Effect of three non-protein thiols on CHO cells exposed to low doses of X-radiation

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Abstract: The goals of this study were to determine whether cysteamine, β-mercaptohetanol and glutathione protect CHO K1 cells against chromosomal aberrations induced by low doses of X-radiation, as well as whether radiation protection is related to physical properties of those thiols. Experimental design included four different treatments: (a) control, (b) cells treated with 5 mM of each thiol, (c) cells treated with 100 mGy of X-rays and (4) cells treated with 5 mM of each thiol and 100 mGy X-rays. In combined treatment, all thiols were added 30 min before irradiation. Thiols remained until cell sacrifice (18 h). Maximum protection was afforded by cysteamine, the minimum protection was produced by glutathione while β-mercaptohetanol did not sow radioprotection effect. These results are consistent with the electrical charges and chemical structure of the three thiols and might be explained by the lower or higher access to DNA.

Keywords: chromosome aberrations; ionising radiation; X-rays; low doses; thiol compounds; CHO K1 cells; cysteamine; Glutathione; β-mercaptohetanol; radioprotection.

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1 Introduction

The main effect of ionising radiation (IR) on living cells is the induction of Reactive Oxygen Species (ROS) (through radiolysis of water which is about of 70% of living organism). The antioxidants such as glutathione, cysteine, β-mercaptoethylamine, cysteamine, propyl gallate, and nordihydroguiaretic acid prevent DNA damage and lethality by IR (Gebhart, 1978; Held, 1988; Levina and Malinowski, 1993).

Non-protein thiols may act as radioprotectors through different mechanisms: radical scavenging, restoration of damaged molecules by hydrogen donation (chemical repair), reduction of peroxides, and maintenance of protein thiols (superoxide dismutase, catalase, and others) in the reduced state and as a partial chemical participator in biochemical repair processes of damaged DNA (Revesz, et al 1984; Chattopadhyay et al., 1999).

Also, it has been proposed that glutathione (GSH) within the cell nucleus and in particular its close proximity to DNA is critical for conferring cellular radioprotection (Edgren and Revesz, 1987; Prise et al., 1992). Furthermore, DNA-bound proteins and other non-protein thiols may be more effective in protecting the DNA, in comparison to soluble compounds Ljungman et al. (1991) and Prise et al. (1992) demonstrated that there is a residual chemical repair capacity in eukaryotic cells that is not dependent upon GSH. This suggests that other reducing agents, such as protein thiols and non-protein thiols, may be more effective in IR-induced free radical scavenging of genomic DNA.

Cysteamine (CSM) has been shown to increase intracellular GSH synthesis in bovine and other animals (Takahashi et al., 1993; De Matos et al., 1995; De Matos et al., 1997; Yamauchi and Nagai, 1999; De Matos et al., 2002a; De Matos et al., 2002b; De Matos et al., 2003; Gasparrini et al., 2003; Rodriguez-Gonzalez et al., 2003; Oyamada and Fukui, 2004; Luciano et al., 2006; Hossein et al., 2007; Kobayashi et al., 2007; Zhou et al., 2008).

The access of thiols to DNA may be controlled by the net charge of these compounds and the negative environment of the DNA. Therefore, positive thiols as CSM should have a better access to DNA than neutral β-mercaptoethanol (BME) and negative ones GSH (Epstein et al., 1997; Lopez-Larraza et al., 2001). Although the radioprotection nature of thiols has been established, all studies were performed using high doses of ionising radiations. It has also been established that low doses of ionising radiations (doses <150 mGy; Bonner, 2003) are not correlated linearly with that of high doses, because the existence of collateral effects can determine that the damage is higher than expected. On the other hand, the radioprotection by non-protein thiols on irradiated cells with low doses of X-radiation has not been studied. The goals of this study were to determine whether CSM, BME and GSH protect CHO K1 cells against chromosomal aberrations induced by low doses of X-radiation 100 mGy, as well as whether radiation protection is related to the chemical structure and the electric charge of the above mentioned thiols.

2 Materials and methods

2.1 Cell cultures

CHO K1 cells were cultured in Ham's F10 medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% foetal bovine serum (Notocor Laboratories, Córdoba,

Argentina) and antibiotics (50 IU penicillin and 50 µg/ml streptomycin) (Bagó Laboratories, Buenos Aires, Argentina) in a humidified atmosphere with 5% CO_2. Cells were cultured in Falcon flask T-25 (Nunc, Roskilde, Denmark); during one cycle (18 h.).

Two hours before sacrifice, cells were exposed to colchicine (0.1 µg/ml final concentration) (Sigma Chemical Co, MO, USA, CAS No. 64-86-8). Chromosome preparations were made by dripping the cell suspension in fresh fixative solution on clean slides, and then stained with Giemsa 5% for 10 min.

2.2 *Experimental design*

Irradiation treatments were performed with non-dividing confluent cell cultures. They were carried out on ice to prevent the repair of strand breaks during the procedure. The irradiation equipment was provided by Dental San Justo Company (Buenos Aires, Argentina) and operated at 65 kV and 5 mA. Doses were determined by a dosimeter (Keithley Digital 35617 EBS microchamber PTW N 2336/414; C-Com Industries, Robertsville, MO, USA) and administered at a dose rate of 50 mGy/min. The experimental design included four different groups: (a) control (cells remained untreated: no X-rays, no thiols); (b) cells treated with 5 mM of GSH (Sigma Chemical Co, MO, USA, CAS No. 70-18-8); (c) cells treated with 100 mGy of X-rays (100 mGy) and (d) cells treated with 5 mM glutathione and 100 mGy X-rays (30 min after treatment with GSH). GSH remained until cell sacrifice. The same protocol was performed for CSM (Sigma Chemical Co, MO, USA, CAS No. 156-57-0) and BME (Sigma Chemical Co, MO, USA, CAS No. 60-24-2). The radiation dose was delivered, taking into account previous experiences in our laboratory (Güerci et al., 2003; Güerci et al., 2004; Seoane et al., 2007) and the dosimetry reported in epidemiological exposures (Barquinero et al., 1993; Antonelli et al., 1995; Paz-y-Miño et al., 1995; Hagelström et al., 1995; Balakrishnan and Roa, 1999; Maluf et al., 2001; He et al., 2000). The concentration of non-protein thiols was chosen taking into account from previous experiments (Mira et al., 2013). The experimental conditions were the same in all the experiments. Two hundred cells were scoring for each experiment and it was repeated three times. As there were no differences among the three experiments they were pooled. Then a total of 600 cells for each group were scored. First of all the existence of significant differences among the different experimental groups of each thiol were determined. After this comparisons were made in pairs (control vs. thiol; control vs. 100 mGy; control vs. thiol plus 100 mGy; thiol vs. 100 mGy, etc.). Finally, significant differences among the combined treatments were calculated. The χ^2 test was used for statistical analysis.

3   Results

Tables 1–3 summarise the results obtained from the cytogenetic analysis. Statistical analysis was carried out by comparing the amount of abnormal cells (cells with at least one chromosomal aberration) induced by the different treatments. Significant differences for the three thiols were found when the different experimental groups were compared ($p < 0.001$). When the results obtained for the different thiols were compared the following results were observed. Significant differences for CSM treatments were found when: control vs. 100 mGy ($p < 0.001$); CSM 5 mM vs. 100 mGy ($p < 0.001$) and 100 mGy vs. CSM 5 mM plus 100 mGy ($p < 0.001$). When BME treatments were compared, significant

differences were found when: control vs. 100 mGy ($p < 0.01$); control vs. mercaptoetanol plus 100 mGy ($p < 0.001$); mercaptoetanol vs. 100 mGy and mercaptoetanol vs. mercaptoetanol plus 100 ($p < 0.001$). When GSH treatments were compared, significant differences were found when: control vs. 100 Ms ($p < 0.001$); control vs. GSH 5 mM plus 100 mGy ($p < 0.05$); GSH 5 mM vs. 100 Ms ($p < 0.001$) and GSH 5 mM vs. GSH 5 mM plus 100 mGy ($p < 0.05$) and 100 mGy vs. GSH 5 mM plus 100 mGy ($p < 0.01$). Finally significant differences among the combined were found ($p < 0.001$)

Table 1 Frequency and standard error of chromosomal aberrations found in the different treatments with cysteamine

Experiment	Metaphases Analysed	Abnormal Cells[1]	AL[2]	B'[3]	B''[4]
Control	600	2.5 ± 0.006	1.2 ± 0.004	0.5 ± 0.002	0.8 ± 0.004
CSM 5 mM	600	1.7 ± 0.005	0.5 ± 0.004	0.5 ± 0.003	0.3 ± 0.067
100 mGy	600	11.0 ± 0.013	6.0 ± 0.009	2.3 ± 0.006	2.6 ± 0.007
CSM 5 mM plus 100 mGy	600	2.2 ± 0.006	2.0 ± 0.004	0.5 ± 0.002	0.5 ± 0.002

Notes: [1]Metaphase with at least one chromosomal aberration; [2]metaphases exhibiting only chromatic lesions (gaps); [3]monochromatid breaks; [4]isochromatid breaks.

Table 2 Frequency and standard error of chromosomal aberrations found in the different treatments with β-mercaptoethanol

Experiment	Metaphases Analysed	Abnormal Cells[1]	AL[2]	B'[3]	B''[4]
Control	600	3.6 ± 0.007	2.0 ± 0.005	0.5 ± 0.003	0.7 ± 0.002
BME 5 mM	600	2.7 ± 0.006	1.5 ± 0.004	0.5 ± 0.002	0.7 ± 0.004
100 mGy	600	7.0 ± 0.010	3.0 ± 0.007	2.8 ± 0.006	0.8 ± 0.004
BME 5 mM plus 100 mGy	600	4.5 ± 0.011	2.5 ± 0.008	0.8 ± 0.007	1.0 ± 0.004

Notes: [1]Metaphase with at least one chromosomal aberration; [2]metaphases exhibiting only chromatic lesions (gaps); [3]monochromatid breaks; [4]isochromatid breaks.

Table 3 Frequency and standard error of chromosomal aberrations found in the different treatments with Gluthathione

Experiment	Metaphases Analysed	Abnormal Cells[1]	AL[2]	B'[3]	B''[4]
Control	600	2.0 ± 0.005	1.6 ± 0.004	0.7 ± 0.002	0.7 ± 0.040
GSH 5 mM	600	2.3 ± 0.006	1.3 ± 0.004	0.7 ± 0.003	0.3 ± 0.067
100 mGy	600	8.7 ± 0.011	3.2 ± 0.007	2.8 ± 0.007	2.7 ± 0.006
GSH 5 mM plus 100 mGy	600	9,0 ± 0.008	4.3 ± 0.006	2.7 ± 0.005	2.0 ± 0.002

Notes: [1]Metaphase with at least one chromosomal aberration; [2]metaphases exhibiting only chromatic lesions (gaps); [3]monochromatid breaks; [4]isochromatid breaks.

4   Discussion

Thiol compounds are able to: (a) free radical scavenging, (b) reduction of cell necrosis, (c) inhibition of apoptosis, (d) stabilisation of the process of DNA repair enzyme and (e) chemical repair of deoxyribose. The processes (b) and (c) could be the result of the process (a). However, none of these three events (a, b and c) explains the decrease of chromosome aberrations. The addition of GSH increased the rate of DNA repair (event d) (Hanigan and Ricketts, 1993; Dutta et al., 2005; Pujari et al., 2010).

This stabilisation of the repair was not demonstrated for CSM and BME. However, it was demonstrated that CSM increases the concentration of GSH. The first event of oxidative damage is the removal of a proton from mainly the positions 4 or 5 of the deoxyribose. It has been shown that several non-enzymatic thiol compounds have the potential to produce chemical repair of deoxyribose (event e). This process is possible thanks to the presence of sulfhydryl radical (-SH) in the thiol compounds. An important chemical barrier able to oppose to the cytotoxic effect of free radical species generated by oxidative stress is the reducing ability of sulfhydryl compounds mainly GSH and its precursor cysteine (CYS). The mechanism by which CYS enters the cell is however complex and involves several steps. In fact, CYS outside the cell is rapidly converted to cystine (its oxidised form) and has to be reduced by GSH to produce c-glutamylcystine and transported across the membrane by GSH reductase to be liberated inside the cell and to also serve as a precursor of GSH (Meister and Anderson, 1983).

These compounds can donate the missing proton of deoxyribose. The results obtained in this study are consistent with the above mentioned chemical repair model.

Previous work has shown that thiol compounds produce this type of repair or a radical reduction of intermediaries in subsequent oxidation reactions (Epstein et al., 1997; Lopez-Larraza et al., 2001). It has also been shown that the intensity of these reductions is related to the electric charge of thiol compounds. Our results showed that glutathione, negatively charged, showed a radioprotector effect. On the other hand, the positively charged CSM proved to be the best radioprotective thiol. BME, neutral in charge, did not show an intermediate effect between the two above mentioned thiols. These results are consistent with the lower or higher access to DNA.

It has been demonstrated that for GSH concentrations (0.5–5 mM), the Oxygen Enhancement Ratios (OERs) for Single Strand Breaks (SSBs) and Double Strand Breaks (DSBs) were very similar (Ayene et al., 1995). It was reported that for the highest GSH concentration (20 mM) in anoxic conditions, the induction efficiencies for either SSBs or DSBs are increased or became saturate when compared to the corresponding values of 5 mM GSH. This indicates that in addition to its protective effects, GSH at high concentrations may also induce DNA strand breaks. Poor protection against CA induced by 4 Gy in GSH pretreated lymphocytes has been observed earlier (Chatterjee and Jacob-Raman, 1986). Conflicting results in the literature, therefore, do not provide a definitive conclusion for the role of GSH either in radiosensitisation or radioprotection. In addition to the –SH group, CSM also possesses an amine group, and protected the cells more than GSH and BME. As an amine, CSM binds to DNA (Trizna, et al., 1993; Hoffmann and Littlefield, 1995; Hoffmann et al., 1995; Hoffmann, 2001) and may alter DNA conformation widening the minor groove of DNA, which may give –SH better access to the site of damage by ionising radiation. That way, the thiol and amine functions of CSM

may act in concert, such that the amine groups may bring the thiol group to the site of oxidative damage on DNA (Hoffmann et al., 2001). CSM and BME are structural analogues, differing only with respect to the amino group. The absence of protection by BME demonstrates the importance of the amino group in the protection against CA induced by ionising radiation.

Ionising radiation is a ubiquitous environmental physical agent that has proved to exert DNA-damaging effects. Radiobiology interpretation accepts that after exposure to DNA damaging agents, mutation frequency increases rapidly at the beginning and declines to background levels when DNA damage repair is completed. This concept on mutagenesis has been challenged in recent years after several findings on radiation-induced genomic instability (Little, 2003; Morgan, 2003). Other studies report an increase of chromosome breaks over chromosome rearrangements with decreasing radiation levels in peripheral lymphocytes of subjects exposed to relatively low doses of ionising radiation, especially when chronic exposure is not higher than 100 mGy (Balakrishnan and Rao, 1999; Maffei et al., 2002; Sari-Minodier et al., 2002; Maffei et al., 2004).

Although the protective effects of GSH and CSM were demonstrated it remains to clarify the neutral thiol β-mercaptoethanol. However the greater accessibility the positively charged CSM the negatively GSH, suggests a role for the charge of the thiols. In a future study, we intend to use more thiols, many of which are neutral. The present study may be a contribution for the prevention of DNA damage induced by chronic exposures to low doses of ionising radiation.

Acknowledgements

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