S-acetyl-glutathione selectively induces apoptosis in human lymphoma cells through a GSH-independent mechanism

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Abstract. Reduced apoptosis is associated to cancer development. Agents able to restore the programmed cell death responsiveness of cancer cells are foreseen as potential effective cancer therapies. In this study, we report that a glutathione-S-derivative, S-acetyl-glutathione (Sag), induces significant apoptosis in three human lymphoma cell lines, including Daudi, Raji and Jurkat cells while it had no or little effect on either Hut-78 lymphoma cells or the normal BT lymphocytes. We used Annexin-V FACS analysis and DNA laddering to demonstrate that Sag activated apoptosis in the three sensitive cell lines in a dose- and time-dependent-fashion. Using mercury orange staining and FACS analysis, we showed that Sag generated an intracellular GSH depletion in Daudi, Raji and Jurkat cells but not in Hut-78 or the normal BT cells. These data provide direct evidence that Sag specifically activates programmed cell death in lymphoma cells through, at least in part, a depletion of intracellular GSH rather than an increase, as previously suggested. Because of its selective effect on cancer cells, Sag appears as a promising new lymphoma cell apoptosis inducer with potential clinical value for lymphoma patients.

Introduction

Reduced glutathione (GSH), a thiol-containing tripeptide, plays a key role in cell protection against radiation, reactive oxygen species and other toxic compounds (1). There are abundant evidence that GSH depletion is involved in the initiation and progression of a wide variety of cancers (2-5). It has been proposed that GSH could be of therapeutic value in prevention and treatment of cancer (6-8). Interestingly, GSH exhibits anti-tumoral activity in *in vitro* (9,10) as well as in *in vivo* models (11-15). However, some authors have failed to demonstrate such an anti-cancer activity for GSH (16). It has been proposed that the anti-cancer activities of GSH are, at least in part, due to the induction of cancer cell apoptosis (9), but the data regarding such a mechanism of action have not been confirmed. Nonetheless, there are abundant data demonstrating that glutathione-S-derivatives are effective apoptosis inducers in human leukemia cells (17-22). In particular, S-D-lactoylglutathione, the physiological intermediate of the cytosolic glyoxalase system (23), stops HL-60 cell growth by an inhibition of *de novo* pyrimidine synthesis and induction of apoptosis (19,20). Exogenous S-D-lactoylglutathione is in fact degraded by membrane-bound γ-glutamyl transpeptidase and dipeptidase, two enzymes involved, in normal conditions, in the extracellular degradation of GSH (24), and converted into intracellular N-D-lactoylcysteine which is the active metabolite responsible for the apoptotic effect.

S-acetyl-glutathione (Sag) is another GSH-S-derivative in which the thiol group of the cysteine residue is acetylated. Among other glutathione S-derivatives, Sag appears as a possible effective apoptosis inducer for two main reasons: i) the presence of a γ-glutamyl bond suggests that Sag may be a substrate for γ-glutamyl transpeptidase (25) and may thus act similarly to S-D-lactoylglutathione; ii) the acetyl group provides protection against oxidation, enhancing the bioavailability *in vivo*.

In the present study, we showed that Sag significantly induces apoptosis in a dose- and time-dependent manner in several types of human lymphoma cells (Daudi, Raji, Jurkat). Normal human BT lymphocytes were significantly less affected by the drug. Moreover, flow cytometry measurements of intracellular GSH concentrations showed that the Sag effect is not mediated through a modification of the cytosolic GSH pool, enforcing the similarity with the S-D-lactoyl-glutathione activity.

Materials and methods

Cell culture and treatments. Jurkat, Daudi, Raji and Hut-78 cells were obtained from the European Collection of Cell

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Abbreviations: γ-GT, gamma-glutamyl-transpeptidase; An-V, Annexin-V; BTL, B and T lymphocytes; DEM, diethylmaleate; FITC, fluorescein isothiocyanate; GSH, reduced glutathione; PBS, phosphate buffer saline; PI, propidium iodide; Sag, S-acetyl-glutathione

Key words: glutathione, apoptosis, lymphoma

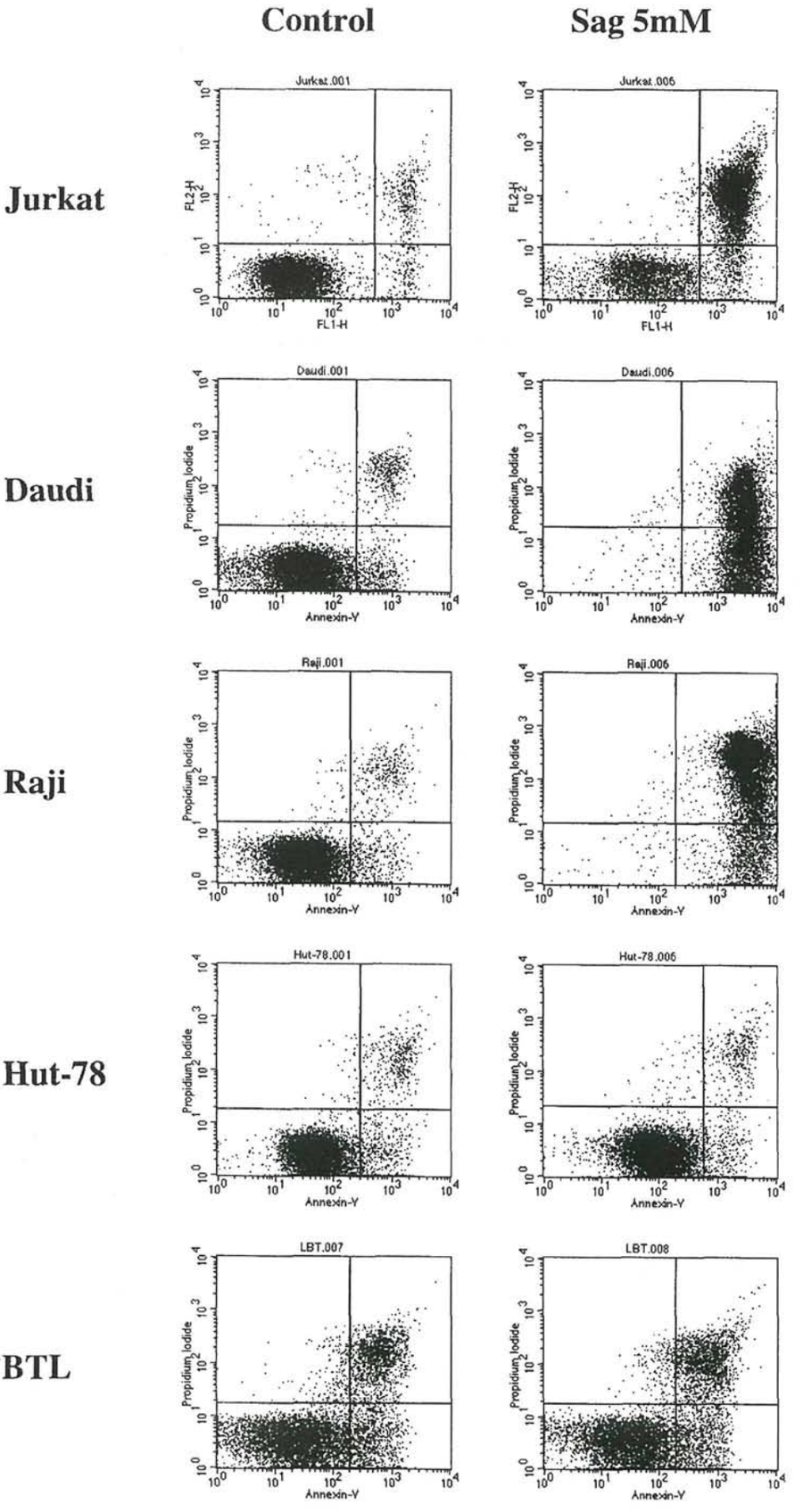

Figure 1. Induction of apoptosis (vs. necrosis) in tumoral and normal human lymphocytes following 5 mM S-acetyl-glutathione (Sag) treatment. Dot-plot fluorescence cytograms obtained after Annexin-V/propidium iodide staining of tumoral (Jurkat, Daudi, Raji and Hut-78) or normal (BTL) human lymphocytes cultured or not for 24 h with 5 mM Sag. X-axis represents the log of the green fluorescence emitted by FITC-conjugated Annexin-V and the Y-axis corresponds to the log of orange fluorescence emitted by propidium iodide.

Cultures (Salisbury, UK). They were maintained in RPMI 1640 medium (Life Technologies, Rockville, MD, USA) supplemented with 10% heat-inactivated fetal bovine serum and 2 mM L-glutamine (Life Technologies). The cell suspensions were kept between $2x10^5$ and 10^6 cells per ml, for a maximum of 15 passages.

Human BT lymphocytes were obtained from the peripheral blood of healthy donors, kindly collected by the Blood Transfusion Department (University Hospital, Liege, Belgium). Blood lymphocytes were purified by Ficoll-Hypaque (Amersham Pharmacia Biotech, Uppsala, Sweden) density gradient centrifugation, following the manufacturer's instructions. The cells were maintained in the medium described above and the proliferation was stimulated by the addition of 5 µg/ml phytohemagglutinin-M (Roche, Mannheim, Germany). Freshly isolated cells were used for each experiment. All cells were incubated at 37°C in an atmosphere containing 5% CO_2 under saturating humidity. For each experiment, the cells were first incubated for 24 h in T25 (flow cytometry) or T75 (DNA fragmentation) culture flasks without treatment, at an initial concentration of $2x10^5$ cells per ml. S-acetyl-glutathione (Sag, Biodynamics, Belgium) and reduced glutathione (GSH, Sigma-Aldrich, Steinheim, Germany) were freshly dissolved in cold PBS before each assay to a concentration of 75 and 150 mM respectively. These solutions were kept on ice until the final dilution in culture medium to obtain the desired concentration.

Annexin-V/propidium iodide apoptosis assay. Apoptotic cell detection was performed using FITC-conjugated Annexin-V-Fluos (Roche) and propidium iodide (Sigma), following the manufacturer's instructions. Briefly, 10^6 cells for each condition were collected, rinsed with 1 ml cold PBS and resuspended in 100 µl incubation buffer (10 mM HEPES/NaOH, pH 7.4; 140 mM NaCl; 6 mM $CaCl_2$) containing 2 µl Annexin-V-Fluos and 1 µg/ml propidium iodide. After 15 min of incubation in the dark and at room temperature, 400 µl incubation buffer were added and the cells were analyzed by flow cytometry (FACSCalibur, BD, Franklin Lakes, NJ, USA), using 488 nm excitation and band pass filters of 530/30 nm (for FITC detection) and 585/42 nm (for PI detection). For each type of cell, appropriate electronic compensation of the instrument was performed to avoid overlapping of the two emission spectra.

DNA fragmentation assay. The detection of fragmented DNA in apoptotic cells was performed as previously described (26). Briefly, around 5.10^6 cells for each condition were collected, washed with PBS and resuspended in 500 µl lysis buffer (5 mM Tris-HCl pH 8.0, 0.25% Nonidet P-40, 1 mM EDTA). Cells were then treated with RNAse A (Sigma, 200 µgml) for 1 h at 37°C, followed by incubation in the same conditions with proteinase K (Sigma) at a final concentration of 300 µg/ml. After phenol/chloroform extraction and ethanol precipitation, the resulting pellet was resuspended in 20 µl TE buffer overnight at 4°C. Samples were then subjected to electrophoresis on a 1.5% agarose gel for 2 h at 90 V, stained with ethidium bromide and observed under UV light.

Determination of the relative intracellular GSH concentration. The method described by O'Connor *et al* (27) was used with minor modifications. Briefly, around $1.5x10^6$ cells for each condition were washed with cold PBS and resuspended in 1 ml mercury orange (Sigma, 75 µM in acetone). After a 5-min incubation on ice, the cells were pelleted, resuspended in 500 µl cold PBS and then analyzed by flow cytometry (FACSCalibur), using a 650 nm long pass filter. To determine the background level due to non-specific binding of the probe, each set of experiments included a condition in which the cells were treated with 10 mM diethylmaleate (DEM, Sigma) for 2 h, in order to deplete intracellular non-protein thiols. The relative intracellular GSH concentrations corresponded to the ratio between experimental vs. DEM mean red fluorescence. All steps were performed on ice or at 4°C to avoid GSH loss during the manipulations.

Statistics. Statistical analysis was carried out using an unpaired Student's t-test. Significance was assumed for p-values <0.05.

Results

Sag selectively induced apoptosis and not necrosis of lymphoma cells. Following 24-h incubation with 5 mM Sag, three out of the four cancer cell lines tested (Jurkat, Daudi, Raji) exhibited morphological changes currently associated with cell death such as reduction of the cell size and chromatin condensation (data not shown). To determine whether these cells underwent apoptosis rather than necrosis, FITC-labeled annexin-V (An-V) and propidium iodide (PI) staining followed by flow cytometry analysis were performed. The translocation of phosphatidylserine (PS), which presents a high affinity for An-V, from the inner to the outer plasma membrane leaflet is a very early event in the apoptotic cascade, occurring long before the classical appearance of DNA fragmentation (28). In our experiments, the total An-V positive population of each condition was separated using simultaneous PI labeling into 'early apoptotic cells' (intact membrane and PI exclusion) and 'necrotic and late apoptotic cells' (permeabilized membrane and PI DNA binding). Fig. 1 shows representative cytograms obtained for three different types of cells treated or not for 24 h with 5 mM Sag. Daudi cells, which are derived from human Negroid Burkitt's lymphoma (NBL), presented a strong increase of the whole An-V positive population comparing to the control. Identical results were obtained for both Jurkat cells and NBL-derived Raji cells (data not shown). Interestingly, neither the human lymphoma cell line Hut-78 nor the normal BT lymphocytes were affected by Sag treatment. These results were reproduced at least in three independent experiments.

DNA laddering experiments were then conducted to further demonstrate the selective Sag induced apoptosis in lymphoma cells. Following 24 h Sag treatments (5 mM), lymphoma and normal cells were analyzed using the An-V/PI technique to quantify the total apoptotic populations (Fig. 2, upper panel). In parallel, cells cultured in the same conditions (excepted Daudi: 48 h of treatment) were collected and submitted to total DNA purification and agarose gel electrophoresis. As shown in Fig. 2 (lower panel), the three cell lines

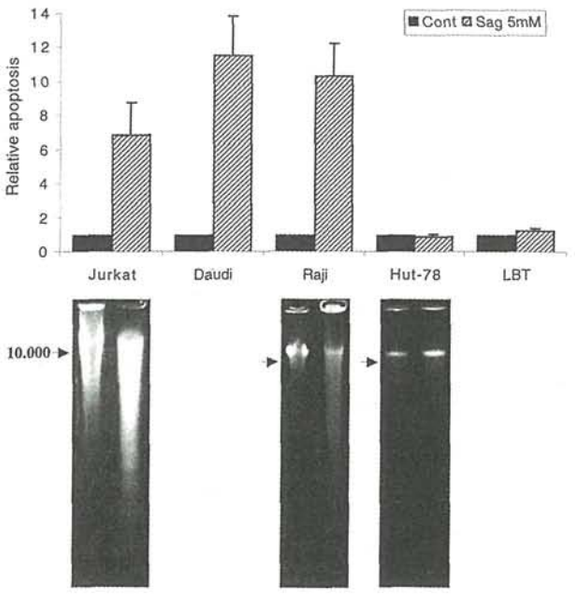

Figure 2. Detection of apoptosis induction in lymphoma cells and normal lymphocytes after Sag treatment by Annexin-V FACS analysis and DNA laddering. In the upper panel, histograms represent the relative total apoptotic populations obtained after An-V/PI FACS staining in Sag-treated (24 h) vs. control cells. Each cytogram was divided into four quadrants according to the control conditions and the percentage of early and late apoptotic cells, i.e. cells belonging to the lower and upper right quadrants, was automatically determined using the FACS analysis software. The values are the mean ± standard deviation of three different experiments. *Significant difference between control and treated cells ($p<0.05$). Lower panel, analysis of DNA fragmentation from cells exposed or not to 5 mM Sag for 24 h (excepted Daudi: 48 h).

exhibited DNA fragmentation, confirming apoptosis as their main type of death.

Sag-mediated induction of apoptosis is dose- and time-dependent. Normal and tumoral lymphocytes were cultured in the presence of increasing Sag concentrations (0-5 mM) for 24 h and then analyzed by An-V/PI FACS staining to evaluate the total apoptotic populations. Significant induction of cell death were obtained at 1 mM (Daudi, Raji) or 2 mM (Jurkat) treatments (Fig. 3A). High concentrations of Sag (2-5 mM) induced 100% apoptosis of Daudi and Raji cells. As expected, Hut-78 and BTL were unaffected by the drug even at high concentrations. Time curve experiments were performed using 5 mM Sag. Fig. 3B clearly shows that the three Sag-sensitive cell lines exhibited significant but not maximal induction of cell death already after 24 h, a plateau being reached for all cell types after a 48-h treatment. Hut-78 was not affected even after a Sag treatment of 96 h while normal BT lymphocytes showed a weak increase in the total apoptotic population after 48 h of treatment.

Sag effect on lymphoma cells is GSH independent. Recently, Donnerstag *et al* (9) described the induction of apoptosis in several tumoral cell lines following treatment with millimolar concentrations of Sag (9). As they observed a similar effect with GSH, they postulated that Sag acts through diffusion across the plasma membrane, intracellular deacetylation and subsequent perturbation of the intracellular GSH pool. We used flow cytometry experiments to explore the possibility that the Sag-induced apoptosis we observed was due to an increase of intracellular GSH concentration (GSHi). Cells were treated either with 5 mM Sag or GSH. The Sag sensitive cell lines Jurkat and Daudi exhibited significant increase in GSHi already 1 h after GSH treatment (Fig. 4, left panel) but did not present any sign of apoptosis induction, as assessed by An-V/PI FACS staining, even after longer delays (Fig. 4, right panel). The same results were obtained with Hut-78 and BTL cells. No GSHi increase was observed in Raji cells following incubation with GSH, suggesting a perturbation of the GSH import enzymatic machinery in these cells (1). In the Sag treatment experiments, a significant decrease of GSHi was measured after 8 h (Raji) or 24 h (Jurkat, Daudi) while Hut-78 and BTL were not affected. Interestingly, the timing of the decrease was correlated with the appearance of cell death (Fig. 4, right panel), suggesting that a decrease of GSHi rather than an increase is associated to enhanced apoptosis. This is more in agreement with the well-admitted observation that apoptosis is associated with intracellular oxidative stress development (29).

Discussion

Resistance to apoptosis is considered to be as important as uncontrolled proliferation in the cellular events leading to cancer development. This new paradigm has stimulated research aiming to identify ways to restore the sensitivity of cancer cells to programmed cell death (30). In the present study, we identified S-acetyl-glutathione (Sag) as a powerful agent to induce apoptosis in three human lymphoma cell lines (Jurkat, Daudi, Raji) while normal human BT lymphocytes were not or only weakly affected. The efficient Sag concentrations belonged to the millimolar range and the effect was already significant after 24 h of treatment. Sag-induced apoptosis was demonstrated both by Annexin-V labeling and DNA laddering.

In their study describing the apoptosis induction by Sag in different type of cancer cells, Donnerstag *et al* (9) proposed the intracellular deacetylation of Sag to form GSH and the subsequent perturbation of the cell redox status as the mechanism of action. Our study does not support this hypothesis as we demonstrated that: i) Sag was not able (excepted for Jurkat) to induce significant GSHi increase, ii) GSH treatment significantly increased GSHi without inducing any sign of apoptosis. Moreover, several recent reports revealed that N-acetyl-L-cysteine, a precursor of GSH and powerful agent to increase GSHi, could induce apoptosis in several cell types, but through a GSH-independent pathway (31,32). Our data demonstrate that the Sag-induced apoptosis is not mediated by increased GSHi. Interestingly, Clelland *et al* have shown that S-D-lactoylglutathione and, to a lesser extent, other S-2-hydroxyacylglutathione substitutes, (two glutathione derivatives) induce cell growth arrest and apoptosis in HL-60 leukemia cells (17). The thioester bond, also present in Sag, was shown to be essential to the apoptosis induction properties of S-D-lactoylglutathione (18). The authors proposed a three step-mechanism of action for the S-D-lactoylglutathione effect: i) external degradation by

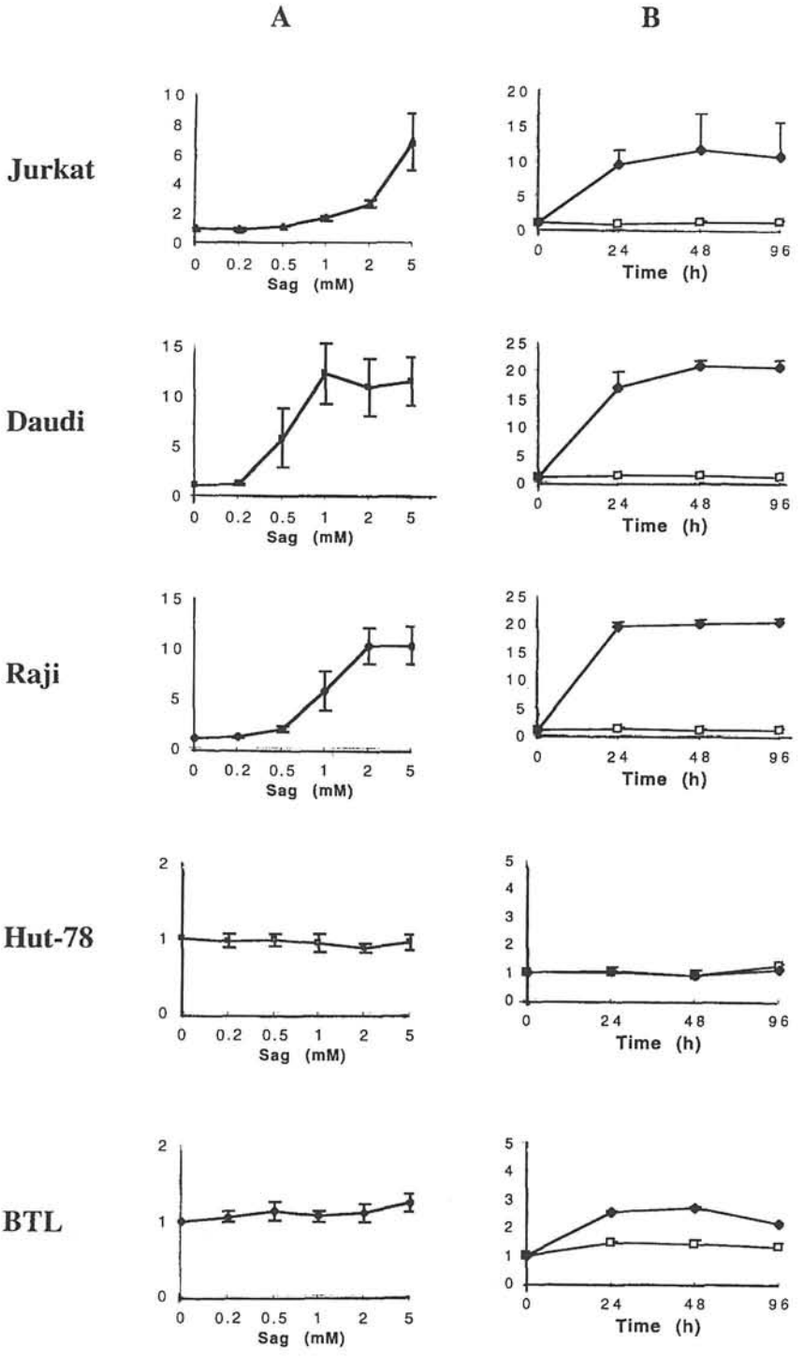

Figure 3. Sag-induced apoptosis is dose- and time-dependent. Lymphoma and normal BT lymphocytes were treated with various concentrations of Sag (A) and for different times (B). Apoptosis was evaluated at each concentration and time point using An-V/PI FACS analysis. Y-axis represents the total apoptotic populations, relative to the control. Values are the mean ± standard deviation of three different assays. No error bars means that they are too small to be represented. *Significant difference, comparing to the control ($p<0.05$).

membrane-bound γ-glutamyl-transpeptidase (γ-GT) and dipeptidase to form N-D-lactoylcysteine; ii) diffusion through the plasma membrane; iii) inhibition of dihydro-orotase, an enzyme implied in *de novo* pyrimidine synthesis (20). The likeliness of such a mechanism was confirmed by experiments in which the effect of S-D-lactoylglutathione was inhibited by the administration of uridine (19). γ-GT catalyses the transfer of a γ-glutamyl moiety of glutathione, but also of its S-substituted derivatives, to a variety of acceptor substrates (25). Sag could be considered as a possible γ-GT substrate and therefore could induce apoptosis using the same mechanism than S-D-lactoylglutathione. Therefore, it is likely that pyrimidine synthesis inhibition is the key event in the Sag-induced apoptosis. An additional mechanism could be associated to the decrease of GSHi induced by Sag. GSH, a powerful reducing molecule, plays a crucial role in the

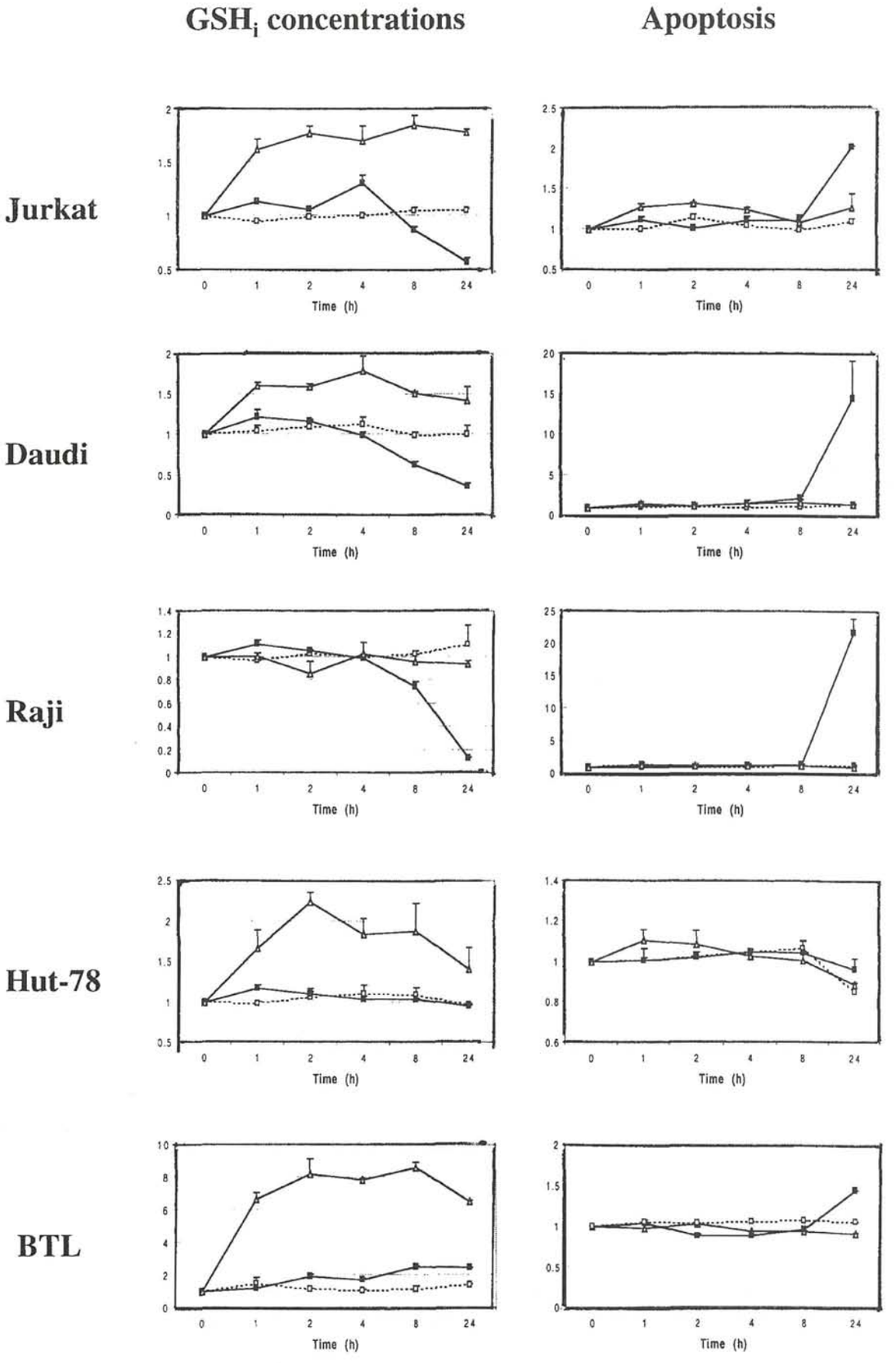

Figure 4. Sag-induced apoptosis is GSH-independent. Left panel, the intracellular concentration of GSH (GSHi) was determined in the lymphoma cell lines and normal BT lymphocytes treated with PBS (□), Sag 5 mM (■) or GSH 5 mM (△) for a given period using a mercury orange staining and Facs analysis. Right panel, time-dependent evolution of the relative total apoptotic populations in the lymphoma cell lines and normal BT lymphocytes treated with PBS (□), Sag 5 mM (■) or GSH 5 mM (△) measured by An-V/PI staining and FACS analysis. Values are the mean ± standard deviation of three different assays. No error bars means that they are too small to be represented. *Significant difference, comparing to the control ($p<0.05$).

scavenging of reactive oxygen species (24). A decrease of GSHi weakened dramatically the oxidative stress defense mechanism of the cell and increased the risk of DNA damage (33). This Sag treatment could induce an oxidative stress condition which, coupled with the potential decrease of pyrimidine synthesis, would lead to alteration of DNA. In this scenario, apoptosis will be triggered by p53 (34). The key role of GSHi depletion in the Sag-induced apoptosis is supported by our observation that in both Hut-78 cells and normal BT lymphocytes (Sag apoptosis resistant), the GSHi remained unchanged after Sag treatment.

While further work is necessary to clarify the exact mechanism of action of Sag and, particularly, to explain its lack of effect towards normal lymphocytes, our study permits to position Sag as a powerful and promising selective apoptosis inducer in human lymphoma with potential valuable clinical applications.

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References

1. Meister A and Anderson ME: Glutathione. Ann Rev Biochem 52: 711-760, 1983.
2. Anasagasti MJ, Martin JJ, Mendoza L, Obrador E, Estrela JM, McCuskey RS and Vidal-Vanaclocha F: Glutathione protects metastatic melanoma cells against oxidative stress in the murine hepatic microvasculature. Hepatology 27: 1249-1256, 1998.
3. Cerutti PA: Prooxidant states and tumor promotion. Science 227: 375-381, 1985.
4. Dekant W and Vamvakas S: Glutathione-dependent bioactivation of xenobiotics. Xenobiotica 23: 873-887, 1993.
5. Ristoff E and Larsson A: Patients with genetic defects in the gamma-glutamyl cycle. Chem Biol Interact 111-112: 113-121, 1998.
6. Chen X, Carystinos GD and Batist G: Potential for selective modulation of glutathione in cancer chemotherapy. Chem Biol Interact 111-112: 263-275, 1998.
7. Kauvar LM, Morgan AS, Sanderson PE and Henner WD: Glutathione based approaches to improving cancer treatment. Chem Biol Interact 111-112: 225-238, 1998.
8. Links M and Lewis C: Chemoprotectants: a review of their clinical pharmacology and therapeutic efficacy. Drugs 57: 293-308, 1999.
9. Donnerstag B, Ohlenschlager G, Cinatl J, Amrani M, Hofmann D, Flindt S, Treusch G and Trager L: Reduced glutathione and S-acetylglutathione as selective apoptosis-inducing agents in cancer therapy [published erratum appears in Cancer Lett 115: 265, 1997]. Cancer Lett 110: 63-70, 1996.
10. Perego P, Paolicchi A, Tongiani R, Pompella A, Tonarelli P, Carenini N, Romanelli S and Zunino F: The cell-specific anti-proliferative effect of reduced glutathione is mediated by gamma-glutamyl transpeptidase-dependent extracellular pro-oxidant reactions. Int J Cancer 71: 246-250, 1997.
11. Karmali RA: Growth inhibition and prostaglandin metabolism in the R323AC mammary adenocarcinoma by GSH. Cancer Biochem Biophys 7: 147-154, 1984.
12. Novi A: Regression of Aflatoxin B1-induced hepatocellular carcinomas by reduced glutathione. Science 212: 541-542, 1981.
13. Novi AM, Flörke R and Stukenkemper M: Glutathione and aflatoxin-B1-induced liver tumors: requirement for regression of malignancy in neoplastic tissue. Ann NY Acad Sci 397: 62-71, 1982.
14. Rotstein JB and Slaga TJ: Effect of exogenous glutathione on tumor progression in the murine skin multistage carcinogenesis model. Carcinogenesis 9: 1547-1551, 1988.
15. Schwartz JL and Shklar G: Glutathione inhibits experimental oral carcinogenesis, p53 expression, and angiogenesis. Nutr Cancer 26: 229-236, 1996.
16. Wagner G, Frenzel H, Wefers H and Sies H: Lack of effect of long-term glutathione administration on aflatoxin B1-induced hepatoma in male rats. Chem Biol Interact 53: 57-68, 1985.
17. Clelland JD, Allen RE and Thornalley PJ: Inhibition of growth of human leukaemia 60 cells by S-2-hydroxyacylglutathiones and monoethyl ester derivatives. Biochem Pharmacol 44: 1953-1959, 1992.
18. Edwards L, Clelland JD and Thornalley PJ: Characteristics of the inhibition of human promyelocytic leukaemia HL60 cell growth by S-D-lactoylglutathione *in vitro*. Leuk Res 17: 305-310, 1993.
19. Edwards LG and Thornalley PJ: Prevention of S-D-lactoyl-glutathione-induced inhibition of human leukaemia 60 cell growth by uridine. Leuk Res 18: 717-722, 1994.
20. Edwards LG, Adesida A and Thornalley PJ: Inhibition of human leukaemia 60 cell growth by S-D-lactoylglutathione *in vitro*. Mediation by metabolism to N-D-lactoylcysteine and induction of apoptosis. Leuk Res 20: 17-26, 1996.
21. Lo TW and Thornalley PJ: Inhibition of proliferation of human leukaemia 60 cells by diethyl esters of glyoxalase inhibitors *in vitro*. Biochem Pharmacol 44: 2357-2363, 1992.
22. McLellan AC, Phillips SA and Thornalley PJ: The assay of S-D-lactoylglutathione in biological systems. Anal Biochem 211: 37-43, 1993.
23. Thornalley PJ: The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life. Biochem J 269: 1-11, 1990.
24. Meister A: Glutathione metabolism and its selective modification. J Biol Chem 263: 17205-17208, 1988.
25. Taniguchi N and Ikeda Y: Gamma-glutamyl transpeptidase: catalytic mechanism and gene expression. Adv Enzymol Relat Areas Mol Biol 72: 239-278, 1998.
26. Wei YQ, Zhao X, Kariya Y, Fukata H, Teshigawara K and Uchida A: Induction of apoptosis by quercetin: involvement of heat shock protein. Cancer Res 54: 4952-4957, 1994.
27. O'Connor JE, Kimler BF, Morgan MC and Tempas KJ: A flow cytometric assay for intracellular nonprotein thiols using mercury orange. Cytometry 9: 529-532, 1988.
28. Martin SJ, Reutelingsperger CP, McGahon AJ, Rader JA, van Schie RC, La Face DM and Green DR: Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. J Exp Med 182: 1545-1556, 1995.
29. Beaver JP and Waring P: A decrease in intracellular glutathione concentration precedes the onset of apoptosis in murine thymocytes. Eur J Cell Biol 68: 47-54, 1995.
30. Thatte U and Dahanukar S: Apoptosis: clinical relevance and pharmacological manipulation. Drugs 54: 511-532, 1997.
31. Jones DP, Maellaro E, Jiang S, Slater AF and Orrenius S: Effects of N-acetyl-L-cysteine on T-cell apoptosis are not mediated by increased cellular glutathione. Immunol Lett 45: 205-209, 1995.
32. Liu M, Pelling JC, Ju J, Chu E and Brash DE: Antioxidant action via p53-mediated apoptosis. Cancer Res 58: 1723-1729, 1998.
33. Pryor WA: Free radical biology: xenobiotics, cancer, and aging. Ann NY Acad Sci 393: 1-22, 1982.
34. Jacks T and Weinberg RA: Cell-cycle control and its watchman. Nature 381: 643-644, 1996.