine	92±4.8(0.166±0.007)	128±5.2(0.222±0.121)	95±2.2(0.055±0.005)	102±3.4(0.059±0.040)
CPZ	103±5.7(0.195±0.016)	91±4.2(0.166±0.018)	107±8.2(0.212±0.020)	98±4.4(0.188±0.014)

Each value represents an average of at least three experiments +standard deviation. The values in the parentheses represent the absolute value of the enzyme activity in units /mg protein

modifying effects of these chemicals were studied at this dose. As shown in Table 2, GSH and caffeine were found to increase the specific activity of GLO I and GLO II. CPZ was shown to either protect or enhance the radiation damage^[22,23]. In the present study, CPZ decrease the radiation-enhanced activity of both enzymes. Thus the effect of radiation on the glyoxalase system can be modified in the presence of chemicals or drugs.

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