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Influence of treatment with aminoglutethimide on plasma and red-blood-cell glutathione status in breast cancer patients

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Abstract *Purpose*: Elevated cellular glutathione has been associated with resistance to cancer chemotherapy. Treatment with the aromatase inhibitor aminoglutethimide increases the concentration of γ-glutamyl transpeptidase (γ-GT) in breast cancer patients. This enzyme catalyzes the first step in the degradation of extracellular glutathione, and the products formed may act as precursors for intracellular glutathione synthesis. *Methods*: Plasma and red-blood-cell glutathione levels were determined in 26 patients suffering from advanced breast cancer before and during treatment with aminoglutethimide ($n = 16$) or the steroidal aromatase inhibitors exemestane or formestane ($n = 10$) and in 5 cancer patients receiving dexamethasone. *Results*: Pretreatment values for γ-GT in the total patient group ($n = 31$) correlated negatively with the level of reduced ($P < 0.0001$), oxidized ($P < 0.025$), and total glutathione ($P < 0.005$) in plasma. Plasma γ-GT levels increased by a mean value of 249% during treatment with aminoglutethimide. The concentration of reduced and oxidized glutathione in plasma decreased to 42.7% ($P < 0.0005$) and 80.6% ($P < 0.005$) of their pretreatment levels, respectively. This fall in reduced plasma glutathione correlated negatively with the increase in γ-GT ($P < 0.001$). The ratio of oxidized to reduced glutathione increased by 88.9% ($P < 0.005$), and this increase correlated positively with the increase in γ-GT ($P < 0.005$). Treatment with the steroidal aromatase inhibitors (exemestane and formestane) or dexamethasone did not influence the plasma thiol status. *Conclusions*: We conclude that aminoglutethimide influences plasma glutathione disposition by mechanisms not related to estrogen suppression or due to glucocorticoids given in concert.

Key words γ-Glutamyl transpeptidase · Glutathione · Aminoglutethimide · Reduction and oxidation · Breast cancer

Abbreviations *DMSO* Dimethyl-sulfoxide · *DTE* dithioerythritol · *GSH* reduced glutathione · *GSSG* glutathione disulfide · *γ-GT* γ-glutamyl transpeptidase · *HBr* hydrogen bromide · *mBrB* monobromobimane · *NEM* *N*-ethylmaleimide · *RBC* red blood cells · *SSA* 5-sulfosalicylic acid dihydrate · *tGSH* total glutathione

Introduction

Aminoglutethimide is widely used for treatment of postmenopausal breast cancer. The drug is a so-called aromatase inhibitor, lowering plasma estrogens by inhibiting peripheral conversion (“aromatization”) of circulating androgens into estrogens [10, 11]. However, the drug also expresses other biochemical effects, such as induction of p-450 mixed-function oxidases [9, 21].

Glutathione exists in a reduced (GSH) and an oxidized (GSSG) form and is the major detoxifying system for free radicals [20]. In addition, several xenobiotics and endogenous substances are conjugated to GSH by the glutathione sulfotransferases (GST) [23]. Elevated levels of intracellular GSH and/or GSTs are thought to play a role in resistance against chemotherapeutic agents by conjugation or detoxification of free radicals [23, 25].

An interesting observation is that the majority of patients treated with aminoglutethimide experience a 2- to 5-fold increase in plasma levels of γ-GT (γ-glutamyl transpeptidase) [11, 22]. γ-GT catalyzes the cleavage of the γ-glutamyl cysteine bond of extracellular glutathione (L-γ-glutamyl-L-cysteinylglycine). The γ-glutamyl moiety is transferred either to an amino acid or to water and can

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be transported into cells [16]. Cysteinylglycine may be hydrolyzed by dipeptidases, and the resulting free amino acids may cross the cellular membrane [4, 7, 15]. Thus, these reactions enable the import of the precursors for glutathione synthesis inside the cell [5, 17].

Many breast cancer patients receive chemotherapy in concert with or shortly after termination of treatment with aminoglutethimide. Alterations in the distribution of glutathione may influence sensitivity to chemotherapy [5, 8]. This study was designed to evaluate the influence of treatment with aminoglutethimide on plasma and red blood cell (RBC) concentrations of GSH and GSSG. Furthermore, we measured the reduced and oxidized forms of the glutathione precursors cysteine and cysteinylglycine in plasma. Because redox conditions change rapidly (within seconds) after blood collection [14, 26], we used a recently developed procedure for determination of the various thiol forms in which the redox conditions in plasma are stabilized by derivatization of the thiols at the time of blood collection [14].

Since any effect of aminoglutethimide could be due to estrogen suppression but also to other biochemical effects of aminoglutethimide as well as of glucocorticoids given routinely in concert, we also evaluated the glutathione status of patients treated with selective aromatase inhibitors not requiring glucocorticoid administration (formestane and exemestane) and in cancer patients receiving short-term treatment with dexamethasone.

Patients and methods

Patients

The study included a total of 31 patients (28 women and 3 men); all were suffering from breast cancer except for 5 patients treated with glucocorticoids alone (3 patients with a diagnosis of primary brain tumors and 2 patients suffering from pulmonary carcinomas with brain metastasis). All patients gave their informed consent.

In all, 1 man and 15 postmenopausal women suffering from advanced breast cancer were treated with aminoglutethimide. The drug schedule was aminoglutethimide given at 250 mg q.i.d. in concert with cortisone acetate given at 50 mg b.i.d. for 2 weeks and then at 25 mg b.i.d. The median age in this patient group was 61.5 (range 28–83) years. Ten postmenopausal women with advanced breast cancer were treated with other aromatase inhibitors (five received formestane given at 250 mg every 2nd week by the intramuscular route and 5 received exemestane given once daily at 25 mg as oral treatment). The median age in this group was 66 (range 48–83) years. Three women and two men were treated with dexamethasone alone at doses varying between 0.5 and 4 mg q.i.d. Their median age was 67 (range 59–76) years.

Materials

Cysteine, dithioerythritol (DTE), *N*-ethylmaleimide (NEM), *N*-ethylmorpholine, glutathione, glutathione disulfide, and oxidized cysteinylglycine were obtained from Sigma Chemical Co. (St. Louis, Mo.). $NaBH_4$ and tetrabutylammonium hydroxide were obtained from Fluka Chemie AG (Buchs, Switzerland). Acetic acid, dimethylsulfoxide (DMSO), hydrogen bromide (HBr), 5-sulfosalicylic acid dihydrate (SSA), perchloric acid, and phosphoric acid were purchased from Merck AG (Darnstadt, Germany). Methanol and acetonitrile were obtained from Labscan Ltd. (Dublin, Ireland). Monobromobimane (mBrB) was purchased from Molecular Probes Inc. (Eugene, Ore.). ODS Hypersil (3-μm particles) column material was obtained from Shandon Southern Ltd. (Chesire, UK). Columns for reverse-phase liquid chromatography (3-μm Hypersil; 150×4.6 mm) were slurry-packed at 62.1 MPa with a Shandon column packer.

Blood sampling

Blood samples were obtained before the commencement of treatment and after a period of 4–16 weeks on treatment with either aminoglutethimide, exemestane, or formestane. Since most patients treated with dexamethasone without any other systemic therapy receive such treatment for a limited period, blood samples in this patient group were taken before treatment started and following 4–22 days on therapy.

All samples were obtained by venipuncture between 8 a.m. and 10 a.m. after an overnight fast. The blood samples were collected into heparinized tubes on ice containing either mBrB (for the determination of reduced thiols), NEM (for oxidized thiols), or no additive except for heparin (total thiol components).

Samples were centrifuged immediately at 1500 *g* for 10 min at 4 °C. Aliquots of 30-μl were taken from the tubes containing heparin only and were mixed with 30 μl 2.0 *M* $NaBH_4$ and 60 μl 20% (w/v) SSA with 100 μ*M* DTE. The mixture was incubated on ice for 20 min before centrifugation (1500 *g* for 2 min). For determination of glutathione in RBC, 100-μl aliquots of the hemoglobin phase were taken from the tubes containing heparin only, and both these and the plasma samples were stored at −20 °C until analysis. Plasma from the other tubes were removed and stored at −20 °C without any further additive.

Determination of reduced, free oxidized, and total glutathione, cysteine, and cysteinylglycine in plasma

Assays for measurements of reduced, free oxidized, and total glutathione, cysteine, and cysteinylglycine have been described in detail elsewhere [14]. The amounts of reduced thiols were determined in the mBrB-treated plasma samples. Reduced thiols react with mBrB and form fluorescent adducts, which were quantified by reversed-phase ion-pair liquid chromatography and fluorescence detection. Free oxidized forms were quantified in NEM-treated plasma. NEM blocks free sulfhydryl groups, enabling the selective determination of oxidized forms. Samples were subsequently treated with SSA. The disulfides were reduced by $NaBH_4$ and then derivatized with mBrB and quantified as described above.

Total glutathione, cysteine, and cysteinylglycine were determined in untreated plasma. Free and protein-bound disulfides in the samples were reduced by $NaBH_4$, thiols were derivatized with mBrB, and the thiol-bimane adducts were quantified as described above. For each of the thiols the ratio of reduced to total species was determined and used as a parameter of the redox status of that thiol. Processed and derivatized samples were stored at −20 °C until chromatographic analysis and were frozen and thawed only once. The various thiol species were routinely measured in duplicate.

Determination of glutathione in RBC

Total blood aliquots (100 μl) were thawed after the addition of 1 ml 5% (w/v) SSA with 50 μ*M* DTE. This was mixed thoroughly and centrifuged at 1500 *g* for 10 min at 4 °C. An aliquot of 250 μl from the supernatant was then diluted with 1 ml 5% (w/v) SSA with 50 μ*M* DTE. Total free glutathione (reduced glutathione + glutathione disulfide + soluble glutathione mixed disulfide; for simplicity referred to as tGSH in the text) and GSH were determined in this diluted acid extract according to a modification [14] of a chromatography procedure described elsewhere [26]. The fraction of oxidized glutathione (glutathione disulfide + soluble glutathione

mixed disulfide) was calculated by the subtraction of GSH from tGSH. All analyses were performed in duplicate.

The hemoglobin concentration in the pellets of ethylenediaminetetraacetic acid (EDTA)-blood samples following centrifugation (1500 *g*) and removal of the plasma fraction was measured by the cyanmethemoglobin method [3] using a T450 Coulter counter.

Determination of γ-GT

The γ-GT activity in plasma was determined routinely by the Technicon Chem Systems method [27].

Statistical analysis

Parameters were tested for goodness-to-fit to a normal distribution with the use of Q-Q plots as raw data and after appropriate linear transformation [6]. All parameters were found to be best fitted to log-normal distribution. Thus, data are presented as geometric mean values with 95% confidence intervals of the means. In addition, data obtained before and during treatment with aminoglutethimide or formestane/exemestane were compared using the paired *t*-test. All *P* values given are two-tailed.

Possible correlations between the concentration of γ-GT, on the one hand, and those of GSH, GSSG, and tGSH in plasma and RBC in the pretreatment situation were tested with use of the Pearson rank-correlation coefficient on log-transformed data. The same test was used to evaluate correlations between percentage alterations in γ-GT levels and the other parameters within the group of patients treated with aminoglutethimide and within the combined group of patients treated with exemestane or formestane.

Three of the patients receiving aminoglutethimide had γ-GT values above the normal limit before the commencement of aminoglutethimide therapy. Thus, possible correlations between alterations in γ-GT and the other parameters from the remaining 13 patients expressing normal γ-GT levels before the start of treatment were tested separately. Similarly, we performed a separate analysis in the group of patients receiving treatment with formestane or exemestane, excluding the two patients with pretreatment values of γ-GT above the normal limit.

A Kruskal-Wallis test was done to evaluate whether there was a difference in the pretreatment levels of total cysteine between the patient group receiving aminoglutethimide, the combined exemestane/formestane group, and the group treated with dexamethasone.

Results

Pretreatment levels of γ-GT in the total patient group ($n = 31$) showed a negative correlation with plasma GSH ($r = -0.827$, $P < 0.0001$), tGSH ($r = -0.537$, $P < 0.005$), and GSSG ($r = -0.442$, $P < 0.025$; Fig. 1) but showed no correlation with the concentration of any of these parameters in RBC.

Table 1 shows plasma and RBC concentrations of the different thiol forms and of γ-GT as determined before and during treatment with aminoglutethimide. Aminoglutethimide treatment suppressed the concentration of GSH in plasma from 1.15 to 0.49 μ*M* (mean suppression to 42.7% of the pretreatment level, $P < 0.0005$) and lowered the GSSG content in plasma from 0.96 to 0.78 μ*M* (80.6% of the pretreatment value, $P < 0.005$; Fig. 2). The ratio of GSSG to GSH in plasma was increased from 0.84 to 1.58 (mean increase of 88.9%, $P < 0.005$),

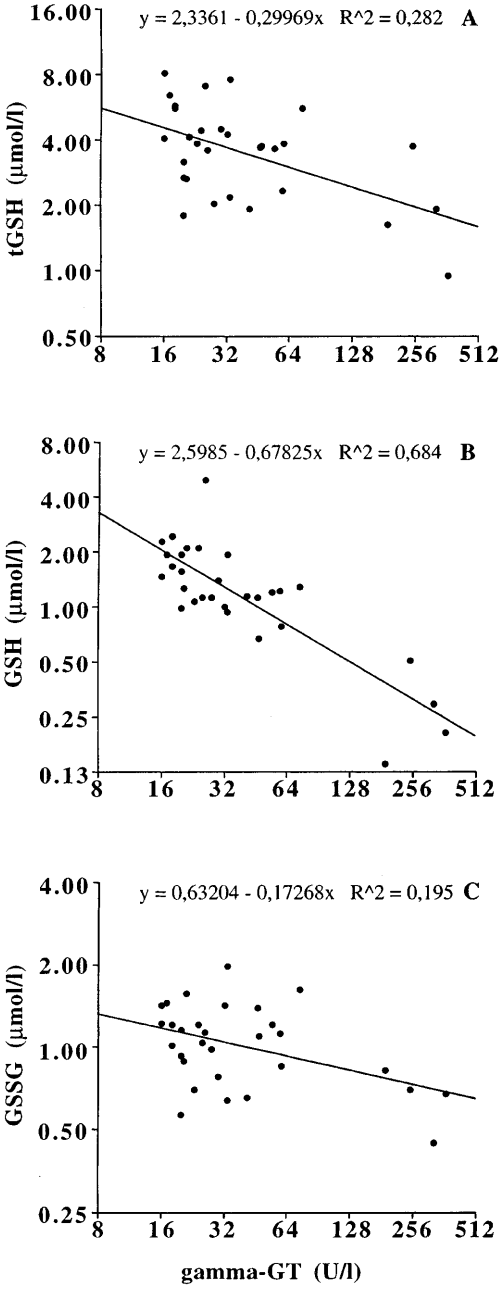

Fig. 1A–C Correlations between the pretreatment levels of γ-GT and the pretreatment levels of **A** total glutathione, **B** reduced glutathione, and **C** oxidized glutathione in plasma from the entire patient group ($n = 31$)

whereas the ratio of total glutathione to total cysteinylglycine decreased from 0.19 to 0.16 (81.1% of the pretreatment value, $P < 0.0025$). A decrease from 1.80 to 0.89 (49.7% of the pretreatment value, $P < 0.005$) was also observed in the ratio of GSH to reduced cysteinylglycine. A small but nonsignificant increase in the RBC level of GSH, GSSG, and tGSH was observed.

Treatment with aminoglutethimide increased the content of γ-GT in plasma by 249% ($P < 0.0001$, Table 1). The increase in γ-GT during treatment with aminoglutethimide correlated negatively with the

Table 1 Effects of aminoglutethimide on the redox status of thiols in plasma and blood cells. Data are given as geometrical mean values with 95% confidence limits of the means

Parameters	Pretreatment values	Treatment with aminoglutethimide	% of pretreatment value
Plasma[a]:			
tGSH	3.94 (3.02–5.21)	3.36 (2.66–4.26)	84.8 (70.8–102.4)
GSH	1.15 (0.79–1.69)	0.49 (0.32–0.76)	42.7 (29.0–62.8)*3
GSSG	0.96 (0.81–1.15)	0.78 (0.63–0.96)	80.6 (70.9–91.6)*1
tCYS	233.4 (205.6–264.8)	240.8 (213.8–278.7)	101.3 (94.3–112.9)
CYS	7.47 (6.25–8.94)	6.73 (5.34–8.49)	90.0 (73.0–115.3)
CSSC	80.5 (73.4–88.2)	80.2 (69.8–92.1)	99.6 (86.8–114.2)
tCYS-GLY	20.6 (16.4–25.7)	21.3 (18.5–24.5)	103.7 (84.8–126.7)
CYS-GLY	0.64 (0.51–0.81)	0.55 (0.42–0.71)	85.8 (69.6–105.9)
OCYS-GLY	7.30 (6.51–8.20)	6.67 (5.59–7.86)	90.80 (77.0–106.9)
GSSG/GSH	0.84 (0.59–1.20)	1.58 (1.16–2.17)	188.9 (128.3–278.4)*1
tGSH/tCYS-GLY	0.19 (0.17–0.22)	0.16 (0.13–0.19)	81.8 (73.6–91.0)*2
GSH/CYS-GLY	1.80 (1.14–2.84)	0.89 (0.61–1.32)	49.7 (31.5–78.3)*1
GSSG/OCYS-GLY	0.13 (0.11–0.16)	0.12 (0.09–0.15)	88.8 (73.3–107.5)
γ-GT	33.3 (21.4–51.7)	116.2 (68.3–197.4)	349.4 (247.2–493.8)*4
RBC[b]:			
tGSH	0.89 (0.60–1.30)	0.91 (0.64–1.51)	110.6 (84.5–144.6)
GSH	0.58 (0.37–0.91)	0.66 (0.44–1.01)	114.7 (89.8–146.7)
GSSG	0.27 (0.17–0.41)	0.33 (0.20–0.55)	125.0 (83.0–188.1)
GSSG/GSH	0.45 (0.25–0.82)	0.49 (0.32–0.74)	107.3 (76.5–150.4)

*1 $P < 0.005$ (paired t-test); *2 $P < 0.0025$ (paired t-test); *3 $P < 0.0005$ (paired t-test); *4 $P < 0.0001$ (paired t-test)

[a] All values in μmol/l except for γ–GT (U/l). Abbreviations: *CSSC* cystine, *CYS* cysteine, *CYS-GLY* reduced cysteinyl glycine, *OCYS-GLY* oxidized cysteinyl glycine, *tCYS* total cysteine, *tCYS-GLY* total cysteinylglycine

[b] All values in μmol/g hemoglobin

alterations in plasma GSH ($r = -0.754$, $P < 0.001$) as well as with the change in the ratio of GSH to reduced cysteinylglycine in plasma ($r = -0.677, P < 0.005$). Although the decrease in plasma GSSG correlated with the increase in γ-GT, this correlation did not reach a level of statistical significance ($r = -0.271, P > 0.20$). On the other hand, the increase in the ratio of GSSG to GSH in plasma correlated positively with the increase in γ-GT ($r = 0.663$, $P < 0.005$). Similar correlations were also observed when patients with pretreatment values of γ-GT above the normal limit (three patients) were excluded from the analysis (γ-GT/GSH: $r = -0.742$, $P < 0.005$; GSH/reduced cysteinylglycine: $r = -0.767, P < 0.0025$; GSSG/GSH: $r = 0.59, P < 0.05$). In this case the increase in γ-GT also correlated negatively with alterations in plasma GSSG ($r = -0.56, P < 0.05$) and with the ratio of plasma GSSG to oxidized cysteinylglycine ($r = -0.812$, $P < 0.001$).

A significant difference between the pretreatment levels of total cysteine was found between the aminoglutethimide group, the combined (exemestane/formestane) group, and the dexamethasone-treated group ($P < 0.05$) when they were compared using Kruskal-Wallis statistics. We have no direct explanation for this observation, which may have occured by chance. Interestingly, no such difference between the groups was found for reduced and oxidized cysteine, which are the free and biologically active forms.

As no difference between patients receiving treatment with exemestane and formestane was observed, data for these two groups were pooled for statistical analysis. Apart from a drop in plasma levels of reduced cysteinylglycine from 0.45 to 0.31 μM (69.1% of the pretreatment value, $P < 0.05$), no significant change in any of the parameters was observed in this group (Table 2).

Patients in the pooled exemestane/formestane group were tested for correlations between individual alterations in plasma γ-GT and other parameters during treatment. A positive correlation between alterations in γ-GT and reduced cysteinylglycine was found ($r = 0.859$, $P < 0.005$). In contrast, alterations in γ-GT correlated negatively with alterations in the ratio of total glutathione to total cysteinylglycine in plasma ($r = -0.857$, $P < 0.0025$), with the ratio of GSH to reduced cysteinylglycine ($r = -0.801$, $P < 0.01$), and with the ratio of GSSG to oxidized cysteinylglycine ($r = -0.723$, $P < 0.05$). Negative correlations were also found between alterations in γ-GT and alterations in plasma GSH ($r = -0.627, P < 0.052$) as well as plasma tGSH ($r = 0.647, P < 0.05$) in the pooled exemestane/formestane group. Exclusion of two pateints expressing γ-GT values above the normal limit in the pretreatment situation from the statistical analysis did not change the result, except for alterations in the ratio between total glutathione and total cysteinylglycine, which no longer correlated significantly with alterations in γ-GT.

No significant change in any of the parameters was observed in the patient group receiving dexamethasone (data not shown).

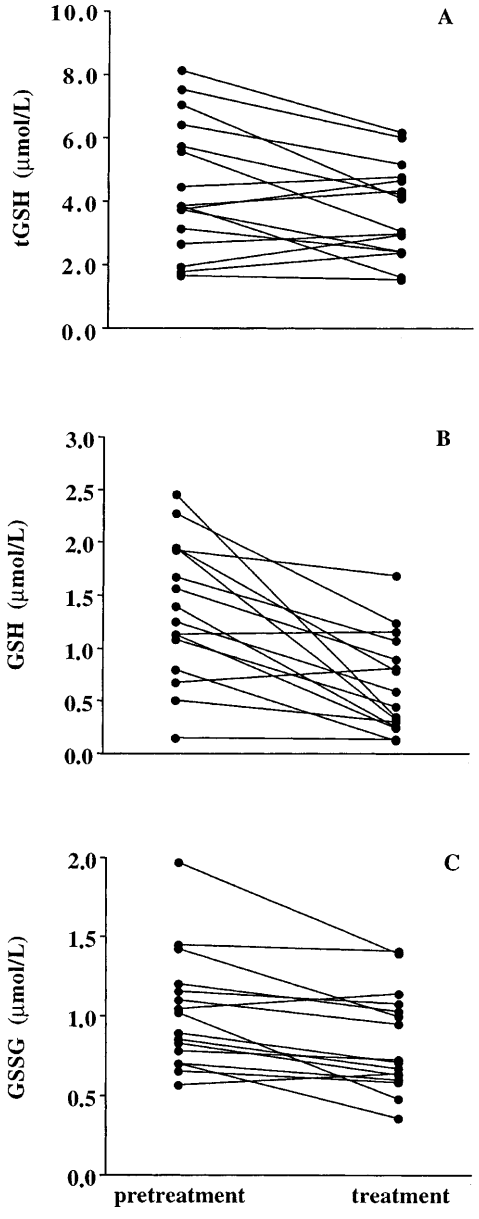

Fig. 2A–C Plasma levels of **A** total glutathione, **B** reduced glutathione, and **C** oxidized glutathione as measured before and during treatment with aminoglutethimide ($n = 16$)

Discussion

This study shows aminoglutethimide to decrease plasma levels of GSH and GSSG in breast cancer patients. Aminoglutethimide, similar to several other xenobiotics such as antipyrine and phenobarbitone, enhances p-450-dependent liver enzymes [24, 28]. Interestingly, all these enzyme inducers also increase plasma levels of γ-GT [21, 22]. The mechanism for aminoglutethimide's induction of γ-GT is, to the knowledge of the authors, not known. The enzyme is responsible for metabolism of extracellular glutathione [16]. The individual drop in plasma GSH and GSSG correlated with the increase in plasma γ-GT. This observation, together with the finding that the other aromatase inhibitors investigated (formestane and exemestane) neither increased γ-GT levels/nor suppressed plasma GSH or GSSG, suggests an increase in plasma γ-GT to be the mechanism by which aminoglutethimide suppresses plasma GSH/GSSG levels. This hypothesis is further supported by the finding of a drop in the glutathione-to-cysteinylglycine ratio and by a study revealing an increased oxidation of glutathione in plasma from patients expressing increased levels of γ-GT [13].

Contrary to what was observed in the plasma, a slight, nonsignificant elevation in the level of GSH, GSSG, and tGSH was observed in RBC during treatment with aminoglutethimide. GSH in the cell is synthesized from extracellular metabolites of GSH obtained by γ-GT cleavage, which may cross the cellular membrane [4, 7, 16]. However, an increase in plasma γ-GT does not necessarily result in an increased cellular level of GSH, as the rate-limiting enzyme in GSH synthesis, γ-glutamylcysteine synthetase, is inhibited by GSH through a negative feedback mechanism [15]. The possibility therefore exists that the two mechanisms, increased delivery of GSH precursors and product inhibition, may counteract each other, balancing the intracellular GSH level. The hypothesis that alterations in plasma γ-GT levels may influence plasma but not RBC thiol levels is further supported by the observation of a negative correlation between the level of γ-GT and the plasma level of GSH, GSSG and tGSH in the pretreatment situation along with the lack of correlation between plasma γ-GT and the same parameters measured in the RBC. Although Michelet et al. [18] reported a lack of correlation between RBC as well as plasma GSH and plasma γ-GT, they used a different analytical method for measurement of the thiols. In addition, in that study [18] the blood samples were not instantly chilled. This is a critical issue, as redox conditions in blood samples may change within seconds of blood sampling [14, 19, 26] in a temperature-dependent manner [1].

One of the patients in the aminoglutethimide group was a man. This should not have any effect on the results, as aminoglutethimide works the same way in men and postmenopausal women [12]. Interestingly, exclusion of the man from the statistical analysis did not influence any of the *P* values.

Despite the observation that the levels of GSH and GSSG in plasma were decreased in patients treated with aminoglutethimide, the ratio of GSSG to GSH increased, and this increase was positively correlated with the increase in γ-GT. One possible explanation could be that an increase in the intracellular level of GSSG may increase the export of GSSG out of the cell to protect the cell from a toxic effect of high GSSG levels [4, 7]. An alternative explanation for the increase in the ratio between GSSG and GSH might be that cysteinylglycine produced from the cleavage of GSH by the action of γ-GT is oxidized nonenzymatically and rapidly to form

Table 2 Effects of exemestane and formestane (control group) on the redox status of thiols in plasma and blood cells. Data are given as geometrical mean values with 95% confidence limits of the means

Parameters	Pretreatment values or formestane	Treatment with exemestane	% of pretreatment value
Plasma[a]:			
tGSH	2.79 (1.96–3.98)	2.61 (1.86–3.65)	93.3 (59.8–145.8)
GSH	1.03 (0.54–1.96)	0.82 (0.50–1.33)	79.4 (45.6–138.4)
GSSG	0.97 (0.74–1.28)	0.91 (0.70–1.18)	93.9 (74.5–118.3)
tCYS	169.2 (143.2–199.5)	199.7 (141.7–281.2)	118.2 (84.6–164.9)
CYS	8.51 (6.13–11.81)	6.79 (5.52–8.37)	80.6 (52.1–124.6)
CSSC	84.5 (67.6–105.7)	93.6 (68.4–127.9)	110.6 (32.7–138.0)
tCYS-GLY	13.6 (9.94–18.5)	15.9 (11.4–22.3)	17.2 (77.5–177.5)
CYS-GLY	0.45 (0.32–0.63)	0.31 (0.22–0.43)	69.1 (49.9–95.8)*
OCYS-GLY	7.49 (6.50–8.62)	7.78 (6.37–9.51)	104.0 (91.9–177.7)
GSSG/GSH	0.94 (0.57–1.56)	1.11 (0.77–1.61)	118.3 (69.3–201.8)
tGSH/tCYS-GLY	0.21 (0.16–0.26)	0.16 (0.12–0.22)	79.5 (59.6–106.2)
GSH/CYS-GLY	2.30 (1.01–5.20)	2.70 (1.35–5.39)	117.6 (52.6–262.7)
GSSG/OCYS-GLY	0.13 (0.10–0.17)	0.12 (0.09–0.15)	90.2 (74.3–109.6)
γ-GT	49.5 (22.9–106.9)	71.7 (26.6–193.6)	145.1 (55.0–383.1)
RBC[b]:			
tGSH	1.88 (1.18–3.00)	1.98 (0.89–4.41)	100.4 (63.7–158.2)
GSH	1.40 (0.81–2.42)	1.50 (0.65–3.47)	102.4 (63.6–164.9)
GSSG	0.41 (0.20–0.84)	0.38 (0.16–0.95)	91.0 (51.6–160.8)
GSSG/GSH	0.29 (0.13–0.66)	0.25 (0.12–0.52)	89.0 (50.3–157.5)

* $P < 0.0005$ (paired t-test)

[a] All values in μmol/l except for γ-GT (U/l). Abbreviations: *CSSC* cystine, *CYS* cysteine, *CYS-GLY* reduced cysteinyl glycine, *OCYS-GLY* oxidized cysteinyl glycine, *tCYS* total cysteine, *tCYS-GLY* total cysteinylglycine

[b] All values in μmol/g hemoglobin

cysteinylbisglycine, which may oxidize GSH in a non-enzymatic transhydrogenation reaction [13].

Aminoglutethimide exerts its antitumor action by suppressing plasma estrogen levels. This mechanism, however, is not likely to have played any role in the effects observed in the present study, as no alteration either in the plasma γ-GT level or in the thiol status was observed among patients receiving other estrogen suppressors.

Although the alterations in γ-GT and thiol status observed in patients treated with aminoglutethimide could have been caused by the glucocorticoids given in concert, this hypothesis is refuted by the finding of no change in any of these parameters among patients treated with dexamethasone alone.

Patients relapsing during treatment with aminoglutethimide often receive subsequent treatment with chemotherapy. The observed increase in γ-GT and the altered thiol status might render these patients more susceptible to chemoresistance [19, 23, 25]. Although we did not find any significant increase in the content of any of the thiols in the blood cells, the conditions for an increased synthesis of glutathione intracellularly are laid by an increase in extracellular glutathione precursors that may cross the cellular membrane. Expression of γ-GT in breast tissue [2] opens the possibility of increased enzyme activity in response to drug treatment within tumor tissue [5]. Our findings raise the possibility that aminoglutethimide and, probably, also other drugs (such as anti-epileptic drugs) increasing γ-GT expression may influence chemoresistance in relation to cancer chemotherapy.

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