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ORIGINAL ARTICLE

The inhibition of glutamine synthetase sensitizes human sarcoma cells to L-asparaginase

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Abstract
Purpose To evaluate the activity of the antitumor enzyme L-asparaginase (ASNase) on tumor cells of mesenchymal origin and the contribution of glutamine synthetase (GS) to the adaptation to the metabolic stress caused by the anti-tumor enzyme.
Methods We studied the effects of ASNase in six human sarcoma cell lines: HT1080 (fibrosarcoma); RD (rhabdomyosarcoma); SW872 (liposarcoma); HOS, SAOS-2, and U2OS (osteosarcoma) in the absence or in the presence of the GS inhibitor methionine L-sulfoximine (MSO).
Results HT1080 and SW872 cells were highly sensitive to ASNase-dependent cytotoxicity. In contrast, RD, SAOS-2, HOS, and U2OS cells exhibited only a partial growth suppression upon treatment with the anti-tumor enzyme. In these cell lines ASNase treatment was associated with increased levels of GS. When ASNase was used together with MSO, the proliferation of the poorly sensitive cell lines was completely blocked and a significant decrease in the IC_{50} for ASNase was observed. Moreover, when ASNase treatment was carried on in the presence of MSO, HOS and U2OS osteosarcoma cells exhibited a marked cytotoxicity, with increased apoptosis.
Conclusions In human sarcoma cells (1) GS markedly contributes to the metabolic adaptation of tumor cells to ASNase and (2) the inhibition of GS activity enhances the antiproliferative and cytotoxic effects of ASNase. The two-step interference with glutamine metabolism, obtained through the combined treatment with ASNase and MSO, may provide a novel therapeutic approach that should be further investigated in human tumors of mesenchymal origin.

Keywords Asparaginase · Glutamine synthetase · HT1080 · Methionine sulfoximine · Osteosarcoma · Rhabdomyosarcoma

Abbreviations

ASNase	L-Asparaginase
AS	Asparagine synthetase
DMEM	Dulbecco's modified Eagle's medium
FBS	Fetal bovine serum
GS	Glutamine synthetase
MSO	Methionine-L-sulfoximine

Introduction

The antitumor enzyme L-asparaginase (L-asparagine amido-hydrolase, E.C. 3.5.1.1, ASNase) has been employed for many years in the treatment of acute lymphoblastic leukemia and specific forms of non-Hodgkin lymphoma (see [10, 15] for review). Recently,

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it has been also found that subsets of human myeloid leukemias and NK cell tumors exhibit a significant sensitivity to the cytotoxic effects of the enzyme [2, 17, 24]. Data on solid tumors are scarce and, in particular, the sensitivity of sarcomas to ASNase has been never investigated.

Although the cytotoxic effects of ASNase have been usually attributed to asparagine starvation in cells with low activity of asparagine synthetase (AS) [4, 13], recent studies in leukemia cells have demonstrated that no exclusive relationship exists between low AS expression and ASNase sensitivity [23] or, consistently, between high AS expression and resistance to ASNase [7, 9]. On the other hand, it is known that ASNase-treated cells respond to the metabolic stress induced by the enzyme with a complex adaptive mechanism [3]. Moreover, genomic analysis has indicated that ASNase-resistant phenotype is associated with the dysregulation of several genes involved in response to amino acid starvation, control of protein synthesis, and activation of apoptotic pathways [6]. These data suggest that additional factors, other than high AS activity, contribute to ASNase-resistant phenotype and that ASNase resistance may be interpreted as the capability to exert an effective metabolic adaptation before ASNase-dependent cytotoxicity ensues.

Most ASNase enzymes used in leukemia therapy are also endowed with glutaminase activity [15] and a marked depletion of extracellular and intracellular glutamine has been indeed observed upon treatment with these ASNase preparations in vitro [5, 25] and in vivo [19, 20, 22]. Glutamine starvation represents a severe metabolic stress that, in many tissues, is followed by the up-regulation of the expression and/or activity of glutamine synthetase (GS), the enzyme that obtains glutamine from glutamate and ammonium [12]. To investigate the role of GS induction in ASNase-resistant phenotypes, our group studied GS expression and activity in a subline of rat Jensen sarcoma cells selected in vitro for ASNase resistance [21]. In this cell model the treatment with the anti-tumor enzyme produces a marked increase in GS expression and a stimulation of GS activity. Moreover, in the same cells the inhibition of GS activity abolishes the resistance to the cytotoxic effects of ASNase leading to massive cell death.

On the basis of those findings, we have decided to investigate ASNase effects and the role of GS in cells lines derived from human tumors of mesenchymal origin. We report here that in cells poorly sensitive to the anti-tumor enzyme the effects of ASNase are significantly enhanced by GS inhibition.

Materials and methods

Cell lines and viability

The human cell lines HT1080, derived from a fibrosarcoma, SW872, derived from a liposarcoma, RD, derived from a rhabdomyosarcoma, SAOS-2 and HOS, both derived from osteosarcomas, were obtained from the Istituto Zooprofilattico Sperimentale (Brescia, Italy). Human osteosarcoma cells U2OS were obtained from ATCC. All cultures were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 4 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin, and maintained at 37°C in an atmosphere of 5% CO_2 in air, pH 7.4.

Unless otherwise stated, the treatments with ASNase and the GS inhibitor methionine-L-sulfoximine (MSO) were performed on cells grown on 75-cm^2 dishes. Concentrations of ASNase (*E. chrisanthemy* ASNase, Erwinase®, Speywood Pharmaceuticals Ltd.) and treatment periods are detailed for each experiment. MSO was used at 1 mM.

The cytotoxic effects of ASNase and MSO on sarcoma cells was assessed with resazurin, a widely used viability indicator [16]. For these experiments, cells were seeded in complete growth medium in 96-well or 24-well plates (Costar Corning, NY, USA), at a density of, respectively, 5×10^3 cells/well or 25×10^3 cells/well, and grown for 24 h. Growth medium was then substituted with fresh medium containing the drug to be tested at the selected concentration. Cell viability was determined replacing medium with a solution of resazurin (44 μM) in complete medium. After 2 h, fluorescence was measured at 572 nm with a fluorimeter (Wallac 1420 Victor^2 Multilabel Counter, Perkin Elmer).

Flow cytometry

Trypsinized cells were centrifuged together with their culture medium. The pellet was washed with PBS and resuspended in 500 μl citrate buffer containing 50 μg/ml propidium iodide, 1 mg/ml ribonuclease A, and 0.1% Nonidet P-40. After 1 h staining in the dark at 4°C, the samples were analyzed by means of a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with a doublet discrimination module. Twenty thousand cells were analyzed for each experimental condition. Flow cytometric data analysis was performed using the Multicycle Cell Cycle Analysis software (Phoenix Flow System, San Diego, CA, USA).

Caspase-3 activity

After a 24-h treatment, the medium was collected and cells were harvested. Cell suspension was centrifuged together with culture medium and the pellet was washed with PBS and resuspended in lysis buffer for 20 min. After a 15-min centrifugation at 18,000*g* at 4°C, caspase-3 activity in the supernatants was assessed with a colorimetric kit (caspase 3 Assay Kit, Sigma) in 96-well plates. The absorbance of the cleaved caspase-3 substrate (Ac-DEVD-pNA) was read at 405 nm with a Wallac 1420 Victor^2 Multilabel Counter and results calculated using a calibration curve of *p*-nitroaniline.

Determination of glutamine synthetase activity

Cell cultures were incubated in EBSS for 1 h, washed twice in ice-cold PBS, scraped off into the same solution, and collected by a low speed centrifugation. Assay of GS activity was performed assaying the γ-glutamyltransferase activity of the enzyme according to the method of Minet et al. [14]. Briefly, after cell scraping and centrifugation, the pellet was lysed in 500 µl of a solution containing 50 mM imidazole–HCl, pH 6.8, 0.5 mM EDTA, 1 mM DTT, supplemented with a cocktail of protease inhibitors (Complete, Mini, EDTA-free, Roche), transferred into a 1.5 ml microtube, disrupted by sonication in ice (Sonicator Ultrasonic Processor XL, Misonix), and clarified by centrifugation at 12,000*g*, 30 min, 4°C. After quantification of the supernatant with the Bio-Rad Protein Assay, protein concentration was adjusted to 1 µg/µl. An aliquot of 150 µl was used for the assay in a mixture (final volume 300 µl) consisting of 50 mM imidazole–HCl (pH 6.8), 50 mM L-glutamine, 25 mM hydroxylamine, 25 mM sodium arsenate, 2 mM $MnCl_2$, and 0.16 mM ADP. After incubation at 37°C for 30 min, the reaction was stopped adding 600 µl of a solution 2.42% $FeCl_3$ and 1.45% TCA in HCl 1.82%. Precipitates were removed by centrifugation (2,000 rpm for 5 min) and supernatants were read at 540 nm using a spectrophotometer (Helios-γ, Spectronic Unicam). Values of specific GS activity, expressed as pmol/min/µg protein of the product γ-glutamyl-hydroxamate, were calculated with a calibration curve using standards of γ-glutamyl-hydroxamate.

Western blot

After the experimental treatment, cells were rinsed twice in PBS with Ca^{++} and Mg^{++} and lysed in 1 ml of RIPA buffer (100 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Igepal CA 630, 1% sodium deoxycholate, 0.1% sodium dodecylsulfate) containing a cocktail of protease inhibitors (Complete, Mini, EDTA-free, Roche) for 1 h at 4°C with vigorous shaking. Lysates were transferred in Eppendorf tubes, sonicated for 15 s, and centrifuged at 16,000*g* for 30 min at 4°C. After quantification with the Bio-Rad protein assay and equilibration of the samples, aliquots of 45 µl, corresponding to 30 µg of proteins, were mixed with 15 µl of Laemmli buffer 4× (250 mM Tris–HCl, pH 6.8, 8% SDS, 40% glycerol, 0.4 M DTT), warmed at 95°C for 5 min, and loaded on a 12% gel for SDS-PAGE. After the electrophoresis, proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA, USA). Non-specific binding sites were blocked with an incubation in Tris–buffer saline (TBS; 50 mM Tris–HCl, pH 7.5, 150 mM NaCl), containing 1% casein, 0.33% gelatin, 3% bovine serum albumin, for 2 h at room temperature. The blots were then exposed to anti-GS monoclonal antibody (1:2,500, BD Transduction Laboratories, Becton & Dickinson, Franklin Lakes, NJ, USA) diluted in blocking solution for 1 h at 37°C. After washing, the blots were exposed to biotin conjugated anti-mouse IgG diluted 1:800 in blocking solution for 1 h and then incubated for 45 min in HRP-conjugated streptavidin at 37°C. For standardization of the total cell lysate, stripped membranes were exposed to a monoclonal antibody against β-tubulin (1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The membranes were washed and the immunoreactivity visualized with enhanced chemiluminescence (Amersham Pharmacia Biotech Italia, Milan, Italy).

Statistical analysis

Data of cell viability are expressed as mean ± standard error (SEM), while caspase and GS activities are given as means ± standard deviation (SD). Two-tail Student's *t* test for unpaired data was used for quantitative data analysis and $P < 0.05$ was considered statistically significant. IC_{50} were evaluated by non-linear regression analysis using the program GraphPad Prism 4.0™.

Materials

Serum was obtained from Euroclone, Milan, Italy. Unless otherwise indicated, Sigma (Milan, Italy) was the source of all the others chemicals, including the caspase-3 Assay Kit, MSO, and culture media.

Results

In a preliminary experiment, we investigated the effects of ASNase on HOS osteosarcoma cells incubated in the

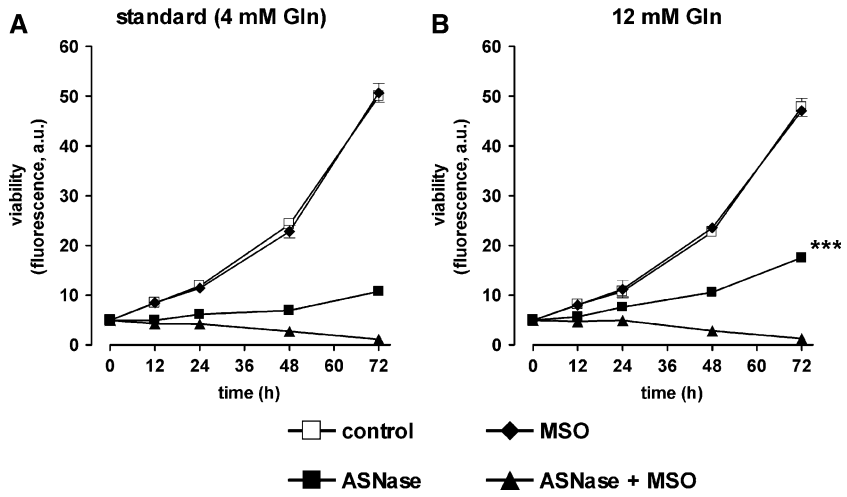

Fig. 1 Effects of ASNase and MSO on the growth of HOS human osteosarcoma cells. HOS cells were seeded on 96-well dishes in standard growth medium (4 mM Gln, *panel a*) or in a modified medium where Gln concentration had been raised at 12 mM (*panel b*). After 24 h ASNase (1 IU/ml) and/or MSO (1 mM) were added to the culture medium, as indicated. Cell viability was assessed at the indicated times as described in Materials and methods. Data are means of six independent determinations with SEM shown when greater than the size of the point. For cells incubated in the presence of ASNase alone for 24, 48, or 72 h ***$P < 0.001$, cells incubated at 4 mM Gln versus cells incubated at 12 mM Gln. For all the other conditions, no significant difference was observed between the two concentrations of extracellular Gln at any time of incubation

absence or in the presence of MSO (Fig. 1, panel a). ASNase alone produced only a partial decrease in cell proliferation. The supplementation of the extracellular medium with ASNase causes the complete hydrolysis of extracellular Gln [5] with the production of equimolar glutamate and ammonium. However, the addition of 4 mM glutamate and/or 4 mM ammonium chloride to the extracellular medium of HOS cells did not affect significantly cell viability (S. Tardito, unpublished results), indicating that the antiproliferative effects of the enzyme are likely referable to Gln depletion rather than ammonium production. MSO alone had no significant effect while the simultaneous treatment with ASNase and MSO produced a clear cut cytotoxicity, already detectable after 24 h of treatment and even more evident at later times. The increase of Gln concentration in the medium from 4–12 mM did not significantly change the effect of the combined treatment with ASNase and MSO (Fig. 1, panel b). However, the decrease in cell proliferation, observed with ASNase alone, was partially rescued by Gln supplementation.

To verify if MSO had similar effects in other cell lines, we determined the dose–response curve for ASNase in the absence or in the presence of the GS inhibitor in HOS cells, in other two lines derived from osteosarcoma (U2OS and SAOS-2 cells), and in three soft tissue sarcoma cell lines (the fibrosarcoma HT1080, the liposarcoma SW872, and the rhabdomyosarcoma RD cells). The results (Fig. 2) indicate that ASNase alone produced a marked decrease of cell viability in HT1080 and SW872 cells. In these lines, cell viability decreased below the initial value determined at the beginning of the treatment, indicating that ASNase caused a significant cell death. Indeed, as expected from previous results obtained in rodent cells [5, 21], in both HT1080 and SW872 cells ASNase triggered an apoptotic pathway as demonstrated by the significant activation of caspase-3 activity (Fig. 3) and the morphologic changes induced by the enzyme (not shown). In both HT1080 and SW872 cells MSO did not alter significantly the IC_{50} values for ASNase.

At variance with HT1080 and SW872 cells, the third line derived from a soft tissue sarcoma, the rhabdomyosarcoma cell line RD, exhibited a significant cell growth even at the highest ASNase concentration used (10 IU/ml), when viability was reduced by only 25% compared to control, untreated cells (Fig. 2). In RD cells the addition of MSO had dramatic effects, with a complete block in cell growth and a more than tenfold decrease of IC_{50}.

The three lines of osteosarcoma cells showed different sensitivities to ASNase. While cell growth was still observed, although lower than in control, untreated cultures, in HOS (see Fig. 1) and SAOS-2 cells, the viability of U2OS cells treated with high ASNase was comparable to that measured before the treatment, indicating that no net cell growth occurred upon incubation with the enzyme in this line. In all the three cell lines, however, the inhibition of GS activity led to an increased sensitivity to ASNase. In particular, impres-

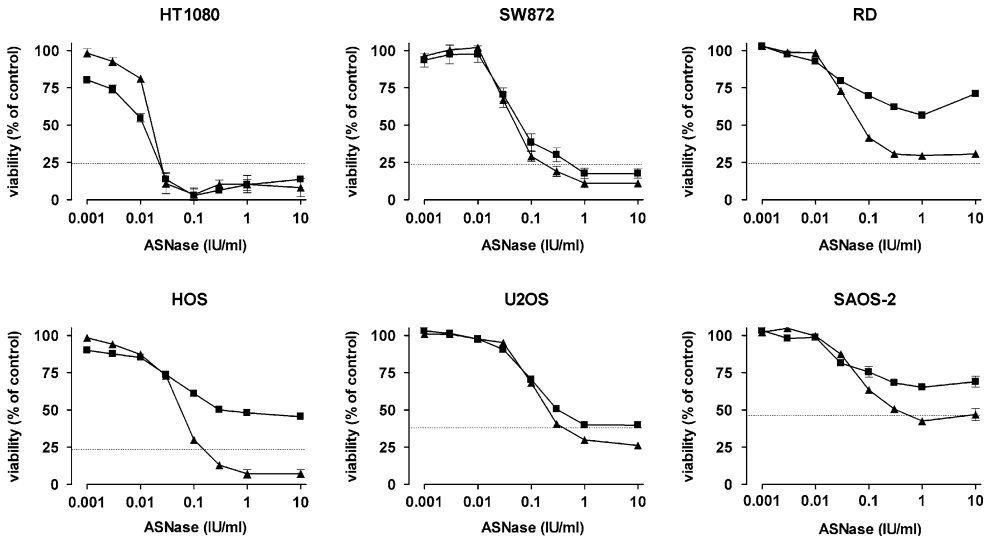

Fig. 2 Dose–response curves for ASNase in human sarcoma cell lines. Cells were seeded on 96-well dishes in complete growth medium. After 24 h ASNase was added at different concentrations, as indicated, in the absence (*filled square*) or in the presence (*filled triangle*) of 1 mM MSO. Cell viability was assessed after 48 h of treatment as described in Materials and methods. The *dotted line* indicates the initial value determined just before the beginning of the treatment. Data are means of six independent determinations with SEM shown when greater than the size of the point. IC_{50} values for ASNase, calculated with the Prism4™ software, were 0.008 (no MSO) and 0.011 IU/ml (+MSO), for HT1080 cells; 0.078 (no MSO) and 0.059 IU/ml (+MSO), for SW872 cells; >10 (no MSO) and 0.089 IU/ml (+MSO), for RD cells; 0.432 (no MSO) and 0.056 IU/ml (+MSO), for HOS cells; 0.382 (no MSO) and 0.221 IU/ml (+MSO), for U2OS cells; >10 (no MSO) and 0.408 IU/ml (+MSO), for SAOS-2 cells

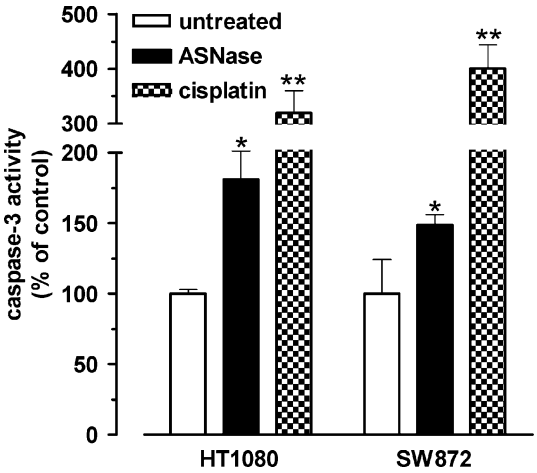

Fig. 3 Caspase activation by ASNase in sarcoma cells. HT1080 and SW872 cells were incubated for 48 h in the absence or in the presence of ASNase (10 IU/ml) or cisplatin (25 μM), as indicated. At the end of the incubation, caspase-3 activity was measured in cell extracts as described in Materials and methods. Data are means of 3 independent determinations with SD shown. *$P < 0.05$ and **$P < 0.01$ versus control, untreated cells

sive decreases in IC_{50} were detected for SAOS-2 cells and HOS cells, while a less marked change was observed for U2OS cells, which, however, in the presence of MSO exhibited marked cytotoxicity at the highest ASNase concentrations used (≥1 IU/ml).

Table 1 reports the results of a cytofluorimetric analysis of apoptosis in human sarcoma cell lines treated with ASNase in the absence or in the presence of MSO. In HT1080, SW872, and SAOS-2 cells the treatment with ASNase caused an increase in apoptotic cells, which was more evident in the presence of MSO. For RD cells, instead, no increase in the percentage of apoptotic cells was detected. Also HOS and U2OS osteosarcoma cells exhibited no marked change in the incidence of apoptosis upon treatment with ASNase alone, but a clear cut increase of apoptotic cells was detectable in these lines if MSO was present. No consistent change in cell cycle was detected in all the cell lines upon treatment with ASNase or ASNase + MSO (results not shown).

The levels of Glutamine Synthetase (GS) activity measured in the extracts of human sarcoma cells are shown in Fig. 4. Very different levels of GS activity were detected in the tested cell lines (panel a). Compared with human fibroblasts treated with dexamethasone, a condition associated with a marked GS induction [26], sarcoma cells exhibited GS activities ranging from 2% (HT1080 fibrosarcoma cells) to nearly 30% (RD rhabdomyosarcoma cells). Osteosarcoma cell lines had intermediate GS activities, with HOS < U2OS < SAOS-2 cells. In Fig. 4, panel b, GS activity was determined in cell extracts of HOS and RD cells after a 48-h incubation in the absence or in the presence of either ASNase or ASNase + MSO. The results indicate that ASNase treatment led to a significant

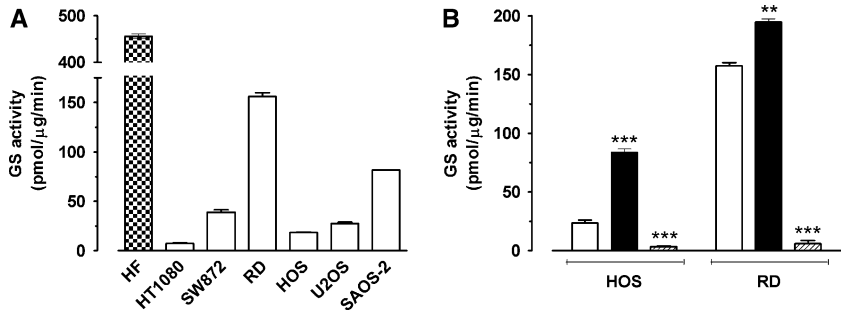

Fig. 4 Activity of Glutamine Synthetase in human sarcoma cells. *Panel a* The activity of glutamine synthetase was measured, as detailed in Materials and methods, in the indicated sarcoma cell lines maintained in complete growth medium (*open bars*). HF, cultured human fibroblasts treated for 48 h with 1 μM dexamethasone (*cross-hatched bar*), used as positive controls of GS activity. *Panel b* HOS cells and RD cells were incubated for 48 h in complete growth medium in the absence (control, *open bars*) or in the presence of ASNase (1 UI/ml, *solid bars*) or ASNase + 1 mM MSO (*hatched bars*). At the end of the incubation cells were lysed and the activity of glutamine synthetase determined. For both panels, data are means of three independent determinations with SD shown. The experiments were repeated twice with comparable results. $^{**}P < 0.01$ and $^{***}P < 0.001$ versus control cells

Table 1 Effects of ASNase and MSO on the percentage of apoptotic cells in human sarcoma cell lines

Cell line	Apoptotic cells (%)		
	Control	+ASNase	+ASNase + MSO
HT1080	2.6	6.8	9.4
SW872	4.6	7.5	21.6
RD	2.5	2.1	1.7
HOS	0.5	1.3	9.7
U2OS	1.2	0.9	23.9
SAOS-2	2.3	6.6	7.2

After a 48 h-incubation in medium supplemented with ASNase (5 IU/ml) or ASNase + MSO (1 mM), or in standard growth medium (control), DNA content was determined by FACS. Data derive from a representative experiment repeated twice with comparable results

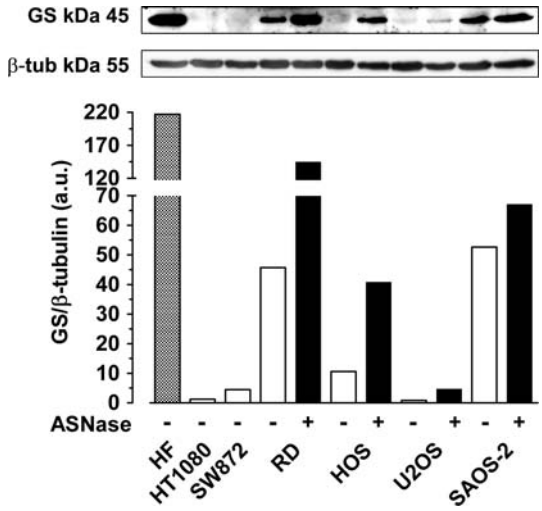

Fig. 5 Expression of glutamine synthetase in human sarcoma cells: effect of ASNase treatment. The indicated cell lines were maintained for 48 h in complete growth medium in the absence (*open bars*) or presence of 1 IU/ml ASNase (*solid bars*). HF, cultured human fibroblasts maintained for 48 h in complete growth medium supplemented with 1 μM dexamethasone (*cross-hatched bar*), used as positive controls of GS expression. *Upper* Western blot of glutamine synthase and β-tubulin in cell lysates. *Lower* Densitometric analysis of the GS abundance, corrected for β-tubulin expression (see Materials and methods for details). A representative experiment, repeated twice with comparable results, is shown

stimulation of GS activity, which is more evident in HOS cells. However, even in ASNase-treated cells, MSO suppressed GS activity in both cell lines.

Also the levels of GS protein were measured in sarcoma cells, using dexamethasone-treated human fibroblasts as positive control (Fig. 5). As for GS activity, GS expression was very different in the various cell lines, with SAOS-2 ≥ RD > HOS > SW872 > HT1080 > U2OS. The effect of a 48 h treatment with ASNase on GS protein abundance was investigated in RD, HOS, U2OS, and SAOS-2 cells since the experiment was not feasible in HT1080 and SW872 cells for the severe cytotoxicity observed upon a prolonged treatment with the anti-tumor enzyme. In all the four cell lines tested, a prolonged ASNase treatment increased the amount of GS protein with relative stimulations ranging from +30%, for SAOS-2 cells, to +470% for U2OS cells. RD and HOS cells exhibited intermediate levels of stimulation of GS expression, with increases of 220 and 280%, respectively.

Discussion

This contribution represents the first study on the effects of L-asparaginase (ASNase) in cells derived

from human tumors of mesenchymal origin. Of the six cell types used, two lines from human soft tissue sarcomas, HT1080 fibrosarcoma cells and SW872 liposarcoma cells, are highly sensitive to the anti-tumor enzyme, with IC_{50} values comparable to those shown by ASNase-sensitive leukaemia cells [6]. Given that no data are available on ASNase effects in human soft tissue sarcomas, these results suggest that a more thorough investigation on the sensitivity of these tumors to the anti-tumor enzyme is worthy to be pursued.

The other four cell lines, three derived from osteosarcomas (HOS, U2OS, and SAOS-2) and one from rhabdomyosarcoma (RD), exhibit, at best, only a partial inhibition of proliferation when treated with ASNase. However, their poor sensitivity to ASNase is significantly enhanced if Glutamine Synthetase (GS) is inhibited during the exposure to the anti-tumor enzyme. This finding suggests that GS activity is essential for the adaptation to the metabolic stress caused by ASNase. The adaptive importance of GS is consistent with the finding that ASNase-treated sarcoma cells exhibit increased levels of GS protein (Fig. 5) associated with an enhanced GS activity (Fig. 4b).

As other ASNase preparations used in leukaemia therapy, ASNase from *E. chrisanthemy*, the enzyme used in this study, is endowed with significant glutaminase activity [1]. Thus, upon its addition to the extracellular medium, a rapid depletion of extracellular glutamine is to be expected, as previously observed for *E. coli* ASNase [5]. Under these conditions, cells fully depend on glutamine synthesized in the intracellular compartment through GS activity and, therefore, GS inhibition by MSO represents a severe nutritional stress that prevents a successful adaptation to ASNase and leads to cell death, as in the case of HOS and U2OS cells, or to the complete proliferative block observed in RD and SAOS-2 cells. Interestingly, in the absence of ASNase, GS inhibition by MSO is without appreciable antiproliferative or cytotoxic effects (see Fig. 1), since extracellular glutamine is available and can be transported into the cell.

The two lines that exhibit the most dramatic increases in sensitivity to ASNase upon MSO treatment, RD and SAOS-2 cells, are also endowed with the highest GS activities and exhibit the lowest sensitivities to ASNase in the absence of MSO. However, these data should not be taken as an evidence for an exclusive role of GS in determining sensitivity or resistance to ASNase. Cell response to the anti-tumor enzyme is, indeed, a complex phenomenon, which includes changes in the expression of many genes [6], as well as diverse biochemical adaptations [3]. Moreover, the nutritional stress imposed by the anti-tumor enzyme involves distinct pathways in different tissues [20], suggesting that the metabolic consequences of ASNase-dependent glutamine depletion are tissue specific. It is possible that increase in GS expression and activity plays an important adaptive role in mesenchymal cells.

All the cell lines sensitized to ASNase by MSO, derived from rhabdomyosarcoma or osteosarcomas, are endowed with a detectable GS activity even under control conditions. As far as osteosarcoma cells are concerned, these results are consistent with recently reported data that demonstrate the presence of GS expression in several lines of osteosarcoma cells as well as in normal osteoblasts [18]. In contrast, this contribution represents the first observation on GS expression in rhabdomyosarcoma cells, although it is well known that the enzyme is expressed in skeletal muscle, where it is finely regulated at both transcriptional and post-transcriptional levels [8, 11].

In conclusion, the results presented in this contribution demonstrate that human sarcoma cells are sensitive to a two-step interference on glutamine fuelling based on the combined treatment with ASNase and MSO. The possibility that this approach may yield a novel therapeutic device deserves further investigations.

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