Cancer Chemother Pharmacol (2000) 45: 192–198

ORIGINAL ARTICLE

Nil Dizdar · Anita Kullman · Bertil Kågedal

Comparison of *N*-acetylcysteine and L-2-oxothiazolidine-4-carboxylate as cysteine deliverers and glutathione precursors in human malignant melanoma transplants in mice

Received: 26 January 1999 / Accepted: 7 September 1999

Abstract *Purpose*: Glutathione is an important cellular compound which affects detoxification of electrophiles and may have direct or indirect effects on pigment formation. It is therefore of importance to study interstitial concentrations in melanoma tissue while decreasing its formation with an enzyme inhibitor and increasing its amount with cysteine deliverers. *Method*: Glutathione formation was inhibited by intraperitoneal (i.p.) injection of BSO. *N*-Acetylcysteine (NAC) and L-2-oxothiazolidine-4-carboxylate (OTC) were then given i.p. to subgroups of the animals. Intratumoral microdialysis was performed during BSO treatment, during BSO treatment combined with NAC or OTC and after discontinuation of BSO but ongoing NAC or OTC treatment. *Results*: Glutathione formation was inhibited during BSO treatment. The dialysate concentrations of both glutathione and cysteine decreased during concomitant treatment with BSO and NAC or OTC. Recovery of the amounts of the two compounds was seen in both groups after discontinuation of BSO treatment. In the NAC group we also observed an acute increase in dialysate concentrations of cysteine after NAC injection. The 5-*S*-cysteinyldopa concentrations were unaffected by variations in glutathione and cysteine concentrations. *Conclusions*: 5-*S*-Cysteinyldopa in melanoma is not formed from glutathione in vivo to any appreciable extent. The intracellular amount of cysteine is probably not a limiting factor for cysteinyldopa formation. It seems that both NAC and OTC can be used as cysteine deliverers to melanoma cells in vivo to produce recovery of glutathione levels after synthesis inhibition by BSO treatment.

N. Dizdar (✉) · A. Kullman · B Kågedal
Department of Clinical Chemistry,
University Hospital, *S*-581 85 Linköping, Sweden
e-mail: Nil.Dizdar@lio.se
Tel: +46-13-223234; Fax: +46-13-223240

Keywords *N*-Acetylcysteine · Athymic mice · Buthionine 4-sulphoximine · 5-*S*-Cysteinyldopa · Microdialysis · Glutathione · L-2-Oxothiazolidine-4-carboxylate

Introduction

Malignant melanoma is a serious tumour which is increasing in incidence in the white population all over the world, and treatment with cytostatic agents or other cytotoxic treatments have limited effects when spread has occurred. Glutathione is an important constituent of the cell and plays an important role in the detoxification of a large number of electrophiles [1]. High intracellular concentrations of glutathione, as is the case in malignant melanoma, may therefore protect tumour cells against cytotoxic drugs, and consequently, inhibition of glutathione synthesis may potentiate the cytotoxic effect of these drugs [2].

Buthionine sulphoximine (BSO) inhibits the enzyme γ-glutamyl-cysteine synthetase, which is the rate-limiting enzyme in the synthesis of glutathione. BSO is therefore very useful for experimentally decreasing glutathione levels [3, 4]. The decrease in intracellular glutathione during treatment with BSO is largely accounted for by its transport out of cells while its production is inhibited [5].

Administration of L-2-oxothiazolidine-4-carboxylate (OTC) or *N*-acetyl-L-cysteine (NAC) increases the levels of glutathione in the liver [6]. By the influence of the enzyme 5-oxo-L-prolinase, OTC is converted to *S*-carboxy-L-cysteine which spontaneously decarboxylates to L-cysteine, the latter being utilized for glutathione synthesis. The bioavailability in humans of NAC given orally is less than 20% [7] probably due to fast metabolism in the gut wall and the liver. The almost complete intestinal absorption of NAC [7] leaves a large amount for in vivo metabolism, e.g. for cellular uptake, deacetylation to cysteine [8] and synthesis of glutathione [9]. Administering the cysteine deliverers OTC or NAC seems to be safer than administering cysteine itself since

cysteine has been reported to have toxic effects on the central nervous system [10, 11]. It has been suggested that OTC should be a more effective cysteine deliverer than NAC [6], but this has not been experimentally studied in comparative investigations. In contrast, Dringen and Hamprecht [12] have shown that NAC, but not OTC, serves as a cysteine donor for the synthesis of glutathione in cultured neurons derived from embryonal rat brain. It is therefore of importance that these two compounds are studied simultaneously in whole animal studies.

Using a microdialysis technique we have studied the in vivo effects of BSO on interstitial glutathione (GSH and GSSG) levels in human melanoma transplants [13]. In a small study on mice we investigated the recovery of the glutathione concentrations following treatment with OTC and NAC, and the results indicate that NAC might be a more potent cysteine deliverer than OTC. We therefore extended our earlier studies to focus on the recovery of both cysteine and glutathione after a period of inhibition of glutathione synthesis in mice.

Glutathione also plays an important role in melanin synthesis, and it has been suggested that glutathione either reacts directly with dopaquinone to give glutathionedopa, which is then degraded to cysteinyldopa [14], or provides cysteine for the formation of 5-*S*-cysteinyldopa [15]. Melanoma tissue is a suitable in vivo model for studies of pigment formation. We therefore also used this model for the investigation of the relationship between glutathione and 5-*S*-cysteinyldopa.

In the present study we investigated the 5-*S*-cysteinyldopa concentration in melanoma tissue microdialysates while decreasing the glutathione concentration of the cell, with the aim of elucidating the importance of glutathione itself for cysteinyldopa production. We produced a sustained cysteine concentration in the animals by giving NAC or OTC simultaneously with BSO inhibition of glutathione synthesis.

Materials and methods

Chemicals

BSO, NAC and OTC were obtained from Sigma Chemical Co. (St. Louis, Mo.) and EDTA was from Merck (Darmstadt, Germany). Ringer acetate and sodium chloride were from Kabi Pharmacia (Uppsala, Sweden), xylazine (Rompun; Bayer, Leverkusen, Germany) and ketamine (Ketalar; Parke-Davis, New Jersey). The latter two compounds were both used for anaesthesia. All chemicals were of analytical quality.

Animals and tumour production

Athymic mice (nu/nu-Balb/cABom), 25 g in weight, were bought from Bomholt Gaard (Ry, Denmark), and a melanoma cell line JKM 86-4 was a gift from Dr Ulrika Stierner, Gothenburg. The study was performed with the approval of the regional ethics committee for animal research of the Faculty of Health Sciences, Linköping University.

About 10^7 melanoma cells from culture were injected at four locations subcutaneously into the backs of the mice. The tumours were about 10×11 mm when first used and about 10×13 mm when the last microdialysis experiments were performed. In a second series of experiments four different melanoma cell lines were injected as described above. The cell lines used were JKM 86-4, M9, Mel 28 and B16F1. The tumours were about 10×11 mm when used.

The total tumour burden was 1.7% in all experiments and none of the animals had any metastasis at autopsy, which was performed on all animals.

Anaesthesia

For general anaesthesia of the mice during the microdialysis 0.1 ml xylazine (20 mg/ml) and 0.4 ml ketamine (50 mg/ml) were mixed with 0.5 ml sodium chloride (154 m*M*) and 0.12 ml of the mixture was injected intraperitoneally (i.p.). The mice were then placed on a temperature-controlled (35–36 °C) electric pad. Maintenance of anaesthesia was by subcutaneous injection of 0.05 ml of the mixture every 30 min.

Microdialysis

A microdialysis set from Carnegie Medicin (Stockholm, Sweden) was used. It consisted of a CMA/100 microinjection pump, microsyringes, and a CMA/130 in vitro stand with accessories. The dialysis membrane of the probes used was a cuprophane membrane CMA/11 with a molecular cut off at 20,000 Da.

The in vitro recoveries obtained with microdialysis of 5-*S*-cysteinyldopa and glutathione have previously been reported [16, 17]. Thus we obtained recoveries of 5-*S*-cysteinyldopa, glutathione and cysteine of 48%, 56%, and 75%, respectively. The data reported here are uncorrected for recoveries.

Experimental design

In the studies of glutathione changes after treatment, 21 athymic mice were used, each with four melanoma transplants approximately 10×11 mm wide. The mice were first treated with 3 mmol/kg BSO by i.p. injection twice a day. After 2 days of BSO treatment the mice were anaesthetized and two probes were inserted into separate transplants. Microdialysis was started and, 60 min from the start, ten animals were given NAC i.p. (2 mmol/kg) and the remaining ten animals were given OTC i.p. (2 mmol/kg). Microdialysis was conducted for 3 h with Ringer acetate pumped through the probes at a flow rate of 1 µl/min. Dialysates were collected in fractions of 15 µl into tubes containing 45 µl of a solution of 10 m*M* HCl and 2 m*M* Na_2EDTA. The mice were then treated twice a day with the combination of BSO and NAC or BSO and OTC in the amounts stated above.

A second microdialysis experiment was performed after 3 days of treatment with these combinations. The microdialysis was conducted for 3 h as described above. The experiment was performed 6 h after the previous injections. The BSO injections were discontinued after a total of 5 days of treatment in both groups and the animals were treated with NAC or OTC for another 4 days (total 7 days). A third and final microdialysis experiment was performed on the last day of NAC or OTC treatment and 9 h after the injections.

In the studies of the relationship between microdialysates and tissue concentrations, 21 athymic mice were used, each with tumours as described above. They were divided into four groups comprising seven mice bearing B16F1 tumours, three bearing M9 tumours, eight bearing JKM 86-4 tumours, and three bearing Mel 28 tumours. When the tumours had reached a size of 10×11 mm microdialysis was performed over 2 h on the anaesthetized animals as described above. After the microdialysis the tumours were removed immediately and cut into two pieces. One piece was homogenized immediately and the other was frozen at −70 °C. Homogenates were made at different times and the results from the analyses were compared.

Analytical methods

A previously reported fluorimetric high-performance liquid chromatographic method for the determination of glutathione (GSH and GSSG) and cysteine in microdialysis samples was used [16]. In the present investigation the same instrumental set-up as recently described was used [13]. Automated analysis of 5-*S*-cysteinyldopa in urine has also been described [18]. This method was used except that microdialysates were injected directly onto the analytical column as described previously [13].

Statistical analysis

Analysis of variance (ANOVA) was carried out with animal and microdialysis set as variables. For adjustment for multiple comparisons the Tukey method was used. Results are expressed as means ± standard deviation (SD). A P-value < 0.05 was considered significant. The results from the second series of experiments were analysed by the partial correlation test with the tumour group set as variable.

Results

As expected, low levels of glutathione were seen during the first microdialysis experiments, performed after 2 days of BSO treatment (Table 1). In the dialysates from the second microdialysis the glutathione levels had decreased even more. After discontinuation of BSO treatment the levels of glutathione increased significantly ($P < 0.001$) in both groups (third microdialysis experiment), although they did not reach the levels from our previous experiments under basal conditions [13].

The initial concentrations of cysteine were also lower than the levels found under basal conditions [13]. However, in the NAC group there was a dramatic increase in cysteine levels approximately 30 min after the NAC injection ($P < 0.001$), reaching a maximum 60 min after injection (Figs. 1A and 2). In the OTC group there was no corresponding increase in the cysteine concentrations (Fig. 1B). During the second microdialysis (stage 2) the cysteine levels were decreased compared to the first stage. During stage 3 the cysteine concentrations were not significantly different from the levels during both stage 1 and 2. During stage 1 (NAC or OTC given just prior to the microdialysis) there was a significant difference between the group given NAC and the group given OTC. This was not the case during stages 2 and 3 when these compounds were given 6 and 9 h, respectively, before the start of microdialysis. The 5-*S*-cysteinyldopa levels remained generally unaltered throughout the experiment in both groups (Table 1).

The results from the tumour homogenates showed that the intracellular concentrations of glutathione, cysteine and 5-*S*-cysteinyldopa were reasonably stable at −70 °C for 2 weeks in all four tumour lines (data not shown). We determined the degree of correlation between the microdialysis concentrations of glutathione, cysteine and 5-*S*-cysteinyldopa and the tumour tissue concentrations. No correlations were found for glutathione and cysteine (data not shown) but with 5-*S*-cysteinyldopa there was a significant correlation between microdialysate and tissue concentrations both for the initial microdialysis and at steady state (Fig. 3).

Discussion

The concentration of a compound in the microdialysate from an organ is thought to reflect the interstitial concentration of the compound. Acute changes from that level, e.g. increases in the concentration during a short experiment or the "artefactually" high level obtained on insertion of a probe into the tissue, may reflect release of the compound from surrounding cells. The rapid increase in NAC in the microdialysate (Fig. 2) from melanoma tissue after i.p. injection is a result of mass transfer from the blood circulation, and since NAC is deacetylated in several other tissues than melanoma tissue to give cysteine [19], the acute changes of cysteine in the microdialysate (Figs. 1 and 2) are also a result of that transfer. This result also illustrates that the conversion of NAC to cysteine is rapid and is in accordance with the short half-life of NAC in blood [7]. The dialysate concentrations of cysteine obtained, well separated in time from acute levels following delivery of NAC or OTC, would represent steady-state concentration levels at that time. It is well recognized that 5-*S*-cysteinyldopa is produced in large amounts in melanoma tissue and via the interstitial fluid reaches the blood circulation and finally the urine. Therefore the microdialysate concentration should be between that of the melanoma cell and the plasma. An unchanged microdialysate concentration as found in the present study thus indicates that cysteinyldopa production is not changed because of a decrease in glutathione.

It has been postulated that the presence of thiols in melanocytes leads to the formation of cysteinyldopas [20], since nucleophilic addition of thiols to dopaquinone is a much faster reaction than the cyclization of dopaquinone. In the present study we were not able to show any substantial change in the interstitial 5-*S*-cysteinyldopa concentration after "cysteine substitution". The observed increase in cysteine after the NAC injection might not have been large enough to stimulate 5-*S*-cysteinyldopa formation. An alternative explanation could be that cysteine is not a limiting factor in the pigment forming compartments of the cell.

What about glutathione? It has been suggested that the formation of glutathionedopa results from the reaction of glutathione and dopaquinone. Glutathionedopa should then be degraded to cysteinyldopa. The results of the present study indicate that glutathione itself does not play a significant role in these reactions.

There is substantial evidence that glutathione in the form of GSH is transported across cell membranes [21]. In one type of transport (inter-organ type) glutathione is transported out of the cells (e.g. the liver) into the plasma and then removed from the plasma by cells equipped with substantial amounts of γ-glutamyl transpeptidase (e.g. the kidney). Thus, some organs, e.g.

Table 1 Concentrations (μmol/l) of glutathione, cysteine and 5-*S*-cysteinyldopa in human malignant melanoma transplants in mice before BSO treatment (stage 0, reference 13) after 2 days of BSO treatment (stage 1), after 5 days of BSO and 3 days of NAC or OTC treatment (stage 2), and after a total of 7 days of NAC or OTC treatment with BSO discontinued for 4 days (stage 3). Data were obtained during the first 15 min, after 1 h, after 2 h and before the end of the microdialysis. The results are expressed as means ± SD

Treatment stage	0–15 min			60–75 min			105–120 min			165–180 min		
	Baseline group	NAC group	OTC group	Baseline group	NAC group	OTC group	Baseline group	NAC group	OTC group	Reference group	NAC group	OTC group
Glutathione												
0	318 ± 87.5 (n = 12)			5.84 ± 2.28 (n = 12)			3.59 ± 1.05 (n = 11)			4.78 ± 4.33 (n = 11)		
1		14.1 ± 6.17	13.0 ± 5.04		2.16 ± 1.31	2.34 ± 3.17		2.55 ± 2.49	1.52 ± 0.62		1.28 ± 0.88	1.32 ± 0.58
2		3.99 ± 2.43	3.06 ± 2.67		1.55 ± 0.72	1.44 ± 0.72		1.39 ± 0.60	1.58 ± 0.69		1.36 ± 0.65	1.45 ± 0.66
3		140 ± 75.3***	130 ± 97.1***		4.95 ± 4.14***	4.22 ± 2.70***		4.19 ± 2.67***	3.58 ± 1.52***		6.88 ± 15.0	3.35 ± 1.72***
Cysteine												
0	33.3 ± 6.53 (n = 12)			6.85 ± 3.48 (n = 12)			6.12 ± 3.37 (n = 11)			4.97 ± 2.91 (n = 11)		
1		16.7 ± 16.2	13.7 ± 13.5		5.96 ± 8.92	3.24 ± 3.72		18.1 ± 13.0	3.28 ± 3.75		11.6 ± 13.1	2.96 ± 3.43
2		9.20 ± 11.0*	6.63 ± 9.19*		1.72 ± 2.11***	1.51 ± 1.37***		1.07 ± 1.29***	1.23 ± 1.00***		0.78 ± 1.49***	0.87 ± 0.72***
3		13.6 ± 10.1	8.06 ± 5.79		3.16 ± 2.06	2.22 ± 1.54		2.29 ± 1.43	1.56 ± 1.28		1.90 ± 1.90	1.58 ± 1.48*
5-*S*-Cysteinyldopa												
0	3.38 ± 1.32 (n = 11)			0.19 ± 0.08 (n = 11)			0.11 ± 0.04 (n = 11)			0.16 ± 0.11 (n = 11)		
1		3.51 ± 1.56	3.53 ± 1.41		0.40 ± 0.43	0.38 ± 0.45		0.19 ± 0.11	0.18 ± 0.11		0.14 ± 0.09	0.13 ± 0.07
2		2.26 ± 1.20**	2.12 ± 0.80**		0.34 ± 0.34	0.42 ± 0.34		0.25 ± 0.26	0.36 ± 0.30**		0.21 ± 0.20	0.28 ± 0.23*
3		1.95 ± 1.43	2.50 ± 1.59		0.14 ± 0.07	0.27 ± 0.17		0.14 ± 0.22	0.19 ± 0.15*		0.17 ± 0.38	0.21 ± 0.21

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, vs next lower stage, ANOVA test

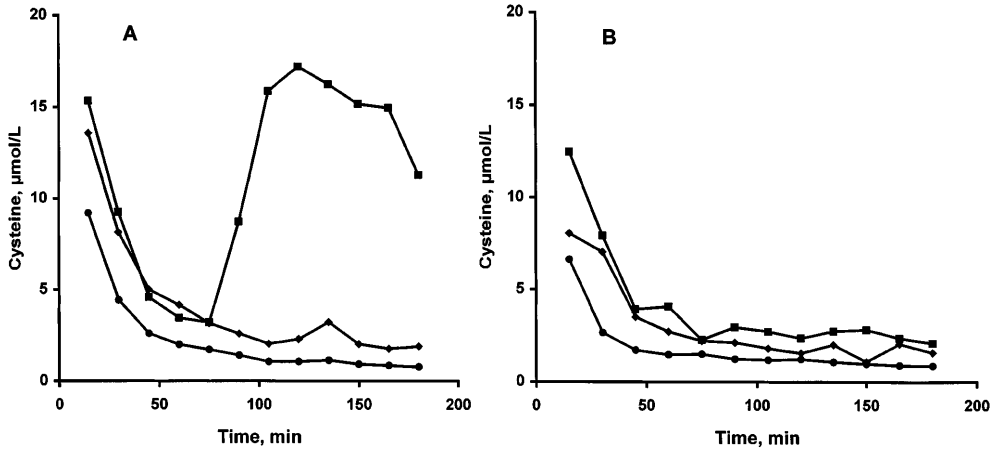

Fig. 1 Cysteine concentrations in microdialysates from malignant melanoma transplants in mice. The mice were treated with i.p. injections of BSO as described in Materials and methods. The first microdialysis was performed after 2 days of BSO treatment and during acute treatment with NAC (**A**) or OTC (**B**) (■). The second measurement was between treatments with BSO and OTC or NAC (●), and the third on continued OTC or NAC treatment but discontinued BSO treatment (◆)

the liver, are net producers and other organs, e.g. the kidney, are extractors. Melanoma tissue has not been classified in this respect, but the fact that the glutathione concentration in melanoma tissue is normally among the highest would indicate that it is a net producer. To determine whether microdialysis measurements mainly reflect melanoma cell or blood circulation concentrations, concomitant intracellular and plasma measurements should be taken simultaneously with microdialysis. However, this is not possible with the small animals we used. We have therefore studied, in patients with Parkinson's disease, the relationship between the concentrations of L-dopa in the subcutaneous extracellular fluid and the blood circulation after oral ingestion [22]. There was a good agreement between the intravenous and the subcutaneous results. Thus, in this case microdialysates from tissue seemed to mainly reflect the interchange with blood.

We found lower glutathione concentrations than previously reported (Table 1) but slightly higher than after 3 days of BSO treatment, e.g. 0.18 ± 0.34 µmol/l at 60–75 min [13]. This is because we started the first microdialysis experiment 2 days after the start of BSO treatment. In the earlier series interstitial glutathione

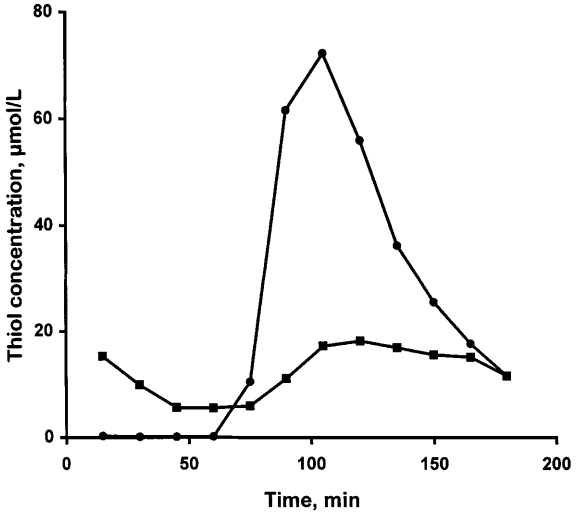

Fig. 2 NAC (●) and cysteine (■) concentrations in microdialysates from malignant melanoma transplants in mice during acute treatment with NAC

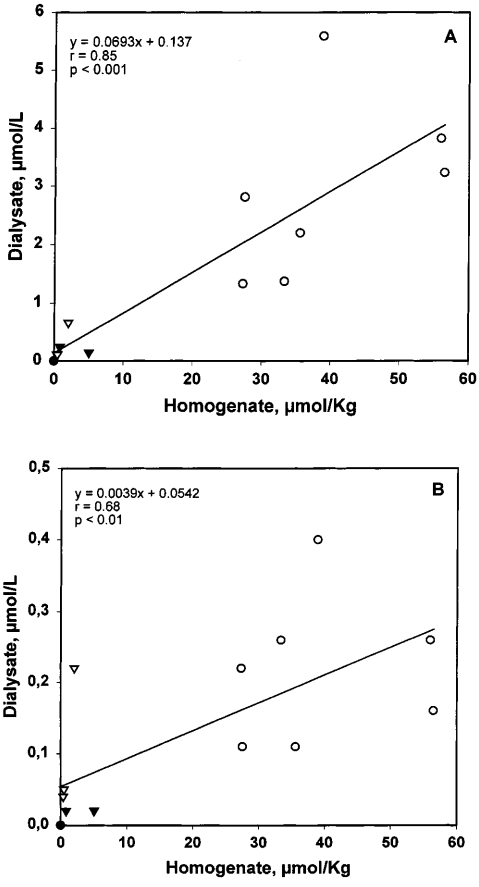

Fig. 3A,B Mice ($n = 17$) were inoculated on the back with melanoma cell lines M9 (●), JKM 86-4 (○), Mel 28 (▼), and B16F1 (▽). The animals, with no drug treatment, were subjected to microdialysis. At the end of the microdialysis period the tumours were taken for analysis of glutathione, cysteine and 5-*S*-cysteinyldopa. The relationships between 5-*S*-cysteinyldopa concentrations in microdialysates and in tissue are shown for two microdialysis periods, 0–15 min (**A**) and 60–75 min (**B**)

was undetectable after 7 days of treatment. Thus it seems that i.p. treatment with BSO decreases the glutathione concentration interstitially most efficiently during the first 3 days of treatment. This is in line with the findings from several organs from mice treated with BSO [23]. Thus, when the mice received BSO in their drinking water for 15 days the glutathione concentrations decreased to less than 10% in the pancreas, kidney and skeletal muscle. In other tissues the decrease was around 50% of the initial level. The decreases were around 80% 2 h after subcutaneous treatment.

It is also of interest to note that there was no difference in microdialysate glutathione concentrations between the animals given OTC or those given NAC 4 days after cessation of the BSO injections. This was also the case with cysteine. Moreover, there was a spontaneous recovery of glutathione levels in animals not given these cysteine deliverers. Thus in earlier studies we found that the glutathione level 7 days after cessation of BSO injections [13] return to about the same levels as in the present study.

From Fig. 2 it can be seen that NAC given i.p. resulted in a rapid interstitial increase in cysteine in melanoma tissue within 30 min of injection. However, an inspection of the concentration–time curves obtained in the OTC-treated mice shows that OTC did not produce an acute effect. Therefore, a comparison of the first and second microdialysis results in the OTC-treated animals indicates a decrease in cysteine as an effect of BSO. This is in line with previous findings [13] of significantly lower interstitial cysteine concentrations after BSO treatment than under basal (untreated) conditions. That cysteine did not increase rapidly after OTC treatment in a similar way to its increase after NAC treatment may be due to the combined oxoprolinase and decarboxylation reactions being slower than the deacetylation of NAC.

The reason why BSO decreased the cysteine concentration interstitially in melanoma tissue is obscure. A hypothesis may be that when the glutathione concentration decreases, there is a decreased production of cysteine from degradation of glutathione. However, it has been reported that cysteine uptake into cells decreases in a concentration-dependent manner during treatment with BSO [24]. The same authors also reported that the decrease in cystine concentrations is more pronounced than the decrease in glutathione production. They further suggest that the decrease in cysteine production is due to both decreased glutathione synthesis and decreased cystine uptake. Overall, if this applies to the whole animal, it means that the total production of cysteine (from the trans-sulphuration pathway) is decreased by BSO treatment, or that cysteine is redistributed into another form such as disulphide.

The levels of glutathione are dependent on the supply of cysteine, predominantly derived from dietary protein and, by trans-sulphuration, from methionine in the liver [25]. However, increasing the glutathione concentration by administration of cysteine is not a suitable strategy because of the cytotoxic effects of this compound. OTC and NAC, on the contrary, are considered to be important and safe cysteine deliverers for the promotion of glutathione synthesis. OTC is metabolized by 5-oxoprolinase to L-cysteine in two steps with the intermediate formation of *S*-carboxy-L-cysteine [6]. NAC, which is widely used in the treatment of, for example, pulmonary diseases, is deacetylated to L-cysteine by *N*-acetylases [8, 26].

We found a significant increase in the cysteine concentrations in the dialysates after NAC and OTC injections when BSO treatment was discontinued. Since the increase in cysteine was slightly higher in animals given NAC, this compound may be a more potent cysteine supplier than OTC in melanoma tissue although it has previously been suggested that OTC is the more potent cysteine supplier in the liver [6]. We gave NAC and OTC to two groups of animals in order to determine which of the compounds is more efficient in increasing glutathione levels after cessation of BSO treatment. As can be seen from Table 1 there seem to be only very slight differences in this respect between the two compounds.

Tumour cells are more vulnerable to different damaging factors than normal cells. Since glutathione has an important protective role [27], decreasing its intracellular levels could result in greater efficacy of chemotherapeutic drugs leading to the use of lower doses with fewer adverse effects. A promising model for in vivo study of the effect of BSO on sensitivity to cytostatic effects has been described by Reverez et al. [28]. They injected B16 mouse melanoma cells into immunocompetent and preirradiated mice. I.p. BSO treatment resulted in prolonged survival of the animals and impaired metastatic spread of the tumour cells. In a study by Prezioso et al. [29], the combination of BSO and melphalan increased the life-span of B16 melanoma-bearing mice by 170% as compared to melphalan alone (80%).

An ultimate goal for investigating antitumour activity of BSO would be to be able to predict the antitumour effect of BSO in individual human subjects with metastatic melanoma. This probably means that the intratumoral glutathione concentration has to be assessed. A proportion of patients with melanomas have cutaneous metastases, and it would be possible to assess their interstitial glutathione concentration by microdialysis.

Acknowledgements This study was supported by grants from the Swedish Cancer Society (Project 2357-B98-13XAC). We greatly acknowledge the valuable help with the statistical analysis of Mats Fredriksson, Ph.D., Department of Health and Environment, Division of Occupational and Environmental Medicine.

References

1. Chasseaud LF (1979) Role of glutathione and glutathione *S*-transferases in the metabolism of chemical carcinogens and other electrophilic agents. Adv Cancer Res 29: 175–274
2. Wendel A (1980) Glutathione peroxidase. In: Jakoby WB (ed) Enzymatic basis of detoxification. Academic Press, New York, pp 333–353

3. Griffith OW, Meister A (1979) Potent and specific inhibition of glutathione synthesis by buthionine sulphoximine (*S*-n-butylhomocysteine-sulphoximine). J Biol Chem 254: 7558–7560
4. Karg E, Brötell H, Rosengren E, Rorsman H (1989) Modulation of glutathione level in cultured human melanoma cells. Acta Derm Venereol 69: 137–141
5. Dethmers JK, Meister A (1981) Glutathione export by human lymphoid cells: depletion of glutathione by inhibition of its synthesis decreases export and increases sensitivity to irradiation. Proc Natl Acad Sci USA 78: 7492–7496
6. Williamson JM, Boettcher B, Meister A (1982) Intracellular cysteine delivery system that protects against toxicity by promoting glutathione synthesis. Proc Natl Acad Sci USA 79: 6246–6249
7. Borgström L, Kågedal B, Paulsen O (1986) Pharmacokinetics of *N*-acetylcysteine in man. Eur J Pharmacol 31: 217–222
8. Sheffner AL, Medler EM, Baily KR, Gallo DG, Mueller AJ, Sarett HP (1966) Metabolic studies with acetylcysteine. Biochem Pharmacol 15: 1523–1535
9. Lauterburg BH, Corcoran GB, Mitchell JR (1983) Mechanism of action of *N*-acetylcysteine in the protection against the hepatotoxicity of acetaminophen in rats in vivo. J Clin Invest 71: 980–991
10. Olney JW, Ho OL, Rhee V (1972) Cytotoxic effects of acidic and sulphur containing amino acids on the infant mouse central nervous system. Exp Brain Res 14: 61–76
11. Karlsen RL, Grofova I, Malthe-Sorensen D, Fonnum F (1981) Morphological changes in rat brain induced by L-cysteine injection in new-born animals. Brain Res 208: 167–180
12. Dringen R, Hamprecht B (1999) *N*-acetylcysteine, but not methionine or 2-oxothiazolidine-4-carboxylate, serves as cysteine donor for the synthesis of glutathione in cultured neurons derived from embryonal rat brain. Neurosci Lett 259: 79–82
13. Dizdar N, Kullman A, Kågedal B, Årstrand K (1997) Effects on interstitial glutathione, cysteine and 5-*S*-cysteinyldopa by buthionine sulfoximine in human melanoma transplants. Melanoma Res 7: 322–328
14. Agrup G, Falck B, Rorsman H, Rosengren A-M, Rosengren E (1977) Glutathionedopa in malignant melanoma. Acta Derm Venereol 57: 221–222
15. Karg E, Tunek A, Brötell H, Hallberg A, Rosengren E, Rorsman H (1990) Glutathione in human melanoma cells. J Dermatol Sci 1: 39–46
16. Dizdar N, Kågedal B, Smeds S, Årstrand K (1991) A high-sensitivity fluorometric high performance liquid chromatographic method for determination of glutathione and other thiols in cultured melanoma cells, microdialysis samples from melanoma tissue, and blood plasma. Melanoma Res 1: 33–34
17. Blomquist L, Dizdar N, Karlsson M, Kågedal B, Ossowicki H, Pettersson A, Smeds S (1991) Microdialysis of 5-*S*-cysteinyldopa from interstitial fluid in cutaneous human melanoma transplanted to athymic mice. Melanoma Res 1: 23–32
18. Kågedal B, Källberg M, Årstrand K (1989) Automated high-performance liquid chromatographic determination of 5-*S*-cysteinyl-3,4-dihydroxyphenylalanine in urine. J Chromatogr 473: 359–370
19. Sjödin K, Nilsson E, Hallberg A, Tunek A (1989) Metabolism of *N*-acetyl-l-cysteine. Biochem Pharmacol 38: 3981–3985
20. Hansson J, Edgren M, Ehrsson H, Ringborg U, Nilsson B (1988) Effect of D,L-buthionine S,R-sulfoximine on cytotoxicity and DNA crosslinking induced by bifunctional DNA-reactive cytostatic drugs in human melanoma cells. Cancer Res 48(1): 19–26
21. Meister A, Griffith OW, Novogrodsky A, Tate SS (1980) New aspects of glutathione metabolism and translocation in mammals. In: Sulphur in biology. CIBA Foundation Symposium 72 (new series). Excerpta Medica, Elsevier/North Holland, Amsterdam, pp 135–161
22. Dizdar N, Kullman A, Norlander B, Olsson J-E, Kågedal B (1999) Human pharmacokinetics of L-3,4-dihydroxyphenylalanine studied with microdialysis. Clin Chem 45(10): 1813–1820
23. Griffith OW, Meister A (1979) Interorgan translocation, turnover, and metabolism. Proc Natl Acad Sci USA 76: 5606–5610
24. Brodie AE, Reed DJ (1985) Buthionine sulfoximine inhibition of cysteine uptake and glutathione biosynthesis in human lung carcinoma cells. Toxicol Appl Pharmacol 77: 381–387
25. Meister A (1983) Selective modification of glutathione metabolism. Science 220: 472–477
26. Birnbaum SM, Levinton L, Kingsley RB, Greenstein JP (1952) Specificity of amino acid acylases. J Biol Chem 194: 455–470
27. Meister A, Griffith OW (1979) Effects of methionine sulfoximine analogues on the synthesis of glutamine and glutathione: possible chemotherapeutic implications. Cancer Treat Rep 63: 1115–1121
28. Reverez L, Edgren MR, Wainson AA (1994) Selective toxicity of buthionine sulfoximine (BSO) to melanoma cells in vitro and in vivo. Int J Radiat Oncol Biol Phys 29: 403–406
29. Prezioso JA, FitzGerald GB, Wick MM (1992) Melanoma cytotoxicity of buthionine sulfoximine (BSO) alone and in combination with 3,4-dihydroxybenzylamine and melphalan. J Invest Dermatol 99: 289–293