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ARTICLES

Modulation of Glutathione Transferase P1–1 Activity by Retinoic Acid in Neuroblastoma Cells

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Abstract The ability of retinoic acid to modulate glutathione S-transferase P1–1 (GSTP1–1) activity has important implications both for cancer prevention and for anticancer therapy. We investigated GSTP1–1 expression and activity in the human neuroblastoma cell line SK-N-BE(2) (genotype *A*/B**) under basal conditions and during 48-h incubation with 0.1 μM all-*trans*-retinoic acid. The steady-state levels of glutathione transferase P1–1 mRNA and protein during 48-h incubation with all-*trans*-retinoic acid did not increase substantially, but we detected a significant reduction of GSTP1–1 specific activity. This reduction in enzymatic activity could not be ascribed to a differential action of retinoic acid on the gene variants *A** and *B**; indeed, the two GSTP1–1 isoforms have different affinities toward 1-chloro-2,4-dinitrobenzene (CDNB), while we found a substantial invariance of the K_m^{CDNB} in the cytosol during retinoid treatment. A modulatory effect of retinoic acid on other enzymes involved in glutathione transferase P1–1 metabolism, such as the retinoic acid-induced tissue *trans*-glutaminase, might be hypothesized, as well as a direct inactivation of GSTP1–1 by the oxidative stress that characterizes the early phases of apoptosis. J. Cell. Biochem. 75:375–381, 1999.

Key words: glutathione transferase P1–1; neuroblastoma; retinoic acid

Glutathione S-transferases (GSTs; EC 2.5.1.18) belong to a large family of functionally different enzymes that catalyze the S-conjugation of glutathione (GSH) with a wide variety of electrophilic compounds, including carcinogens and anticancer drugs. The soluble GSTs in human tissues are classified in four main classes—alpha, mu, pi, and theta—some of which are present in multiple isoforms.

GSTs are enzymes functionally active as homodimers or heterodimers formed between subunits of the same class [Armstrong, 1997]. These enzymes catalyze the conjugation of GSH with a variety of noxious compounds following the general reaction scheme:

$$GSH + RX \rightarrow GS\text{-}R + HX$$

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Even though these reactions protect the cell from cytotoxic and carcinogenic agents, there is considerable evidence showing that some classes might be considered tumor markers and may be involved in anticancer drug resistance [Tew, 1994; Tsucida and Sato, 1992; Zhang et al., 1994]. In particular, elevated levels of GSTP1–1 have been found in stomach, colon, bladder, oral, breast, skin, and lung tumors, compared with corresponding normal tissues [Gaffey et al., 1995; Gilbert et al., 1993; Helzlsover et al., 1998; Russo et al., 1994].

Many studies have been focused on GSTP1–1 to elucidate its role in both normal and neoplastic cells. For many years, GSTP1–1 has been described as a single isoform; more recently, some allelic variants have been described that differ from each other by single conservative amino acid substitutions [Zimniak et al., 1994]. Molecular cloning and expression of cDNAs of GSTP1–1 gene variants showed the presence of three main isoforms: *A** (Ile^{104}), *B**(Val^{104}), and *C**(Val^{104}; Ala→Val^{113}) characterized by point mutations involving exon 5 (A→G +313) and exon 6 (C→T +341) [Ali-Osman et al., 1997]. The amino acid substitutions residing in the

xenobiotic substrate binding site (H site) result in steric modification of this region; consequently, the three isoforms show different cosubstrate specificity, as well as different thermostability [Ali-Osman et al., 1997; Sundeberg et al., 1998; Zimniak et al., 1994].

By means of restriction endonuclease mapping, the GSTP1–1 polymorphism may be easily and rapidly assessed. Recent epidemiological studies seem to suggest a possible association between the presence of isoform *C** and the occurrence of breast carcinoma [Helzlsover et al., 1998], glioblastoma [Ali-Osman et al., 1997], bladder, and testicular cancer [Harries et al., 1997]. Nevertheless, the allelic frequency of this polymorphism in different populations has not been extensively studied. A recent report shows important ethnic differences in the GSTP1–1 allele expression, suggesting a genotype-associated cancer susceptibility [Watson et al., 1998].

Recently, inducibility of GSTP1–1 expression by all-*trans*-retinoic acid (ATRA) in a human glioblastoma cell line and the presence of retinoic acid-responsive elements (RARE) in intron 5 of the GSTP1–1 gene have been described. In view of the important role played by GSTP1–1 in the ability of tumor cells to inactivate anticancer agents, Lo and Ali-Osman [1997] suggest that pretreatment with retinoic acid might increase tumor resistance to alkylating agent chemotherapy. By contrast, suppression of GSTP1–1 promoter activity by retinoic acid has also been reported, independent of RARE, and possibly mediated through an anti-AP-1 effect. [Xia et al., 1996]. Nevertheless, in both studies, modulation of the GSTP1–1 catalytic activity by retinoic acid has not been investigated.

In recent years, we have focused our attention on the retinoic acid effects on human neuroblastoma cell lines. Retinoic acid induces different degrees of neuronal differentiation in these cell lines and is accompanied by a marked growth inhibition [Melino et al., 1993a]. Retinoic acid acts through a complex network of gene transactivations modulating the expression of growth factors and related proteins such as the insulin-like growth factor II [Melino et al., 1993b], the insulin-like growth factor binding proteins -2 and -4 [Bernardini et al., 1994], the insulin degrading enzyme [Melino et al., 1996], as well as genes involved in apoptosis [Melino et al., 1997] and in retinoic acid receptor bioavailability [Bernardini et al., 1997]. The aim of our study was to analyze the role of ATRA in the GSTP1–1 expression and catalytic activity in the human neuroblastoma cell line SK-N-BE(2).

MATERIALS AND METHODS

Reagents

Media, reagents, and plastics for cell culture, trypsin, EDTA, HEPES, L-glutamine, sodium bicarbonate, phosphate-buffered saline (PBS), fetal bovine serum (FBS), and nonessential amino acid were from Flow Laboratories Ltd. (Herts, UK). ATRA, GSH, and 1-chloro-2,4-dinitrobenzene (CDNB) were from Sigma Chemical Co. (St. Louis, MO). Electrophoresis reagents were from Bio-Rad (Richmond, CA).

Rabbit polyclonal antibodies NCL-GSTpi, NCL-GSTmuM2, NCL-GSTα, were from Novocastra (Newcastle, UK); the monoclonal anti TGase Ab-3 was from Neomarkers (Fremont, CA).

Taq DNA polymerase and dNTPs were from Pharmacia-Biotech (Stockholm, Sweden), Trizol reagent and primers were from Life Technology (Milan, Italy), the 1st Strand cDNA Synthesis kit and DNA marker V were from Boehringer Mannheim (Indianapolis, IN).

Cell Cultures

SK-N-BE(2) human neuroblastoma cells were provided by Dr. June Biedler (Memorial Sloan-Kettering Cancer Center, NY). Cells were grown in monolayer culture in a mixture of minimum essential medium (MEM) and Ham's F-12 media (50:50, v/v) supplemented with 15% heat-inactivated FBS, sodium bicarbonate (1.2 mg/ml), Hepes buffer (15 mM), L-glutamine (2 mM), and NEAA (1% v/v). Cells were routinely evaluated for *Mycoplasma* infection. Cells were fed every 3–4 days and split weekly at a ratio of 1:5, 1:10 using trypsin (0.025%)-EDTA (0.02%). Cells were routinely fed 24 h before being harvested for experiments. The cells were incubated for 6, 24, and 48 h with 0.1 μM all-*trans*-retinoic acid (5 mM stock solution in 70% ethanol) each sample in triplicate; 0.07% ethanol was added to the control cultures. After removal of the culture medium, cells were centrifuged at 800*g* for 10 min. The pellets, suspended in 100 μl of 0.1 M potassium phosphate buffer pH 6.5, were sonicated by Sonics Vibra Cell (Danbury, CT) and then centrifuged at 12,000*g* for 5 min, 4°C; the resulting supernatants were assayed for GST activity. Pellets were immediately stored

at −80°C to be subsequently utilized for immunoblot or RNA extraction.

Glutathione Transferase Activity and Kinetic Measurements

Glutathione transferase activity was determined using CDNB as co-substrate as previously described [Habig aand Jacoby, 1981]; in a typical experiment, 20 µl of the cell supernatant (about 50 µg of protein) was added to 1 ml (final volume) of 0.1 M potassium phosphate buffer, 0.1 M EDTA at pH 6.5, containing 1 mM CDNB, and 1 mM GSH. The reaction was monitored at 340 nm, where the product absorbs (ϵ = 9,600 $M^{-1}cm^{-1}$), using a double beam Uvikon 940 spectrophotometer Kontron equipped with a thermostated cuvette holder at 25°C. The K_m^{CDNB} was calculated at fixed GSH 2.5 mM and variable CDNB within the range 0.1–2 mM. The K_m values were obtained from a Lineweaver-Burk double reciprocal plot.

Total protein content was determined by the bicinchoninic acid (BCA) method. The GSTP1–1 content was determined from immunoblotting analysis by comparing the densitometric value of each sample band with that of a standard curve obtained by the immunoblotting of a different amount of purified GSTP1–1 (1–100 ng).

Western Blot Analysis

Cytosol protein was electrophoresed at 30 µg/lane in 12% sodium dodecyl sulfate (SDS) polyacrylamide gel, transferred onto nitrocellulose membrane (0.45 µm), and probed with rabbit polyclonal GSTP1–1, muM2, and α-specific antibodies. A goat antirabbit IgG-peroxidase conjugate was then added and the GSTs visualized by a peroxidase substrate (4 chloro-1-naphtolo). The Immuno Blot for the transglutaminase was perfomed as above described except for a different resolving gel (10.5% SDS polyacrylamide gel), and a monoclonal anti tTG first antibody and a goat anti mouse IgG-peroxidase-conjugated second antibody.

Endonuclease Restriction Mapping

Total RNA was purified using a standard method and reverse transcribed with AMV reverse transcriptase, according to the protocol of Boehringer Mannheim. A 484-bp cDNA fragment spanning position +112 to +596 of the GSTP1–1 cDNA was amplified by polymerase chain reaction (PCR), using primers and conditions previously described [Ali-Osman et al., 1997]. The cDNA product was purified and, after restriction digestion with *Mae*II and *Xcm*I, was electrophoresed in 2% agarose, 0.01% ethidium bromide.

RT-PCR Analysis

The cDNA was amplified by differential PCR, using β2-microglobulin as an internal standard whose expression is not modulated by retinoic acid. Kinetics and titration analysis were performed to determine the condition in which data could be obtained before the amplification reaction reached the plateau phase. PCR was performed with a DNA Thermal cycler (Perkin Elmer). PCR for GSTP1–1/β2-M was carried out after a preheating step at 94°C for 5 min through 25 cycles (denaturation at 94°C for 1 min, annealing at 59°C for 1 min, extention at 72°C for 1 min), and a final extention at 72°C for 7 min.

The reaction mixture for a final volume of 50 µl was as follows: 10 µl of the RT reaction mixture, PCR buffer 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1.25 U *Taq* polymerase, and 0.5 µM of the listed primers:

GSTP1–1 sense:5′ TCA CTC AAA GCC TCC TGC CTA T 3′
GSTP1–1 antisense: 5′ CAG TGC CTT CAC ATA GTC ATC C 3′
β2-M sense: 5′ ACC CCC ACT GAA AAA GAT G 3′
β2-M antisense: 5′ ATC TTC AAA CCT CCA TGA TG 3′

β2-M primer sequence generated a 110-bp product; the GSTP1–1 primers a 242-bp product, including exons 5 and 6 internal to the 484-bp previously reported amplification product. RT-PCR product (20 µl) was separated on 2% agarose and 0.01% ethidium bromide gels and visualized on a transilluminator. The ratio of target to control β2-M gene products was determined by bidimensional densitometry on a GS-670 Densitometer (Bio-Rad).

RESULTS

Western immunoblotting of the cytosol of human neuroblastoma cell line SK-N-BE(2), performed using specific polyclonal antibodies, showed the presence of a single band corresponding to GSTP1–1, while the α and mu classes were absent (Fig. 1).

By means of endonuclease restriction mapping of GSTP1–1 cDNA, we showed the presence of a heterozygosity *A**/*B** demonstrated by lack of digestion with *Xcm*I (which recognizes a restriction site specific of variant *C**), the pres-

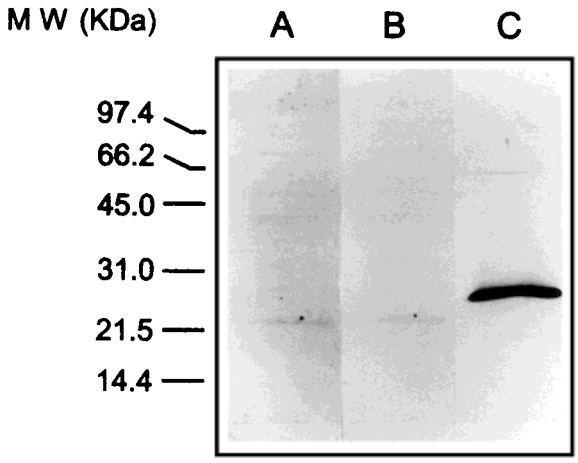

Fig. 1. Western immunoblotting of the cytosol of human neuroblastoma cell line SK-N-BE(2). The blotted sample was incubated with anti GSTα Ab **(lane A)**, anti GSTmu Ab **(lane B)**, and anti GSTP1–1 Ab **(lane C)**.

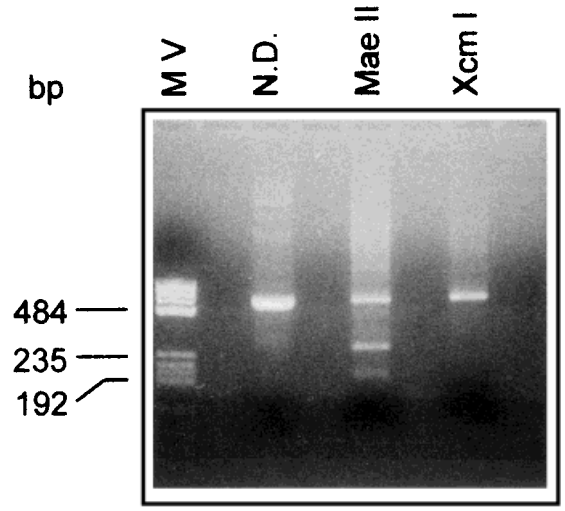

Fig. 2. *Mae*II and *Xcm*I endonuclease restriction mapping of GSTP1–1 cDNA, including exons 5 and 6 (amplification product of 484 bp) in SK-N-BE(2) neuroblastoma cell line. MV, DNA molecular-weight Marker V (587–8 bp) (Boehringer Mannheim). ND, not digested sample.

ence of two bands digested with *Mae*II (specific for variants *B** or *C**) and one undigest upper band (specific for wild variant *A**) (Fig. 2).

The immunoblotting assay after 48 h of incubation with 0.1 μM ATRA showed a slight increase of GSTP1–1 relative to control (Fig. 3). This increase was only apparent because the same amount of cytosolic proteins was electrophoresed in each lane. Total protein concentration in ATRA-treated cells, however, showed a 50% reduction relative to control (2.3 and 4.8 mg/ml for ATRA-treated cells and control cells respectively). In fact, the absolute GSTP1–1

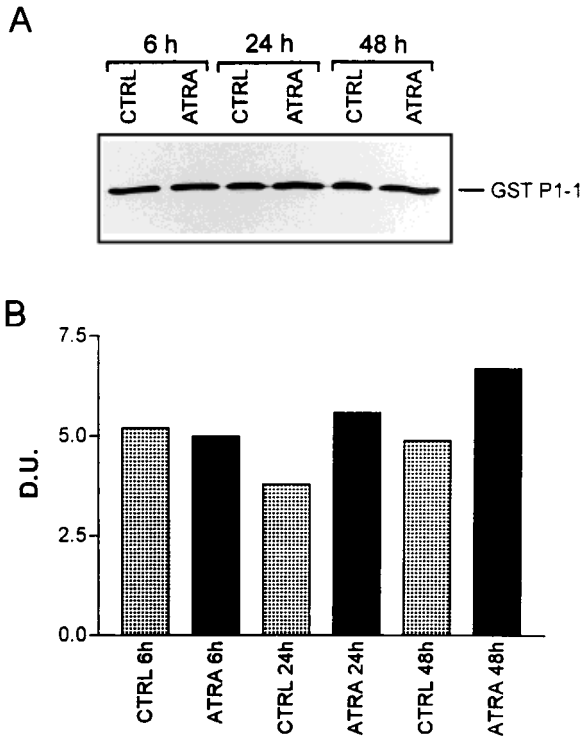

Fig. 3. **A:** Immunoblotting of GSTP1–1 in basal conditions and after incubation with 0.1 μM ATRA for 6, 24, 48 h. **B:** Bidimensional densitometric analysis.

TABLE I. GSTP1-1 Specific Activity Toward CDNB Expressed as μmol/min/mg of GSTP1–1 During 48-h Incubation With 0.1 μM ATRA[a]

	6 h	24 h	48 h
CTRL	75 ± 7.5	133 ± 3.5	133 ± 5.2
ATRA	89 ± 8.7	106 ± 2.9	88 ± 8.7*

[a]Each reported value is the mean of the activity obtained from three different cell flasks; standard error is reported.
*$p < 0.05$; Student's *t*-test.

cytosolic concentration, calculated by means of a standard curve obtained by the immunoblotting of different amounts of purified GSTP1–1 (1–100 ng), was substantially decreased after 48 h of ATRA incubation (12 and 7 μg/ml for control samples and ATRA-treated cells, respectively).

The GSTP1–1 specific activity using CDNB as substrate showed a significant decrease after 48 h of incubation with 0.1 μM ATRA (X±SE from 133 ± 5.2 to 88 ± 8.7 μmol/min/mg of GSTP1–1; $P < 0.05$) (Table I).

The K_m^{CDNB} during ATRA treatment did not change substantially ($K_{m\ CTRL}$=1.5 mM; $K_{m\ 0.1\ \mu M\ ATRA}$=1.2 mM; $K_{m\ 1\ \mu M\ ATRA}$=1.3 mM). The expression of GSTP1–1 mRNA, calculated as

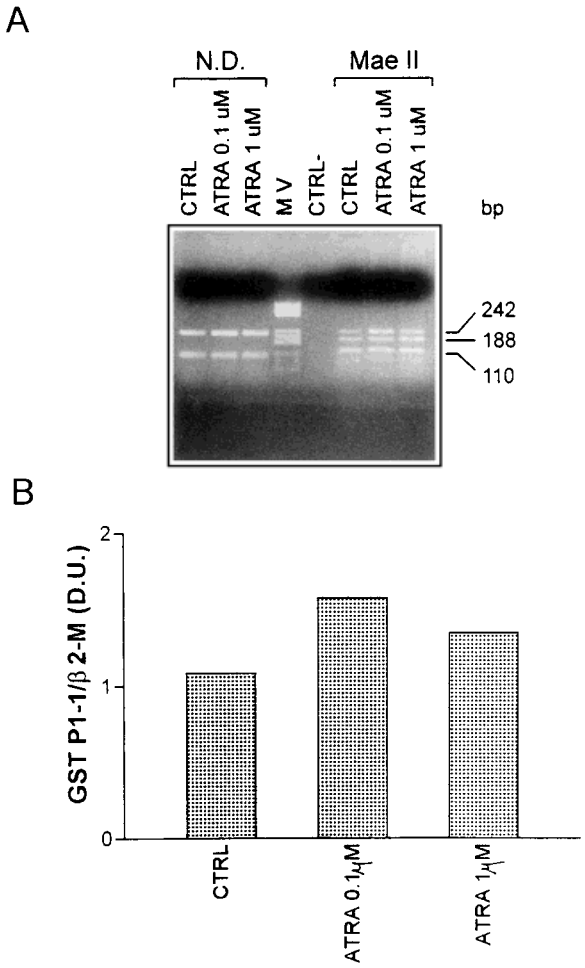

Fig. 4. **A:** Differential reverse transcription-polymerase chain reaction (RT-PCR) with coamplification of GSTP1–1 and β2-microglobulin in basal conditions and after incubation with ATRA 0.1 and 1.0 μM. The sample was not digested (ND) and restricted with *Mae*II. **B:** Bidimensional analysis of GSTP1–1/β2-M of nondigested samples.

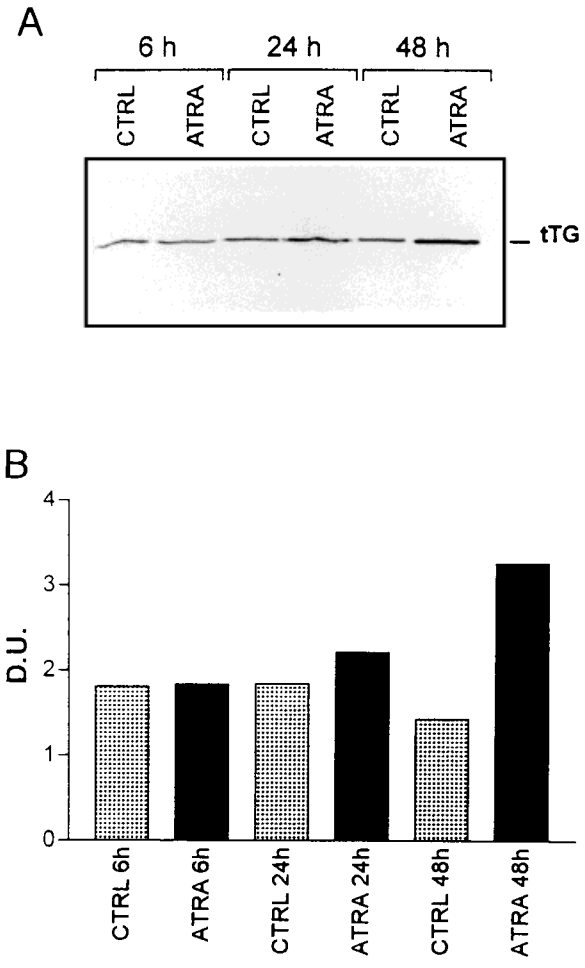

Fig. 5. **A:** Immunoblotting of tTG in basal conditions and after 0.1 μM ATRA after 6, 24, and 48 h. **B:** Bidimensional densitometric analysis.

GSTP1–1/β2-M ratio in differential RT-PCR, did not change substantially when using a 1-μM concentration of ATRA (Fig. 4). The immunoblotting assay for tissue transglutaminase showed increased protein levels after 48 h of incubation with 0.1 μM ATRA, which remains higher after correction for total protein levels (Fig. 5).

DISCUSSION

High levels of GSTP1–1 have been associated with malignancy, as well as with antineoplastic drug resistance. For many years, GSTP1–1 was assumed to be present as a single isoform and expressed from a single gene. More recently, three allelic variants (*A**, *B**, and *C**) have been described that differ in catalytic efficiency toward different substrates [Ali-Osman et al., 1997; Zimniak et al., 1994].

Recently, a modulation of the GSTP1–1 expression by retinoic acid (RA) has been described; this is of potential interest, considering the role of retinoids in cell differentiation and apoptosis and their implication in anticancer therapy. In particular, a suppression of GSTP1–1 expression by RA, in the presence of its receptor (RAR), has been shown as the result of decreased transcription. Chloramphenicol acetyltransferase (CAT) reporter assay indicates that the effect of RA on the *GSTP1* promoter-CAT fusion gene is mediated by a region which co-localizes with a consensus activator protein-1 (AP-1) and that the sequence essential for repression is not a retinoic acid-responsive element (RARE). Consequently the RAR-mediated RA repression of the *GSTP1* promoter may occur independent of the binding of

an RAR-RA complex to DNA, and a direct inhibition mediated by the formation of a functional RA-RAR-AP-1 complex has been suggested [Xia et al., 1996]. At the same time, an induction of GSTP1–1 expression by RA, mediated by RARE, present in intron 5, has been described, suggesting different mechanisms of action for RA in the modulation of GSTP1–1 expression [Lo and Ali-Osman, 1997].

This complex model of *GSTP1* gene regulation, based on the antagonism between the AP-1 site and the classical RA gene transactivation, might be cell type-dependent or a sensitive mean to modulate GSTP1–1 activity in different functional moments of the cell. The ability of RA to modulate GSTP1–1 activity has important implications for both cancer prevention and anticancer therapy. Indeed, retinoids are usually employed as adjuvants in anticancer therapy and the induction of detoxication enzymes could be an unwanted side effect. It should also be considered that, in previous studies, there is evidence that many anticancer drugs are direct substrates for GSTP1–1 [Tew, 1994]. The polymorphism of the *GSTP1* gene affecting the functional properties of the "H" site of the protein might further interfere with this mechanism.

We focused our attention on the modulation of GST activity by RA in human neuroblastoma cell line SK-N-BE(2), previously studied in our laboratory in relation to the action of RA in the modulation of differentiation, apoptosis, and growth factor expression.

According to data obtained by other investigators [Ali-Osman et al., 1997] in a glioblastoma cell line, we demostrated a slight relative increase of GSTP1–1 protein levels in immunoblotting assay during 48 h of incubation, using 10 times less ATRA to reach more physiological conditions; nevertheless the absolute GSTP1–1 concentration was substantially decreased.

Moreover, we tested the activity of the enzyme and observed a significant decrease in GSTP1–1 specific activity in 48-h ATRA-treated cells, as compared with control cells. The reduction of enzymatic activity probably could not be ascribed to a different action of ATRA on gene variants *A** and *B**, even though the two GSTP1–1 isoforms have different affinities toward CDNB [Ali-Osman et al., 1997], because we found a lack of variation of the K_m^{CDNB} in the cytosol during ATRA treatment.

An alternative explanation for the decreased specific activity, might be offered by the recently reported role of the GSTP1–1 as substrate for the human tissue transglutaminase (tTG), leading to polymerization of GSTP1–1 and functional inactivation [Piredda et al., 1999]. The tTG is known to be induced by ATRA, as previously reported [Melino et al., 1997] and confirmed in the present study.

Furthermore, it is known that tTG has a priming effect on apoptosis [Melino et al., 1997], and our data could point to a linkage with the glutathione transferase activity. Indeed, it is well known that massive glutathione depletion and oxidative stress characterize the early phases of apoptosis [Kane et al., 1993; Kletsa et al., 1998; Van den Dobbelstein, 1996]. The tTG-dependent post-translational modification of GSTP1–1 might occur at an increased rate in cases of GSH depletion, GSTP1–1 becoming more available as substrate for tTG [Piredda et al., 1999].

Furthermore, the reduction of catalytic activity might also be a consequence of a specific response of GSTP1–1 to oxidative stress, through a direct reaction of the -SH groups with reactive oxygen species with subsequent disulfide formation and inactivation [Di Ilio et al., 1996; Ricci et al., 1991].

As a final result, the decreased GSTP1–1 activity induced by ATRA, as demonstrated in our model, might contribute to blunt the mechanisms of drug resistance possibly mediated by the GSTP1–1, which are likely to be triggered during the course of anticancer therapy.

Our results should be interpreted with caution, since in our model we used CDNB as substrate for GSTP1–1, which does not necessarily reflect the activity in the presence of other substrates, such as antineoplastic drugs. Furthermore, our neuroblastoma cell line displayed an *A**/*B** genotype, which might behave differently from the other possible genotypes.

Further studies are warranted that use all the possible allelic combinations in neuroblastoma cell lines, as well other human tumor cell lines that might be more or less responsive to retinoic acid treatment.

REFERENCES

Ali-Osman F, Akade O, Antoun G, Mao JX, Boulamwini J. 1997. Molecular cloning, characterization, and expression in *Escherichia coli* of full-length cDNAs of three human glutathione S-transferase P1 gene variants. J Biol Chem 272:10004–10012.

Armstrong RN. 1997. Structure, catalytic mechanism, and evolution of the glutathione transferase. Chem Res Toxicol 10:2–18.

Bernardini S, Cianfarani S, Spagnoli A, Annicchiarico-Petruzzelli M, Melino G, Massoud R, Boschenini B, Finazzi-Agnò A, Rosenfeldt RG, Federici G. 1994. Expression and down regulation by retinoic acid of IGF binding protein-2 and -4 in medium from human neuroblastoma cells. J Neuroendocrinology 6:409–413.

Bernardini S, Melino G, Saura F, Annicchiarico-Petruzzelli M, Motti C, Cortese C, Federici G. 1997. Expression of co-factors (SMRT and Trip-I) for retinoic acid receptors in human neuroectodermal cell lines. Biochem Biophys Res Commun 234:278–282.

Di Ilio C, Sacchetta P, Angelini S, Bucciarelli T, Pennelli A, Mazzetti AP, Lo Bello M, Aceto A. 1996. Interaction of glutathione transferase P1–1 with Captan and Captafol. Biochem Pharmacol 52:43–48.

Gaffey MJ, Iezzoni JC, Meredith SD, Boyd JC, Stoler MH, Weiss LM. 1995. Cyclin D1 (PRAD1, CCND1) and glutathione S-transferase pi gene expression in head and neck squamous cell carcinoma. Hum Pathol 26:1221–1226.

Gilbert L, Elwood LJ, Merino M, Masood S, Barnes R, Steinberg SM. 1993. A pilot study of pi-class glutathione transferase expression and prognosis in node-negative breast cancer. J Clin Oncol 11:49–58.

Habig WH, Jacoby WB. 1981. Assays for differentiation of glutathione S-transferases. Methods Enzymol 77:398–405.

Harries LW, Stubbins MJ, Forman D, Howard G, Wolf CR. 1997. Identification of genetic polymorphism at the glutathione S-transferase P1–1 locus and association with susceptibility to bladder, testicular and prostate cancer. Carcinogenesis 18:641–644.

Helzlsover KJ, Selmin O, Huang HY, Strickland PT, Hoffman S, Alberg AJ, Watson M, Comstock GW, Bell D. 1998. Association between glutathione S-transferase M1, P1, and T1 genetic polymorphism and development of breast cancer. J Natl Cancer Inst 90:512–518.

Hui-Wen Lo, Ali-Osman F. 1997. Genomic cloning of hGSTP1*C, an allelic human pi class glutathione S-transferase gene variant and functional characterization of its retinoic acid responsive elements. J Biol Chem 272:32743–32749.

Kane DJ, Sarafian TA, Anton R, Hahn H, Gralla EB, Severstone Valentine J, Ord T, Bredesen DE. 1993. Bcl-2 inhibition of neuronal death: decreased generation of reactive oxygen species. Science 262:1274–1276.

Kletsa D, Barbieri D, Stathakos D, Botti B, Bergamini S, Tomasi A, Monti D, Malorni W, Franceschi C. 1998. The highly reducing sugar 2-deoxy-D-ribose induces apoptosis in human fibroblasts by reduced glutathione depletion and cytoskeletal disruption. Biochem Biophys Res Commun 243:416–425.

Melino G, Knight RA, Thiele CJ. 1993a. New insight on the biology of neuroectodermal tumours. Cancer Res 53:926–928.

Melino G, Stephanou A, Annicchirico-Petruzzelli M, Knight RA,Finazzi Agrò A, Lightman SL. 1993b. Modulation of IGF-II expression during growth and differentiation of human neuroblastoma cells: retinoic acid may induce IGF-II. Neurosci Lett 151:187–191.

Melino G, Draoui M, Bernardini S, Bellincampi L, Reichert O, Cohen P. 1996. Regulation by retinoic acid of insulin degrading enzyme (IDE) and of a related endoprotease in human neuroblastoma cell lines: implication in the IGF-II autocrine loop. Cell Growth Diff 7:787–796.

Melino G, Draoui M, Bellincampi L, Bernassola F, Bernardini S, Piacentini M, Reichert U, Cohen P. 1997. Retinoic acid α and γ mediate the induction of tissue transglutaminase activity and apoptosis in human neuroblastoma cells. Exp Cell Res 235:55–61.

Piredda L, Farrace MG, Lo Bello M, Malorni W, Melino G, Petruzzelli R, Piacentini M. 1999. Identification of "tissue transglutaminase binding proteins" in neural cells committed to apoptosis. FASEB J 13:355–364.

Ricci G, Del Boccio C, Pennelli A, Lo Bello M, Petruzzelli R, Barra D, Caccari AM, Federici G. 1991. Redox forms of placental human glutathione transferase. J Biol Chem 266:21409–21415.

Russo D, Marie JP, Zhou DC, Faussat AM, Delmer A, Maisonneuve L. 1994. Coexpression of anionic glutathione S-transferase (GST pi) and multidrug resistance (mdr 1) genes in acute myeloid and lymphoid leukemias. Leukemia 8:881–884.

Sundeberg K, Johansson AS, Stenberg G, Widersten M, Seidel A, Mannervik B, Jennström B. 1998. Differences in the catalytic efficiences of allelic variants of glutathione transferase P1–1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons. Carcinogenesis 19: 433–436.

Tew KD. 1994. Glutathione associated enzymes in anticancer drug resistance. Cancer Res 54:4313–4320.

Tsucida S, Sato K. 1992. Glutathione transferases and cancer. Crit Rev Biochem Mol Biol 27:337–384.

Van den Dobbelsteen DJ, Nobel SCI, Schlegel J, Cotgreave IA, Orrenius S, Slater AR. 1996. Rapid and specific efflux of reduced glutathione during apoptosis induced by anti-Fas/APO-1 antibody. J Biol Chem 28:15420–15427.

Watson MA, Stewart RK, Smith GBJ, Massey TE, Bell DA. 1998. Human glutathione S-transferase P1–1 polymorphism: relationship to lung tissue enzyme activity and population distribution. Carcinogenesis 19:275–280.

Xia C, Hu J, Ketterer B, Taylor JB. 1996. The organization of the human glutathione S-transferase P1–1 gene promoter and its response to retinoic acid and redox status. Biochem J 298:385–390.

Zhang K, Mack P, Wong KP. 1998. Glutathione related mechanisms in cellular resistance to anticancer drugs. Int J Oncol 12:871–882.

Zimniak P, Nanduri B, Pikula S, Bandorowicz-Pikula J, Singhal S, Srivastava SK, Awastni S, Awastni Y. 1994. Naturally occurring human glutathione S-transferase GSTP1–1 isoforms which isoleucine and valine in position 104 differ in enzymatic properties. Eur J Biochem 224:893–899.