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Thiols and selenium: protective effect on human skin fibroblasts exposed to UVA radiation

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Abstract

The sensitivity of human dermal fibroblasts to UVA radiation has been linked to a decrease in intracellular glutathione (GSH) levels. GSH (γ-glutamyl-cysteinyl-glycine) is a radical scavenger and a cofactor for protective enzymes such as selenium-dependent GSH peroxidases. In this study, we examine the possibility of a cooperative interaction between three cysteine delivery systems and selenium in protecting human cultured fibroblasts exposed to UVA radiation. Cells were irradiated (9, 15 and 20 J cm^{-2}) following incubation with *N*-acetyl-cysteine (NAC, 5 mM), *N*-acetyl-homocysteine-thiolactone (citiolone (CIT), 1 mM) or *L*-2-oxothiazolidine-4-carboxylate (OTC, 1 mM). The modulation of the intracellular GSH levels by the addition of the different compounds was determined by enzymatic and separative methods. Cells were harvested for survival analysis by measuring the ability of the cell to adhere and proliferate. Treatments with NAC and CIT resulted in a significant rise in GSH levels compared with control cells, with protection against UVA radiation. OTC did not induce any rise in GSH level; nevertheless, the protective effect afforded by OTC is similar to that observed with NAC and CIT. Moreover, selenium (0.1 mg l^{-1}), as sodium selenite, significantly increased the protective efficiency of NAC and CIT, but not of OTC. Although the precise mechanism is not known, thiol molecules can inhibit the deleterious effects of UVA radiation. These results provide evidence that compounds capable of inducing GSH synthesis can act with selenium to protect cells against UVA damage. © 1997 Elsevier Science S.A.

Keywords: Cultured cells; Glutathione; Selenium; Thiols; UVA radiation

1. Introduction

UVA radiation (320–380 nm) induces significant photodamaging effects, including carcinogenesis and aging [1], due to the action of reactive oxygen species [2,3]. Moreover, solar radiation induces the depletion of enzymatic and non-enzymatic antioxidant compounds in skin cells in situ, which leads to the enhancement of free radical reactions [4,5]. Of the non enzymatic compounds, it is noteworthy that UVA radiation decreases cellular glutathione (GSH, γ-glutamyl-cysteinyl-glycine) levels [6] and total protein sulphydryl group content [3]. Furthermore, GSH provides an important protective system [7,8]. Its antioxidant ability may be related to direct free radical quenching. However, in the UVA range, the protective role of GSH also seems to be mediated via selenium-dependent GSH peroxidases (EC 1.11.1.9., Se-GPX), which detoxify hydrogen peroxide and organic peroxides using GSH as hydrogen donor [9,10], and whose activity depends on the selenium (Se) status [11]. Interestingly, Se, when added directly to the culture medium, has been shown to have a protective effect on human skin fibroblasts exposed to UVA radiation [12,13].

Various methods have been used to increase the total GSH content in different tissues for protection against oxidative stress [14–16]. GSH synthesis is dependent on the cysteine level; however, this amino acid can be toxic and is rapidly oxidized to cystine. A more effective approach involving other thiol compounds can be used to increase the intracellular GSH content. In this regard, we have tested three different molecules which can act as efficient cysteine delivery systems. The first is the aminothiol *N*-acetyl-cysteine (NAC), which enters biological reactions involving free radicals through thiol metabolism into GSH and through its intrinsic scavenger properties [17,18]. The second, L-2-oxothiazoli-

Abbreviations: CIT, *N*-acetyl-homocysteine-thiolactone or citiolone; GSH, glutathione; NAC, *N*-acetyl-cysteine; OTC, L-2-oxothiazolidine-4-carboxylate; Se, selenium; Se-GPX, selenium-dependent GSH peroxidase; UVA, ultraviolet radiation in the A region (320–380 nm)

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dine-4-carboxylate (OTC), is converted intracellularly to cysteine by the enzyme 5-L-oxoprolinase (EC 3.5.2.9.) [19]. OTC markedly enhances endothelial cell GSH concentration [20]. Moreover, administration of OTC to mice produces a substantial increase in liver GSH levels [21]. The third, *N*-acetyl-homocysteine-thiolactone or citiolone (CIT), is an SH donor, normally used in the treatment of hepatic disorders [22], and has been reported to act as a free radical scavenger [23].

The objectives of this study are as follows:

1. to test the hypothesis that NAC, OTC and CIT additions are effective in maintaining the intracellular GSH levels of human skin fibroblasts exposed to UVA radiation;
2. to determine their putative antioxidant efficiencies in preventing UVA cytotoxicity;
3. to study the efficiencies of thiol–Se combinations as antioxidants by comparing their capacity to protect cultured human skin fibroblasts against UVA radiation.

2. Materials and methods

2.1. Culture media and routine equipment

RPMI-1640 and foetal calf serum (FCS) were purchased from ATGC (ATGC Biotechnologie, Noisy-le-Grand, France). L-glutamine and fungizone were obtained from Boehringer (Mannheim, Germany), penicillin and streptomycin from Polylabo (Paul Block and Cie, Strasbourg, France) and kanamycin and Puck's saline from Gibco (Grand Island, USA). Phosphate-buffered saline (PBS), containing calcium and magnesium, was purchased from Eurobio (Les Ullis, France). Sodium selenite, NAC, OTC, CIT, monobromobimane (mBrB), *N*-ethylmorpholine, dithioerythritol, GSH, GSH reductase and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). 5-Sulphosalicylic acid, acetonitrile and perchloric acid were purchased from PROLABO (Fontenay Sous-Bois, France).

2.2. Cell treatment prior to irradiation

Cultures of normal human skin fibroblasts were established from excess tissues of breast plastic surgery specimens and were further propagated in RPMI-1640 supplemented with 10% FCS, 300 μg ml^{-1} L-glutamine, 0.5 μg ml^{-1} fungizone, 0.17 μg ml^{-1} penicillin and streptomycin and 54 μg ml^{-1} kanamycin. For each study, cells between population doubling levels 7 and 14 were seeded in 9 cm^2 Petri dishes (NUNC, Gibco, Grand Island, USA) and grown for 5 days to near confluency. The culture medium was replaced by fresh medium 48 h before the experiment. According to previous experiments performed in our laboratory, and to the literature, thiol compounds were added directly to the culture medium, 4 h before UVA irradiation, and also to the PBS used for the experiment. The final concentrations of thiol compounds were chosen after studying their cytotoxicity and ability to provide an optimum level of GSH at the time of irradiation. The concentrations used were as follows: 5 mM for NAC and 1 mM for OTC [21] and CIT [24]. Se treatment (0.1 mg l^{-1} as sodium selenite) was performed during three subcultures as described previously [12].

2.3. UVA irradiation

The UVA source was a high-pressure Tecimex apparatus (Dixwell, St. Symphorien d'Ozon, France). The spectrum was centred on a maximum intensity of 372 nm. The UVA doses effectively received from the bottom by the cells were evaluated with a radiometer (Dixwell, St. Symphorien d'Ozon, France). Prior to irradiation, the culture medium was removed and reserved. Cells were rinsed twice with 1.5 ml of PBS and left in 1 ml of PBS. Control cells were similarly treated and left in the dark while irradiation was carried out. Three UVA doses were used: 9, 15 and 20 J cm^{-2}.

2.4. Biochemical analysis

2.4.1. Cell viability

Cell viability was measured using the adhesion–proliferation method. This technique was compared with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method to ensure that it was suitable for the evaluation of oxidative injury [25]. After the stress, cells were washed twice with Puck's saline, trypsinized, transferred to new dishes with their reserved medium and placed in an incubator. After 18 h, non-viable cells were removed by rinsing vigorously with NaCl (0.9%). The total cell proteins were determined according to the procedure described by Shopsis and Mackay [26]. Two dishes for each condition were tested, and each condition was run three times.

2.4.2. Total GSH measurement

After treatment, the cells were returned to their original medium and incubated for 2 h. They were rinsed twice with NaCl (0.9%) and scraped in deionized water. Cell suspensions were deproteinized by adding metaphosphoric acid (6%) (lysate–metaphosphoric acid (5 : 1, v/v)). After shaking, the mixtures were centrifuged for 10 min at 4000 rev min^{-1} at 4 °C. One Petri dish was kept for total protein determination [26]. The supernatants were stored at −80 °C until analysis. The total intracellular GSH contents were evaluated according to the method of Akerboom and Sies [27], slightly modified as follows. The reduction rate of DTNB into 5-thio-2-nitrobenzoate (TNB) was evaluated spectrophotometrically at 412 nm in the following mixture: 1 ml of 0.4 M *N*-morpholinopropanesulphonic acid (MOPS) buffer, pH 6.75, containing 2 mM Na-EDTA, 50 μl of reduced nicotinamide adenine dinucleotide phosphate (NADPH) solution (4 mg ml^{-1} in $NaHCO_3$ (0.5%)), 20 μl of GSH reductase (6 U ml^{-1} in MOPS buffer), 20 μl DTNB (1.5 mg ml^{-1} in $NaHCO_3$ (0.5%)) and 100 μl of sample. The values were

determined by comparing the reduction rate against a standard curve of GSH. Oxidized GSH (GSSG) was determined in the same conditions after adjusting the pH with ethanolamine and trapping GSH with 3-vinylpyridine. In addition, reduced thiol determinations were performed using a separative technique adapted from Mansoor et al. [28], and based on the derivatization of free thiols with mBrB. The thiol–bimane adducts were quantified by reverse-phase liquid chromatography. Confluent cells were irradiated in 75 cm^2 culture flasks (NUNC, Grand Island, USA). The cells were rinsed and scraped in 4 ml of deionized water as described previously. A portion (250 μl) of the cell suspension was kept for total protein determination [26]. mBrB (56.25 μl; 180 mmol l^{-1}) and phosphate buffer (pH 7.4) (375 μl) were added to the remaining cell suspension. The proteins were then precipitated by the addition of 50% sulphosalicylic acid solution containing 500 μM dithioerythritol (DTE). The precipitated proteins were removed by centrifugation and filtration. To 30 μl of the supernatant were added 30 μl of sulphosalicylic acid (5%) plus 50 μM of DTE, 50 μl of 0.1 M *N*-ethylmorpholine, 10 μl of acetonitrile and 160 μl of distilled water. After 10 min incubation in the dark at room temperature, 20 μl of a 70% solution of perchloric acid was added. Samples of 50 μl were injected into a 250 mm × 4.6 mm column packed with 5 μm particles of ODS-Hypersil (C18). Elution solvent A (1 l) contained 12.5 mM phosphate buffer (pH 3.4) and 9 ml of tetrahydrofuran. Solvent B (1 l) contained 530 ml of phosphate buffer (12.5 mM, pH 3.4), 400 ml of acetonitrile and 70 ml of tetrahydrofuran. The excitation and emission wavelengths were 400 nm and 475 nm respectively.

2.5. Treatments of results

The results represent the means of three experiments with two Petri dishes for each condition (Cc, control cells; ICc, irradiated control cells; Tc, treated cells; Tlc, treated irradiated cells). In Fig. 1, the results are expressed as the viability (%) evaluated from the formulae (ICc/Cc) × 100 and (Tlc/Tc) × 100.

The protective effect of each thiol–Se combination was evaluated quantitatively from the equation [29]

$$\text{Antioxidant activity (\%)} = (1 - A/B) \times 100$$

where

$$A = [(1 - \text{ICc})/\text{Cc}] \times 100;\ B = [(1 - \text{Tlc})/\text{Tc}] \times 100$$

2.6. Statistics

The results are presented as the mean ± standard deviation. All data were processed statistically using Student's t-test. Differences were considered to be significant when $p < 0.05$.

3. Results

3.1. Modulation of endogenous GSH levels by the addition of thiol molecules and UVA irradiation

As shown in Table 1, the total intracellular GSH (GSH + GSSG) content in control cells is 16.30 ± 2.65 μmol (g protein)$^{-1}$ (mean ± SD; $n = 3$; $p < 0.05$); GSSG is not detectable in our analytical conditions. Cells treated with NAC (5 mM) and CIT (1 mM) exhibit threefold and 1.6-fold increases respectively in the total intracellular GSH content compared with controls. It is noteworthy that no significant difference in the total intracellular GSH concentration is observed between controls and cells pretreated with OTC (1 mM). We also report in Table 1 the GSH concentrations determined by reverse-phase liquid chromatography [26]. The results indicate that the modifications in the total intracellular GSH content with NAC and CIT are mainly due to an increase in GSH synthesis.

The oxidation of intracellular GSH has been reported on exposure to UVA radiation [6]; thus we followed the modulation of endogenous GSH in thiol-supplemented cells exposed to UVA radiation. The results are presented in Fig. 1. Irradiation of control cells leads to a typical decrease in GSH level to 2.15 ± 1.60 μmol (g protein)$^{-1}$ for 20 J cm^{-2}. With NAC and CIT, the decrease recorded is statistically significant only for the high UVA dose (20 J cm^{-2}). Furthermore, with OTC, the slight decrease in intracellular GSH content observed is not significant. No detectable change in the relative amount of intracellular GSSG is observed.

3.2. Effect of NAC, CIT and OTC on the UVA cytotoxicity

The results listed in Fig. 2 illustrate the influence of the addition of thiol compounds on the UVA cytotoxicity. UVA radiation from 9 to 20 J cm^{-2} decreases the percentage of living control cells in a dose-dependent manner. Cells treated with 5 mM NAC, 1 mM CIT or 1 mM OTC are significantly less sensitive to UVA radiation compared with the controls. Interestingly, there is no significant difference in the efficiency of NAC, CIT or OTC in protecting fibroblasts against UVA radiation from 9 to 15 J cm^{-2}. However, at a fluence

Table 1
Effect of thiol supplementation (NAC, 5 mM; OTC, 1 mM; CIT, 1 mM) on the total GSH (GSH + GSSG, μmol (g total protein)$^{-1}$) and GSH (μmol (g total protein)$^{-1}$) contents in human skin fibroblasts (Cc, control cells; NAC, NAC-treated cells; CIT, CIT-treated cells; OTC, OTC-treated cells)

	Cc	NAC	CIT	OTC
GSH + GSSG[a]	16.30 ± 2.65	51.30 ± 8.10*	26.70 ± 2.50*	13.56 ± 3
GSH[b]	24	77.25	30.15	12.80

[a]Method of Akerboom and Sies [27]. The results are presented as mean ± standard deviation ($n = 3$).
[b]Determined by high performance liquid chromatography [28]. The results are from one representative experiment.
*$p < 0.05$ vs. Cc.

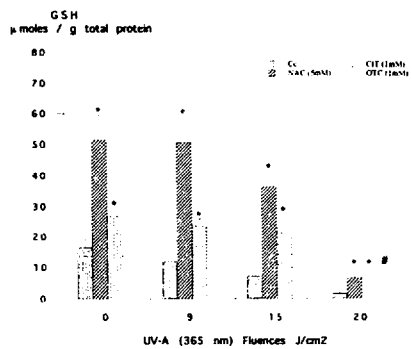

Fig. 1. Influence of thiol supplementation (NAC, 5 mM; OTC, 1 mM; CIT, 1 mM) on the GSH content (µmol (g total protein)$^{-1}$) of human skin fibroblasts exposed to increasing UVA doses. GSH content was determined 3 h after UVA exposure by the method of Akerboom and Sies [27]. The results are presented as mean ± standard deviation ($n = 3$). $^{*}p < 0.05$ vs. Cc; $^{\#}p < 0.05$ vs. OTC-treated and irradiated cells (UVA doses, less than 20 J cm^{-2}).

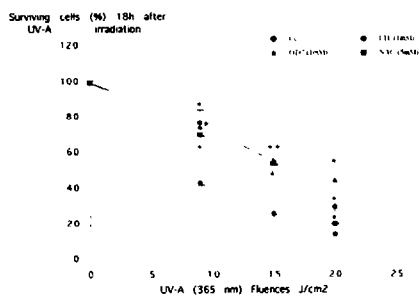

Fig. 2. Influence of thiol supplementation (NAC, 5 mM; OTC, 1 mM; CIT, 1 mM) on the survival of human skin fibroblasts exposed to increasing UVA doses. We determined the percentage survival by measuring the ability of cells to proliferate [25]. The percentage survival for each supplementation and each dose was calculated using the formulae (ICc/Cc) × 100 and (Tlc/Tc) × 100 (ICc, irradiated control cells; Cc, control cells; Tlc, treated irradiated cells; Tc, treated cells). $^{*}p < 0.05$ vs. Cc.

of 20 J cm^{-2}, 1 mM OTC increases the amount of surviving cells by a factor of three, 1 mM CIT by a factor of two and NAC by a factor of 1.4 compared with control cells.

3.3. Protective role of the thiol–selenium combination

We have investigated the possibility of enhancing the basal protective effect of NAC, CIT and OTC against UVA radiation using selenium (0.1 mg l^{-1} as sodium selenite). To assess the efficiency of the thiol–Se combination, the percentage of antioxidant activity was determined for all treatments as listed in Table 2. Treatment with the combination NAC–Se leads to a remarkable increase in the efficiency of NAC for protecting human fibroblasts against UVA radiation ($p < 0.05$). Combining Se with CIT also produces an increase ($p < 0.05$) in the protecting properties of CIT, except at 9 J cm^{-2}. Interestingly, the percentage of antioxidant activity of OTC remains unchanged.

The next experiment addressed the question of whether Se can influence fibroblast GSH content. The GSH concentrations were estimated in non-irradiated Se and Se–thiol-treated cells. From the data listed in Table 3, it is clear that Se does not affect the total intracellular GSH content. In Se-treated cells, NAC and CIT treatments lead to an increase in the GSH level. Irradiation of the cells with increasing UVA doses leads to the same pattern of evolution of total endogenous GSH irrespective of whether cells are treated with thiols alone or with one of the thiol–Se combinations (data not shown).

4. Discussion

GSH depletion has been found previously to sensitize human skin fibroblasts or epidermal keratinocytes to the damaging effects of UVB and UVA [30]. Moreover, Tyrrell and Pidoux [31] have reported a quantitative correlation between the cellular GSH content and the sensitivity of skin cells to radiation at defined wavelengths in the solar UV range. This investigation was undertaken to determine the putative effect of NAC, CIT and OTC on GSH synthesis and UVA cytotoxicity in human skin fibroblasts.

NAC (5 mM) and CIT (1 mM), but not OTC (1 mM), can increase significantly GSH synthesis in cultured human skin fibroblasts. We can conclude that NAC and CIT, which

Table 2
Comparison of the antioxidant activity (%) of NAC (5 mM), OTC (1 mM) and CIT (1 mM) vs. thiol–Se-treated cells (Se, 0.1 mg l^{-1}) exposed to increasing UVA doses (NAC, NAC-treated cells; NAC–Se, NAC- and Se-treated cells; CIT, CIT-treated cells; CIT–Se, CIT- and Se-treated cells; OTC, OTC-treated cells; OTC–Se, OTC- and Se-treated cells). The results are presented as mean ± standard deviation ($n = 3$)

UVA fluence (J cm^{-2})	Antioxidant activity (%)						
	Se	NAC	NAC–Se	CIT	CIT–Se	OTC	OTC–Se
9	28 ± 3	48 ± 4	70.55 ± 5.10^{a}	59.40 ± 9.65	57.15 ± 5.60	55.60 ± 10.55	49.20 ± 3.60
15	16.80 ± 3.30	36 ± 5	68.40 ± 6.56^{a}	39 ± 6.90	60.40 ± 8.20^{a}	39.80 ± 5.30	49.30 ± 6.65
20	3.40 ± 0.20	7.10 ± 0.40	11.10 ± 0.70^{a}	18 ± 1.80	25.70 ± 4.15^{a}	35.50 ± 6.30	35.20 ± 9

$^{a}p < 0.05$ (NAC–Se vs. NAC, CIT–Se vs. CIT, OTC–Se vs. OTC).

Table 3
Modulation by selenium or by a combination of both selenium (0.1 mg l^{-1}) and thiol of total GSH content (GSH + GSSG, μmol (g total protein)$^{-1}$)

	Cc	Se	NAC–Se	CIT–Se	OTC–Se
GSH + GSSG[a]	16.30 ± 2.65	19.30 ± 6.80	58.70 ± 16.75[b]	30.90 ± 15[b]	17.25 ± 2.90

[a]Method of Akerboom and Sies [27]. The results are presented as mean ± standard deviation ($n = 3$).
[b]$p < 0.05$ (Se vs. Cc, NAC–Se vs. NAC, CIT–Se vs. CIT, OTC–Se vs. OTC).

are transported into cells and deacetylated, can replenish the cellular cysteine level required to produce GSH. Under the conditions used, NAC is more effective than CIT in increasing the intracellular GSH levels. This result may partly be explained by the difference in the concentrations used. However, it should be noted that 3 mM CIT is cytotoxic to human skin fibroblasts (data not shown). According to the literature, OTC is hydrolysed to cysteine in vitro [21] and in vivo [22], which may lead to an increase in GSH synthesis. However, in our model, we were unable to reproduce the induction of GSH synthesis by this thiazolidine. No clear explanation can be given. It has been shown that OTC can increase GSH concentration, but only when GSH has been previously depleted [32]. This suggests that the conversion of OTC to cysteine by the enzyme 5-oxoprolinase, in our model, may be delayed in time, according to GSH depletion on UVA irradiation. It should be noted that the intracellular GSH levels determined by reverse-phase liquid chromatography [28] are in agreement with the results obtained enzymatically using the method of Akerboom and Sies [27]. In addition to the increase in intracellular GSH concentration, NAC and CIT have the capacity to prevent the dramatic fall in GSH recorded on exposure to UVA radiation, except at 20 J cm^{-2}. This decrease is not significant in the cells treated with OTC. The latter finding reinforces the speculation presented above concerning the modulation of GSH metabolism by OTC. In all of these experiments, fresh medium was not added after the stress in order to maintain constant cell culture conditions.

In human fibroblasts, the increase and stability of the intracellular GSH concentration, by two of the thiol-modulating agents, are closely correlated with the decrease in UVA cytotoxicity. Such a correlation suggests that NAC and CIT can protect human fibroblasts mainly by enhancing GSH synthesis. Moreover, the large depletion in GSH content observed at 20 J cm^{-2} correlates with a dramatic rise in the cytotoxic efficiency of UVA radiation, reinforcing the hypothesis that the cytoprotection afforded by NAC is mediated via a GSH pathway. An additional hypothesis is that NAC protects against UVA-induced cytotoxicity by the prevention of the loss of protein sulphydryl groups, as described in isolated hepatocytes exposed to allyl alcohol cytotoxicity [33]. Evidence exists to suggest the involvement of oxygen free radicals in UVA-mediated damage to skin cells. Thus we cannot exclude the possibility that radical scavenging or the quenching of oxygen products directly by NAC is involved in the inhibition of the cytotoxicity of UVA [18,34]. In the same way, CIT scavenges the hydroxyl free radical $^{\cdot}OH$ [24], which is involved in UVA-induced DNA damage, as clearly demonstrated by Peak and Peak [35]. In addition, CIT has been reported to enhance superoxide dismutase (SOD) activity in rat islets [36], but adverse results have been found on one cell line [24]. Although the importance of SOD activity in cells exposed to UV radiation remains unclear [4,37,38], we cannot exclude the possibility that, in our work, the protective role of CIT may be partly related to SOD activity and/or to regulation of the balance between antioxidant enzymes, as described by Amstad et al. [39]. In conclusion, the present findings suggest that NAC, CIT and OTC protect cultured human skin fibroblasts against UVA damage with a similar efficiency. The property of these three compounds to protect skin cells from UV radiation damage adds further information to the protective effect of thiols described previously [40–42], and this investigation underlines the protective properties of NAC against UV radiation [43,44]. Although NAC is a well-known antioxidant molecule, to our knowledge, this is the first time that the potential protective efficiency of CIT and OTC against UV radiation has been described. Moreover, it seems that the protective effect of these molecules is mainly mediated through the GSH pathway, indicating that intracellular GSH plays a crucial role in the stress response. Nevertheless, it would be interesting to define precisely the mechanisms by which these molecules counteract UVA damage, particularly in the case of OTC.

Selenium is an essential trace element, which is involved in cytoprotection, mainly through the activity of Se-GPX. Previous work performed in the laboratory demonstrated that the addition of Se protected cultured cells from UVA irradiation through an increase in Se-GPX activity (245.33 ± 33.86 U (g protein)$^{-1}$ in Se-treated fibroblasts vs. 132.33 ± 34.82 U (g protein)$^{-1}$ in control cells). Pence et al. [45] have recently reported a correlation between the selenium level in the diet and both the number of skin tumours and the activity of antioxidant enzymes, such as catalase, SOD or Se-GPX, on UV-irradiated skin. Thus it was of interest to determine the interaction between thiol molecules and Se in UVA-induced cytotoxicity. In this work, a significant increase in the antioxidant capacity of NAC and CIT was observed in Se-treated cells. The interaction between these antioxidants seems to be related both to the increase in GSH status observed with NAC and CIT and to an increase in Se-GPX activity. These two mechanisms could lead to an inhibition of lipid peroxidation and a limited rate of GSH depletion which have both been shown to be immediate consequences of UVA irradiation [37,46]. Our data and evidence in the

literature support this hypothesis [47,48]. Moreover, selenium uptake [49] and retention may be facilitated by GSH [50]. It is interesting to note that the protective role of the combination of OTC and selenium is similar to that obtained with the thiazolidine alone. This may be related to the unchanged GSH synthesis observed with this molecule.

In summary, the deleterious effects of UVA are significantly reduced by pretreatment with NAC, CIT or OTC, which can limit GSH depletion. Our data clearly show an additional protective benefit of the combinations NAC–Se and CIT–Se. Because several selenoproteins e.g. thioredoxine reductase [51], exist in mammalian cells, further studies are required to elucidate the mechanism of interaction between NAC or CIT and Se in protecting human skin fibroblasts from UVA oxidative damage.

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