Experimental Chemotherapy

Chemotherapy

Chemotherapy 2007;53:210–217
DOI: 10.1159/000100812

Received: October 10, 2005
Accepted after revision: February 28, 2006
Published online: March 15, 2007

Sulfasalazine-Induced Reduction of Glutathione Levels in Breast Cancer Cells: Enhancement of Growth-Inhibitory Activity of Doxorubicin

Vishal S. Narang[a] Giovanni M. Pauletti[a] Peter W. Gout[b] Donna J. Buckley[a] Arthur R. Buckley[a]

[a]College of Pharmacy, University of Cincinnati, Cincinnati, Ohio, USA, and [b]Department of Cancer Endocrinology, BC Cancer Agency, Vancouver, Canada

Key Words

Doxorubicin · Glutathione · Mammary cancer · Methotrexate · Multidrug-resistant proteins · Sulfasalazine · x_c^- cystine transporter

Abstract

Background: We previously showed that the anti-inflammatory drug, sulfasalazine (salicylazosulfapyridine, SASP), can arrest proliferation of MCF-7 and MDA-MB-231 mammary cancer cells by inhibiting uptake of cystine via the x_c^- cystine/glutamate antiporter. Here we examined SASP with regard to reduction of cellular glutathione (GSH) levels and drug efficacy-enhancing ability. ***Methods:*** GSH levels were measured spectrophotometrically. Cellular drug retention was determined with ^{3}H-labeled methotrexate, and drug efficacy with a colony formation assay. ***Results:*** Incubation of the mammary cancer cells with SASP (0.3–0.5 mM) led to reduction of their GSH content in a time- and concentration-dependent manner. Similar to MK-571, a multidrug resistance-associated protein inhibitor, SASP increased intracellular accumulation of methotrexate. Preincubation of cells with SASP (0.3 mM) significantly enhanced the potency of the anticancer agent doxorubicin (2.5 nM). ***Conclusions:*** SASP-induced reduction of cellular GSH levels can lead to growth arrest of mammary cancer cells and enhancement of anticancer drug efficacy.

Introduction

Drug resistance is a major impediment to successful chemotherapy of a variety of malignancies, including breast cancer. While hormone-sensitive breast cancer may initially respond to endocrine therapy, most treated tumors return in a hormone-independent form resistant to further hormonal therapy and many other regimens [1]. Although the nature of drug resistance in breast cancer is likely to be multifactorial, there is evidence for a major role for multidrug resistance (MDR), an intrinsic or acquired cross-resistance to multiple, structurally and functionally unrelated drugs [2, 3]. MDR is often associated with a decrease in cellular drug accumulation that is attributed to increased activity of unidirectional ATP-binding cassette (ABC) transporter proteins involved in excretion of drugs from cells [4]. Such transporters include multidrug resistance-associated proteins, MRP1–MRP7, which exhibit high affinity toward anionic molecules including the anticancer agent, methotrexate (MTX), and neutral drugs conjugated to acidic ligands such as glutathione (GSH) [3, 5, 6]. This compound, a tripeptide thiol best known as a cellular antioxidant, also has a major role in drug resistance, as it is involved in drug detoxification and elimination [7]. GSH can combine with anticancer drugs to form less toxic and more water-soluble GSH conjugates (a reaction catalyzed by GSH *S*-transferases), which can then be exported from cells by multidrug-re-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/che

Dr. Arthur R. Buckley
3225 Eden Avenue
Cincinnati, OH 45267-0004 (USA)
Tel. +1 513 558 2575, Fax +1 513 558 0978
E-Mail Arthur.buckley@uc.edu

sistant proteins (MRPs) [8]. There is increasing evidence that depletion of GSH in cancer cells can inhibit drug efflux capability of MRPs, leading to increased intracellular accumulation of drugs and reversal of drug resistance [9–11]. Recent studies indicate that GSH induces a conformational change at a site within MRP1 that is indispensable for interaction of this efflux protein with its substrates [12]. GSH therefore appears to play a critical role in MDR, and it is recognized that GSH depletion can enhance therapeutic efficacy of anticancer agents and could provide a new strategy urgently needed in cancer therapy [8, 13].

The x_c^- cystine/glutamate antiporter is a plasma membrane transporter that mediates cellular uptake of the amino acid, cystine [14]. Intracellularly, cystine is reduced to cysteine, which is an immediate precursor of GSH (i.e. γ-glutamyl-cysteine-glycine). The x_c^- transporter plays an important role in cancer cells that depend on uptake of cystine from their microenvironment for growth [15–17]. Its activity should also enhance GSH production and hence MDR [18, 19]. We have previously proposed that malignant progression of breast cancers, as well as lymphomas, is associated with acquisition of the x_c^- transporter and increased reliance on its mediation of cystine uptake [15, 17]. We also showed, for the first time, that sulfasalazine (salicylazosulfapyridine, SASP), an anti-inflammatory drug used against inflammatory bowel disease and rheumatoid arthritis, is a potent inhibitor of x_c^--mediated cystine uptake [16], and that it can markedly inhibit proliferation of human breast carcinoma cells at relatively low concentrations (0.2–0.5 mM) via cystine starvation [17]. Since availability of reduced cystine is a rate-limiting step for synthesis of GSH, which has a short half-life [7], SASP-induced cystine starvation could readily lead to a decline in GSH levels and possibly to lower MDR. In the present study we examined this possibility using MCF-7 and MDA-MB-231 human breast cancer cells. It was found that SASP treatment can indeed lead to a marked reduction in the GSH levels of the cells and can enhance the intracellular accumulation of MTX and efficacy of doxorubicin (DOX), a drug important in therapy of metastatic breast cancer [1].

Materials and Methods

Materials

The human breast carcinoma cell lines, MCF-7 (estrogen receptor positive) and MDA-MB-231 (estrogen receptor negative, highly invasive), were obtained from the American Type Culture Collection (Manassas, Va., USA). They were maintained at 37°C in modified essential Dul-becco's medium containing 10% fetal bovine serum, penicillin (50 units/ml) and streptomycin (50 μg/ml), as previously described [17]. Culture media and fetal bovine serum were obtained from Life Technologies (Carlsbad, Calif., USA). Tritium-labeled MTX (^{3}H-MTX) was obtained from Amersham (Piscataway, N.J., USA). The MRP antagonist, MK-571, was purchased from Biomol (Plymouth, Pa., USA). Unless otherwise noted, all other reagents were of molecular biological grade and obtained from Sigma (St. Louis, Mo., USA).

GSH Assay

Cells (1×10^6), incubated with SASP for up to 72 h, were sonicated with 0.3% metaphosphoric acid and 1 mM thiourea. Reduction of Ellman's reagent DTNB [5,5′-dithiobis(2-nitrobenzoic acid) to TNB in the presence of GSH reductase, an NADPH utilizing enzyme, was monitored spectrophotometrically at 412 nm [20]. A blank containing all reagents but no sample was included to determine nonspecific reduction of DTNB. A GSH standard curve ($r^2 > 0.99$) was used to calculate the total intracellular GSH content.

Intracellular MTX Accumulation

MDA-MB-231 cells (1×10^6/well) were allowed to adhere in 12-well plates for 24 h. The culture medium was then aspirated and the cells washed twice with uptake buffer: 25 mM HEPES/Tris (pH 7.5), 140 mM N-methyl-D-glucamine, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$ and 5 mM glucose. Cells were then preincubated in uptake medium (1,000 μl) with SASP or MK-571 for 30 min prior to addition of ^{3}H-MTX (250 nM; in uptake buffer). Intracellular accumulation of ^{3}H-MTX was determined after 120 min by scintillation counting. Total protein was determined using Bradford reagent.

Colony Formation Assay

Approximately 600 cells were allowed to adhere in 35-mm Petri dishes overnight. In experiments in which SASP and DOX were combined, cells were first incubated with SASP for 72 h, and immediately following removal of SASP exposed to DOX for another 24 h. The culture medium was then changed to fresh, drug-free medium and the cells allowed to form colonies for 8–10 days. A cluster of 50 cells was defined as a colony. Colonies were stained with crystal violet and counted using software Gel Expert (Nucleotech, San Mateo, Calif., USA). To determine the concentrations of the individual drugs that suppressed cell proliferation by 50% (IC_{50}), parallel cultures were incubated with SASP and DOX alone for 72 and 24 h, respectively.

Data Analysis

Results are presented from experiments replicated at least two times. Where applicable, data are presented as means ± SEM. Statistically significant differences among treatment groups were evaluated by ANOVA followed by the Student's Newman Keul's post-hoc test for multiple comparisons.

Results

Reduction of Intracellular GSH Content by SASP

GSH exists either in the reduced (GSH) or oxidized form (GSSG). Under steady-state conditions the kinetics

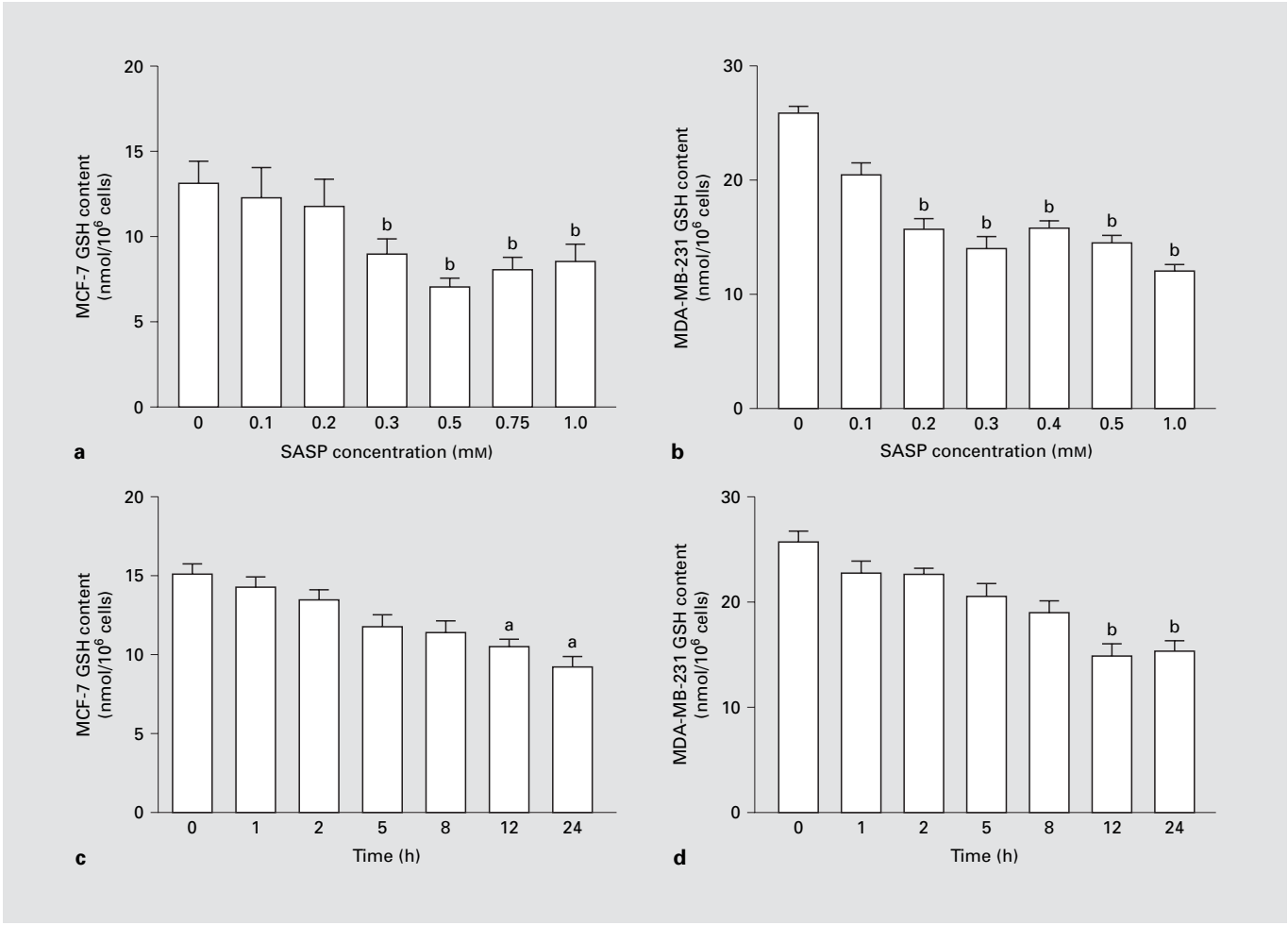

Fig. 1. Effect of SASP on GSH content in breast carcinoma cells. Data are presented as means ± SEM of triplicate samples obtained from experiments replicated at least two times. CTL represents cells cultured in the absence of SASP. **a**, **b** Concentration-dependent reduction of total GSH content by SASP determined after 24 h of incubation. **c**, **d** Time-dependent reduction of total GSH content by 0.5 and 0.3 mM SASP in MCF-7 and MDA-MB-231 cells, respectively. [a] $p < 0.05$, [b] $p < 0.01$ vs. CTL.

favor maintenance of cellular GSH in its reduced state (GSH > 99%) [20]. The effect of SASP on total intracellular GSH levels of MCF-7 and MDA-MB-231 cells was first determined using a range of SASP concentrations (0–1 mM) and a 24-hour incubation time. In both cases SASP reduced the GSH levels in a concentration-dependent manner, with maximal reductions of about 50% obtained with 0.5 and 0.3 mM SASP for MCF-7 and MDA-MB-231 cells, respectively (fig. 1a, b). In time-course experiments, MCF-7 and MDA-MB-231 cells were incubated with 0.5 and 0.3 mM SASP, respectively, for intervals of up to 72 h. SASP induced maximal reduction of total GSH (~50%) within 12–24 h (fig. 1c, d). A similar time- and concentration-dependent decrease in GSSG content of SASP-treated cultures was also observed (data not shown). The steady-state levels of GSH were substantially higher in MDA-MB-231 than in MCF-7 cells. This is consistent with a much greater expression of the x_c^--transporter observed for MDA-MB-231 cells [17].

The SASP-induced reduction in cellular GSH content was likely due to an intracellular cysteine deficiency created by inhibition by SASP of x_c^--mediated cystine uptake [16]. This was investigated using 2-mercaptoethanol (2-ME) which at ~60 μM allows cellular uptake of cystine via the leucine transporter in the form of a 2-ME-cysteine mixed disulfide [21]. MCF-7 and MDA-MB-231 cells

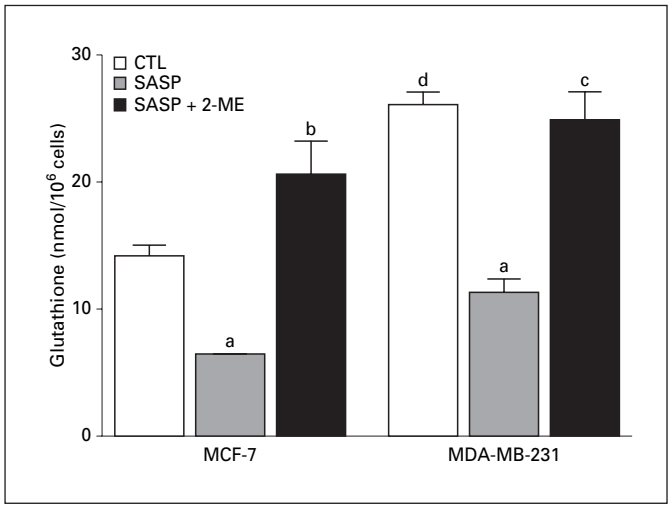

Fig. 2. Prevention by 2-ME of SASP-induced reduction of total intracellular GSH. MCF-7 and MDA-MB-231 cells were cultured with 0.5 and 0.3 mM SASP, respectively, in the absence and presence of 2-ME (60 μM) for 24 h. CTL represents untreated cells. Data are presented as means ± SEM of triplicate samples obtained from experiments replicated two times. [a] $p < 0.01$ vs. CTL; [b] $p < 0.05$, [c] $p < 0.01$ vs. SASP, [d] $p < 0.05$ vs. MCF-7 CTL.

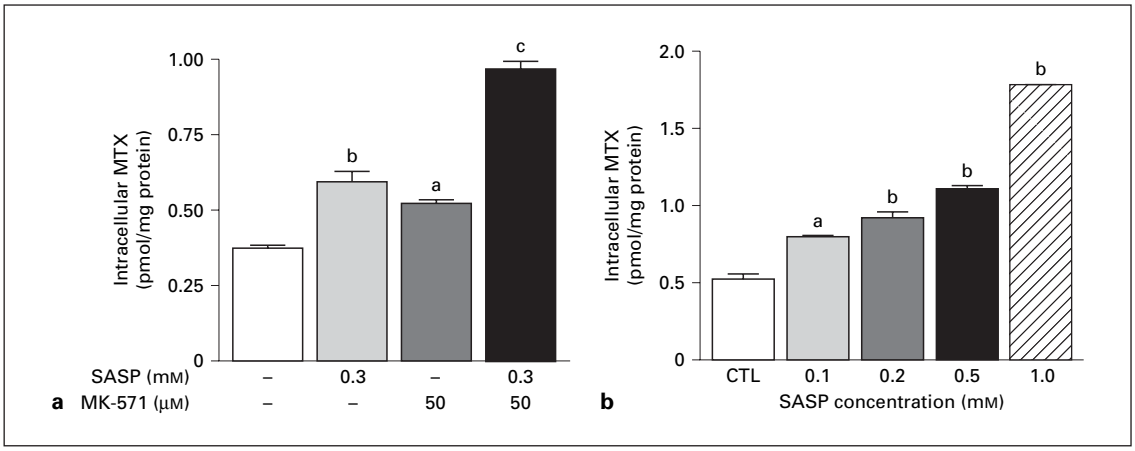

Fig. 3. Effect of SASP and MK-571 on accumulation of ^{3}H-MTX in MDA-MB-231 cells. **a** Effect of SASP, MK-571, and SASP + MK-571 on cell-associated ^{3}H-MTX in MDA-MB-231 cultures. Cells were preincubated with 0.3 mM SASP, 50 μM MK-571, or SASP + MK-571 for 30 min. Cell-associated radioactivity was determined after 120 min of incubation with ^{3}H-MTX (250 nM). CTL represents uptake of ^{3}H-MTX in the absence of SASP or MK-571. **b** Effect of increasing concentrations of SASP on amount of cell-associated ^{3}H-MTX. Cells were preincubated with SASP for 30 min followed by a 120-min incubation with ^{3}H-MTX (250 nM). [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ vs. CTL.

were incubated with 0.5 and 0.3 mM SASP, respectively, in the absence and presence of 60 μM 2-ME. As shown in figure 2, SASP alone substantially reduced the intracellular GSH content of both cell lines (>50%). However, when 2-ME was present in addition to SASP, the GSH levels were of the same order, or higher, than those in control cells. This shows that the SASP-induced reduction in intracellular GSH levels was due to inhibition of the x_c^- transporter leading to intracellular cysteine deficiency.

Effect of SASP and MK-571 on Intracellular MTX Accumulation

The efficacy of drugs can be enhanced by preventing their extrusion from target cells. The effect of SASP on retention of MTX by MDA-MB-231 cells was determined using intracellular accumulation of ^{3}H-MTX, a well-characterized MRP substrate [5], as an index. In addition, the effect of MK-571, a highly potent and specific MRP1 and MRP2 antagonist [22], was evaluated. Utilizing gene-specific primers, expression of MRP1 and MRP2 mRNA in

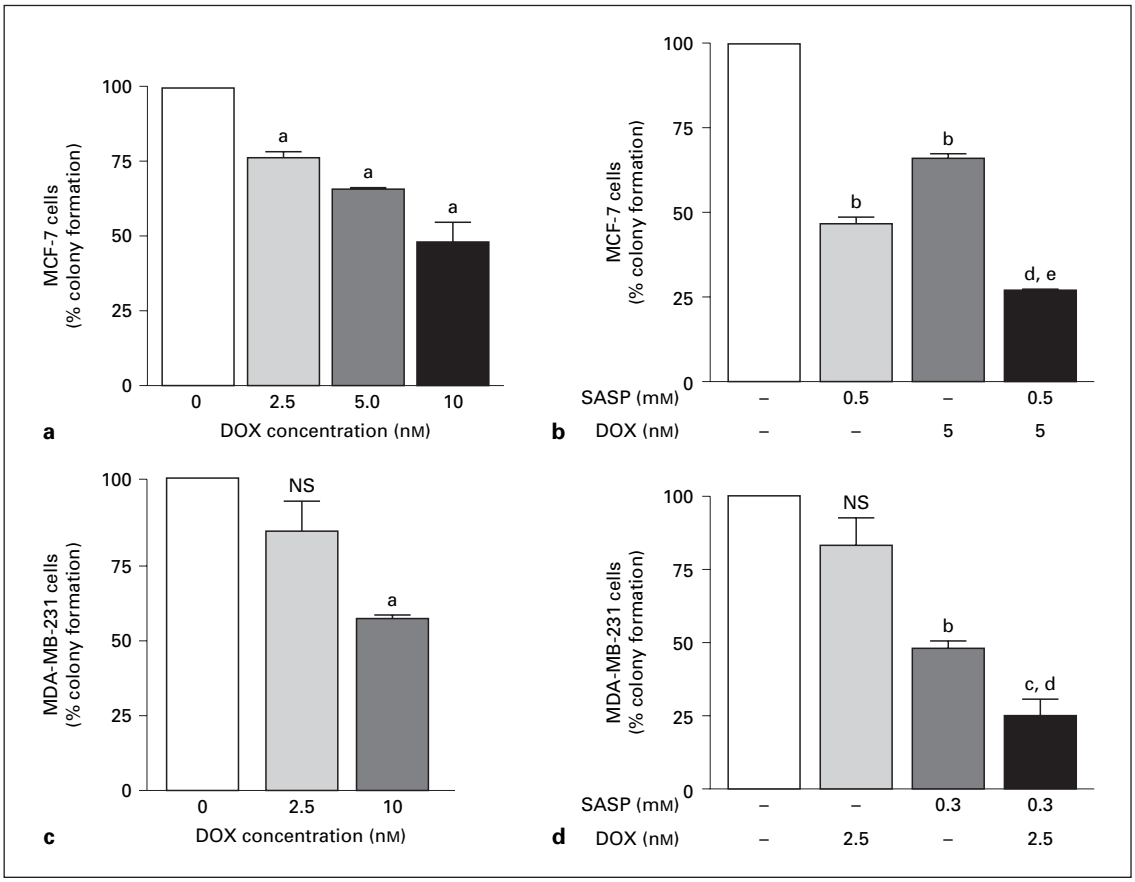

Fig. 4. Effects of SASP and DOX on colony formation in MCF-7 (a, b) and MDA-MB-231 (c, d) cultures. **a**, **c** Effect of DOX alone. Cells were incubated with increasing concentrations of DOX for 24 h. The drug was then removed and fresh medium added. Colonies >50 cells/colony that formed after 8–10 days were counted. **b**, **d** Effect of SASP and DOX. MCF-7 and MDA-MB-231 cells were preincubated with 0.5 and 0.3 mM SASP, respectively, for 72 h. The drug was then removed and the cells further cultured with DOX for 24 h. The drug was replaced with fresh medium and colonies that formed after 8–10 days in fresh medium were enumerated. For comparison, effects on colony formation of 0.5 and 0.3 mM SASP alone are shown. CTL represents cells cultured in the absence of SASP and DOX. [a] $p < 0.01$ vs. 0 nM DOX, [b] $p < 0.01$ vs. CTL, [c] $p < 0.001$ vs. 2.5 nM DOX, [d] $p < 0.01$ vs. SASP alone, [e] $p < 0.01$ vs. 5 nM DOX; NS = Nonsignificant.

MDA-MB-231 cells was confirmed by semiquantitative RT-PCR (data not shown). SASP (0.3 mM) significantly enhanced accumulation of ^{3}H-MTX ($p< 0.01$; fig. 3a). Importantly, this enhancing effect of SASP was comparable to that of MK-571 (50 μM). Also, SASP and MK-571 in combination further increased ^{3}H-MTX accumulation in an additive manner. In a companion experiment, SASP enhanced MTX accumulation in a concentration-dependent fashion (fig. 3b).

Effect of SASP and DOX on Colony Formation

DOX, a member of the anthracycline class of antibiotics, is actively used in breast cancer therapy. However, its routine application is hampered by toxic side effects and emergence of MDR following repetitive administrations [1]. Broader use of DOX would be facilitated by improving both its potency and dosage regimen, possibly by reduction of intracellular GSH levels in target cells [10]. With this in mind it was investigated whether pretreatment with SASP could enhance the inhibitory effect of DOX on the colony formation of MCF-7 and MDA-MB-231 breast carcinoma cells.

The growth-inhibitory effects of DOX (0–10 nM) on the two cell lines are shown in figure 4a and 4c. IC_{50}s of ~5 and 10 nM DOX were established for MCF-7 and MDA-MB-231 cells, respectively. In a parallel experiment,

the IC_{50}s for SASP were found to be 0.5 mM for MCF-7 and 0.3 mM for MDA-MB-231 cells (fig. 4b, d). The inhibitory effects of SASP could in large part be prevented by addition of 2-ME (60 μM) indicating that the SASP-treated cultures had become deficient in cysteine and GSH (data not shown). The effects of pretreatment of cells with SASP followed by treatment with DOX are shown in figure 4b and 4d. While in the MDA-MB-231 cultures SASP alone (0.3 mM) inhibited colony formation by 50%, further treatment with DOX (2.5 nM) inhibited colony formation by an additional 25%. The inhibition obtained with the two drugs (75%) was significantly higher ($p < 0.001$) than the null effect observed in MDA-MB-231 cultures treated with 2.5 nM DOX as a single agent (fig. 4c, d). In contrast, the degree of inhibition in MCF-7 cultures by 0.5 mM SASP alone (50%) or by 5 nM DOX alone (50%) was only modestly increased to 75% by combining the drugs (fig. 4a, b). This observation suggests that the SASP-DOX combination was superior in arresting colony formation in MDA-MB-231 than in MCF-7 cultures.

Discussion

Development of new, effective strategies for therapy of refractive cancers remains a most important task in the field of oncology, and many different approaches have been initiated, including nutrient starvation of target cells [23]. Asparaginase treatment, aimed at depletion in the body of the amino acid, asparagine, has been used for decades in combination chemotherapy of acute lymphocytic leukemia of children [24]. As previously reported by us, SASP-induced cystine starvation can be used to markedly inhibit the proliferation of MCF-7 and MDA-MB-231 human breast cancer cells [17]. In the present study we found that SASP treatment led to substantial decreases in the GSH content of these mammary cancer cells in a time- and concentration-dependent manner (fig. 1). A maximal reduction in GSH content (50–60%) occurred within 24 h at the same SASP concentrations (0.3–0.5 mM) used to markedly inhibit cell population growth [17]. The reduction in cellular GSH content stemmed from inhibition of x_c^--mediated cystine uptake, since it was completely prevented by cystine supplied to the cells via 60 μM 2-ME (fig. 2). GSH is known to act as a growth regulator, with GSH deficiency leading to growth arrest [25]. It therefore appears that the predominant mechanism by which SASP suppressed the proliferation of the breast cancer cells involved reduction of their GSH levels with a consequent decrease in their resistance to oxidative stress. Incubation with SASP exceeding 24 h did not result in further reduction of intracellular GSH levels (data not shown). This could be due to intracellular production of cysteine, via the methionine transsulfuration pathway [26], maintaining threshold levels of GSH adequate for survival but insufficient for replication.

Potential for use of SASP as an inhibitor of drug efflux was indicated by the finding that it significantly enhanced accumulation of MTX in MRP-expressing MDA-MB-231 cells (fig. 3). An increase in intracellular MTX accumulation similar to that obtained with 50 μM MK-571, a potent MRP inhibitor [22], was reached with 0.3 mM SASP, a patient-tolerated concentration [27, 28]. Although the precise mechanism underlying the action of SASP was not investigated, a plausible explanation would be provided by the strong interaction reported between SASP and MRP [29] that could interfere with extrusion of MTX. This would also explain the essentially additive effects of SASP and MK-571 on cellular MTX accumulation when these drugs were used in combination (fig. 3a). The SASP-induced reduction in cellular GSH levels during the 2.5-hour assay was marginal (fig. 1) and probably too low to interfere with the extrusion of MTX. It therefore appears that the SASP-induced increase in MTX accumulation stemmed from an SASP-MRP interaction interfering with the extrusion of MTX.

A potential role for SASP as a drug efficacy enhancer was further investigated by evaluating its use in combination with DOX, one of the more valuable antineoplastic drugs currently used in breast cancer therapy [1]. As found with the highly malignant MDA-MB-231 cell line, pretreatment with 0.3 mM SASP significantly enhanced the growth-inhibitory activity of 2.5 nM DOX (fig. 4). This was likely due to SASP-induced deficiency in GSH, since a similar drug efficacy enhancement was achieved for DOX via reduction of GSH levels in target tumor cells by DL-buthionine-(S,R)-sulfoximine, a potent inhibitor of GSH synthesis [10]. GSH has a critical role in GSH conjugation of drugs for their detoxification and elimination [7] and, as recently reported, in enabling interaction of MRP with its substrates [12]. A decrease in GSH levels would attenuate detoxification of DOX, decrease its efflux and lead to its intracellular accumulation, thereby enhancing its cytotoxic action. In addition, the reduction in GSH levels would decrease the resistance of the cells to oxidative stress generated by DOX and eventually lead to cell death. The difference in effective concentrations of SASP (0.3 mM) and DOX (2.5 nM) likely reflects differences in the mechanisms of action and target affinities of these drugs. Supporting evidence for drug efficacy-en-

hancing properties of SASP comes from a study showing similar enhancement by SASP of cisplatin cytotoxicity in human small cell lung cancer cell cultures [30]. More recently Muerköster et al. [31] showed that SASP may be used as a sensitizer of human pancreatic cancer cells by pretreating them with 0.5 mM SASP, a sensitizing ability linked to inhibition of NFκB activation. SASP is known to inhibit activation of this anti-apoptotic transcription factor, but at concentrations ≥0.5 mM [31, 32], i.e. concentrations exceeding SASP levels that inhibit cystine uptake via the x_c^- cystine transporter, i.e. 0.1–0.5 mM [16, 17], or as low as 0.05 mM in our recent studies with pancreatic cancer cell cultures [33]. In the study by Muerköster et al. [31] the effect of SASP on GSH levels in the pancreatic cells was not determined and, on the basis of the presented data, it cannot be excluded that the sensitizing property of SASP in these cells stemmed, at least in part, from a decrease in intracellular GSH levels. In the present study, the observed drug efficacy-enhancing activity of SASP is not likely due to inhibition of NFκB activation since, as previously reported, SASP at 0.3 mM did not inhibit activation of the transcription factor in MDA-MB-231 cells [17].

The SASP-DOX combination was comparatively less effective in MCF-7 cultures. Differences among cell lines in GSH-recycling abilities that could rapidly compensate for SASP-induced GSH deficiencies may possibly explain the differences in the sensitizing effects of SASP in the two cell lines. Nonetheless, the observation further underlines the relative differences in the degree of reliance of the cell lines on the supply of cystine via the x_c^- antiporter.

The IC_{50}s for SASP observed in the present study are in the mM range and as such usually not considered pharmacologically effective. However, SASP plasma levels in the range of 0.08–0.5 mM have been reported for patients treated with SASP for severe inflammatory diseases [27, 28]. Moreover, experimental in vivo studies have shown that SASP can be effective as a sensitizing agent in mice carrying human pancreatic cancer xenografts [31], or as a single agent, inhibiting growth of rat lymphoma transplants [16, 34] and, very recently, growth of primary human glioma xenografts [35]. In view of this, SASP could be useful in a clinical setting, both as an anticancer therapeutic and as a drug efficacy-enhancing agent, for therapy of mammary and other cancers depending on the x_c^- cystine transporter for growth and/or drug resistance. Although the present study utilized DOX as an example for underlining the potential utility of SASP as a sensitizing agent in combination chemotherapy, it is likely that SASP can be used in combination with a multitude of cytotoxic agents whose actions are adversely affected by intracellular GSH content and MRP activity. While oral administration of SASP would lead to its degradation by intestinal bacteria to sulfapyridine and 5-aminosalicylic acid, and hence to loss of x_c^--inhibitory activity [16], this problem could likely be reduced by oral administration of SASP in combination with antibiotics, reported to diminish bacterial cleavage of the drug [36]. The fact that SASP is a relatively nontoxic, inexpensive and FDA-approved drug should facilitate its clinical use as a new anticancer agent.

Support for our original proposal that SASP may be useful as an anticancer agent by inhibiting cellular cystine uptake via the x_c^- cystine transporter [16] has recently come from the study by Chung et al. [35] in which SASP-induced inhibition of cystine uptake led to disruption of the growth of primary human brain tumor xenografts in mice. Also, the x_c^- cystine/glutamate antiporter is recently attracting increasing amounts of interest as a mediator of GSH-based drug resistance [37].

Acknowledgments

This study was supported in part by RO1-DK53452 from the National Institutes of Health and the University of Cincinnati Cancer Center (A.R.B.), DAMD 17-00-1-0202 from the Department of Defense Breast Cancer Program (G.M.P.), and a grant from the Canadian Institutes of Health Research (P.W.G.).

References

1 Sundar S, Decatris MP, O'Byrne KJ: Management of endocrine resistant breast cancer. J Br Menopause Soc 2004;10:16–23.
2 Leonessa F, Clarke R: ATP binding cassette transporters and drug resistance in breast cancer. Endocr Relat Cancer 2003;10:43–73.
3 Filipits M, Pohl G, Rudas M, Dietze O, Lax S, Grill R, Pirker R, Zielinski CC, Hausmaninger H, Kubista E, Samonigg H, Jakesz R: Clinical role of multidrug resistance protein 1 expression in chemotherapy resistance in early-stage breast cancer: the Austrian Breast and Colorectal Cancer Study Group. J Clin Oncol 2005;23:1161–1168.
4 Leslie EM, Deeley RG, Cole SP: Multidrug resistance proteins: role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. Toxicol Appl Pharmacol 2005;204:216–237.
5 Haimeur A, Deeley RG, Cole SP: Charged amino acids in the sixth transmembrane helix of multidrug resistance protein 1 (MRP1/ABCC1) are critical determinants of transport activity. J Biol Chem 2002;277:41326–41333.
6 Schneider J, Efferth T, Rodriguez-Escudero FJ, Volm M: Intrinsic overexpression of two different mechanisms of resistance to chemotherapy (P-glycoprotein and GST-pi) in human endometrial carcinoma. Chemotherapy 1994;40:265–271.
7 Hamilton D, Batist G: Glutathione analogues in cancer treatment. Curr Oncol Rep 2004;6:116–122.
8 Zhang K, Mack P, Wong KP: Glutathione-related mechanisms in cellular resistance to anticancer drugs. Int J Oncol 1998;12:871–882.
9 Benderra Z, Trussardi A, Morjani H, Villa AM, Doglia SM, Manfait M: Regulation of cellular glutathione modulates nuclear accumulation of daunorubicin in human MCF-7 cells overexpressing multidrug resistance associated protein. Eur J Cancer 2000;36:428–434.
10 Vanhoefer U, Cao S, Minderman H, Toth K, Skenderis BS, Slovak ML, Rustum YM: D,L-buthionine-(S,R)-sulfoximine potentiates in vivo the therapeutic efficacy of doxorubicin against multidrug resistance protein-expressing tumors. Clin Cancer Res 1996;2:1961–1968.
11 Rudin CM, Yang Z, Schumaker LM, VanderWeele DJ, Newkirk K, Egorin MJ, Zuhowski EG, Cullen KJ: Inhibition of glutathione synthesis reverses Bcl-2-mediated cisplatin resistance. Cancer Res 2003;63:312–318.
12 Ren XQ, Furukawa T, Nakajima Y, Takahashi H, Aoki S, Sumizawa T, Haraguchi M, Kobayashi M, Chijiiwa K, Akiyama S: GSH inhibits trypsinization of the C-terminal half of human MRP1. J Biol Chem 2005;280:6231–6237.
13 Fojo T, Bates S: Strategies for reversing drug resistance. Oncogene 2003;22:7512–7523.
14 Bannai S: Exchange of cystine and glutamate across plasma membrane of human fibroblasts. J Biol Chem 1986;261:2256–2263.
15 Gout PW, Kang YJ, Buckley DJ, Bruchovsky N, Buckley AR: Increased cystine uptake capability associated with malignant progression of Nb2 lymphoma cells. Leukemia 1997;11:1329–1337.
16 Gout PW, Buckley AR, Simms CR, Bruchovsky N: Sulfasalazine, a potent suppressor of lymphoma growth by inhibition of the x_c^- cystine transporter: a new action for an old drug. Leukemia 2001;15:1633–1640.
17 Narang VS, Pauletti GM, Gout PW, Buckley DJ, Buckley AR: Suppression of cystine uptake by sulfasalazine inhibits proliferation of human mammary carcinoma cells. Anticancer Res 2003;23:4571–4579.
18 Sato H, Kuriyama-Matsumura K, Siow RC, Ishii T, Bannai S, Mann GE: Induction of cystine transport via system x-c and maintenance of intracellular glutathione levels in pancreatic acinar and islet cell lines. Biochim Biophys Acta 1998;1414:85–94.
19 Okuno S, Sato H, Kuriyama-Matsumura K, Tamba M, Wang H, Sohda S, Hamada H, Yoshikawa H, Kondo T, Bannai S: Role of cystine transport in intracellular glutathione level and cisplatin resistance in human ovarian cancer cell lines. Br J Cancer 2003;88:951–956.
20 Griffith OW: Biologic and pharmacologic regulation of mammalian glutathione synthesis. Free Radic Biol Med 1999;27:922–935.
21 Ishii T, Hishinuma I, Bannai S, Sugita Y: Mechanism of growth promotion of mouse lymphoma L1210 cells in vitro by feeder layer or 2-mercaptoethanol. J Cell Physiol 1981;107:283-293.
22 Gekeler V, Ise W, Sanders KH, Ulrich WR, Beck J: The leukotriene LTD4 receptor antagonist MK571 specifically modulates MRP associated multidrug resistance. Biochem Biophys Res Commun 1995;208:345–352.
23 Hori K: Antineoplastic strategy: irreversible tumor blood flow stasis induced by the combretastatin A-4 derivative AVE8062 (AC7700). Chemotherapy 2005;51:357–360.
24 Keating MJ, Holmes R, Lerner S, Ho DH: L-asparaginase and PEG asparaginase – past, present, and future. Leuk Lymphoma 1993;10(suppl):153–157.
25 Iwata S, Hori T, Sato N, Hirota K, Sasada T, Mitsui A, Hirakawa T, Yodoi J: Adult T cell leukemia (ATL)-derived factor/human thioredoxin prevents apoptosis of lymphoid cells induced by L-cystine and glutathione depletion. J Immunol 1997;158:3108–3117.
26 Stabler SP, Lindenbaum J, Savage DG, Allen RH: Elevation of serum cystathionine levels in patients with cobalamin and folate deficiency. Blood 1993;81:3404–3413.
27 Peppercorn MA, Goldman P: Distribution studies of salicylazosulfapyridine and its metabolites. Gastroenterology 1973;64:240–245.
28 Guastavino E, Litwin NH, Nahmod LH, Licastro R: Ulcerative colitis in children. Levels of salicylazosulfapyridine and sulfapyridine during treatment. Acta Gastroenterol Latinoam 1988;18:107–113.
29 Liang E, Proudfoot J, Yazdanian M: Mechanisms of transport and structure-permeability relationship of sulfasalazine and its analogs in Caco-2 cell monolayers. Pharm Res 2000;17:1168–1174.
30 Awasthi S, Sharma R, Singhal SS, Herzog NK, Chaubey M, Awasthi YC: Modulation of cisplatin cytotoxicity by sulphasalazine. Br J Cancer 1994;70:190–194.
31 Muerköster S, Arlt A, Witt M, Gehrz A, Haye S, March C, Grohmann F, Wegehenkel K, Kalthoff H, Fölsch UR, Schäfer H: Usage of the NF-κB inhibitor sulfasalazine as sensitizing agent in combined chemotherapy of pancreatic cancer. Int J Cancer 2003;104:469–476.
32 Wahl C, Liptay S, Adler G, Schmid RM: Sulfasalazine, a potent and specific inhibitor of nuclear factor kappa B. J Clin Invest 1998;101:1163–1174.
33 Lo M, Doxsee D, Wang Y, Xue H, Ling V, Wang YZ, Gout PW: Sulfasalazine: potential use of an old drug for treatment of pancreatic cancer. Proc Annu Meet Am Assoc Cancer Res, Washington, 2006, abstract No. 4705.
34 Gout PW, Simms CR, Robertson MC: In vitro studies on the lymphoma growth-inhibitory activity of sulfasalazine. A preclinical report. Anticancer Drugs 2003;14:21–29.
35 Chung WJ, Lyons SA, Nelson GM, Hamza H, Gladson CL, Gillespie GY, Sontheimer H: Inhibition of cystine uptake disrupts the growth of primary brain tumors. J Neurosci 2005;25:7101–7110.
36 Houston JB, Day J, Walker J: Azo reduction of sulphasalazine in healthy volunteers. Br J Clin Pharmacol 1982;14:395–398.
37 Huang Y, Dai Z, Barbacioru C, Sadée W: Cystine-glutamate transporter *SLC7A11* in cancer chemosensitivity and chemoresistance. Cancer Res 2005;65:7446–7454.