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Promotion of Cystine Uptake, Increase of Glutathione Biosynthesis, and Modulation of Glutathione Status by *S*-2-(3-Aminopropylamino)ethyl Phosphorothioic Acid (WR-2721) in Chinese Hamster Cells[1]

Rolf D. Issels[2] and Arno Nagele

Gesellschaft für Strahlen- und Umweltforschung (GSF), Institut für Haematologie, Abteilung für Klinische Haematologie [R. D. I.] and Klinikum Grosshadern, Medizinische Klinik III, Ludwig-Maximilians-Universität München [R. D. I., A. N.], 8000 Munich, West Germany

ABSTRACT

We recently found that exposure of cells to different aminothiols promotes cystine uptake and leads to an increase of cellular glutathione by new biosynthesis (Issels *et al.*, Biochem. Pharmacol., *37*: 881–888, 1988). Therefore, we further investigated whether the known radioprotective and chemoprotective aminothiol derivative *S*-2-(3-aminopropylamino)ethylphosphorothioic acid (WR-2721) or its dephosphorylated form (WR-1065) will lead to similar effects. In order to convert WR-2721 to the free thiol compound (WR-1065) *in vitro*, the medium also contained 20 U/ml alkaline phosphatase (AP). For uptake studies a modified McCoy's 5A medium supplemented with 0.1 mM [^{35}S]cystine was used. In Chinese hamster ovary (CHO) and Chinese hamster ovarian carcinoma (OvCa) cells, WR-2721 exposure alone did not increase the cystine uptake relative to that of control (untreated) cells, while WR-2721 + AP enhanced the uptake of cystine more than twofold in both cell lines. The increase of cystine uptake was dependent on the time of exposure (0–60 min) and the concentrations of WR-2721 (0–8 mM) + AP. Half-maximal uptake of cystine was observed at concentrations of 0.69 and 0.57 mM WR-2721 in CHO and OvCa cells, respectively. Determination of both reduced (GSH) and oxidized (GSSG) cellular glutathione levels after the exposure (0–300 min) to WR-2721 + AP in CHO cells showed a depletion of GSH to less than 10% of the pretreatment value and a 4-fold reduction of the GSH/GSSG ratio. In contrast, in OvCa cells the amount of total glutathione rather increased with no significant change of the GSH/GSSG ratio by the exposure to WR-2721 + AP. Further analysis using high-performance liquid chromatography of cell extracts revealed that the relative amount of incorporated [^{35}S]-cystine into glutathione was increased similarly in both cell lines. The data show that precursor availability and new biosynthesis of glutathione is enhanced by the exposure to WR-2721 + AP *in vitro* despite the differential modulation of the cellular glutathione status in the two cell lines. These findings may have important implications for the use of aminothiols like WR-2721 in various cells and tissues in regard of their response to chemotherapeutic agents, ionizing radiation and/or hyperthermia.

INTRODUCTION

WR-2721[3] is a sulfhydryl compound that, in the animal model, may protect selectively normal tissues against the cytotoxicity of radiation and alkylating agent chemotherapy (1). Subsequently, results of clinical trials showed that WR-2721 provides significant protection against cyclophosphamide-induced granulocytopenia (2–4), cisplatinum-induced nephrotoxicity (5, 6), and against radiation-induced gastrointestinal damage in experimental animal studies (7). Damage produced by alkylating agents is mediated through the covalent bonding of alkyl groups to cellular molecules (8). It is known that the presence of oxygen or other electrophilic compounds increases the cytotoxic effects of such alkylating agents via radical formation (9) and also increases the fixation of damage produced by radiation (10). Currently, the mechanism of chemo- and/or radioprotection by aminothiols like WR-2721 at the cellular and molecular levels is not completely understood. It is thought to result from scavenging free radicals either directly by the SH-compounds themselves (11) or indirectly by GSH released from protein-bound mixed disulfide (12).

The modulation of the glutathione status of cells by exogenous aminothiols (13–15) and its substantial effects upon cellular sensitivity to alkylating agents (16), ionizing radiation (17), and hyperthermia (18) have been reported by several authors. We could demonstrate that exposure of CHO cells to aminothiols like cysteamine and *N*-acetylcysteine leads to a promoted uptake of cystine and its rapid utilization for GSH biosynthesis (19). The objective of this study was to investigate whether WR-2721 or its active dephosphorylated metabolite (WR-1065) influences the cellular glutathione status by similar mechanisms.

MATERIALS AND METHODS

Chemicals

L-[^{35}S]Cystine (specific activity, 50–120 mCi/mmol) was obtained from Amersham Buchler (Braunschweig, FRG). WR-2721 was a gift of Dr. Leo Gerweck of the Department of Radiation Medicine, Massachusetts General Hospital, Boston, MA. Trypsin (0.25%), newborn and fetal calf serum, McCoy's 5A medium and McCoy's 5A medium without cystine, glutathione and methionine were purchased from GIBCO BRL (Eggenstein, FRG). Alkaline phosphatase, type VII-NL from bovine intestine was obtained from Sigma Chemical Co. (Deisenhofen, FRG). Other enzymes and NADPH were from Boehringer Mannheim (Mannheim, FRG). Reagents for liquid scintillation counting were obtained from New England Nuclear (NEN, Dreieich, FRG) and from Roth (Karlsruhe, FRG). All other chemicals were purchased from E. Merck (Darmstadt, FRG).

Cell Culture

CHO cells were routinely grown and subcultured in McCoy's 5A medium supplemented with 10% newborn and 5% fetal calf serum at 37°C and in an atmosphere with 5% CO_2. Colony forming efficiency was 80 to 90%, and the population doubling time was 12 to 15 h. OvCa cells were kindly supplied by Dr. Dennis Leeper of the Department of Radiation Therapy and Nuclear Medicine, Thomas Jefferson University Hospital, Philadelphia, PA. OvCa cells were grown in McCoy's 5A medium supplemented with 10% fetal calf serum. Colony forming efficiency of these cells was 70–80%, and the population doubling time was 11 to 13 h. For uptake studies, CHO or OvCa cells were trypsinized and plated in 35-mm plastic Petri dishes (0.5×10^6 cells) containing 2 ml complete medium per dish. At the time of the experiment (≈24 h after plating) the cell number was $1.5–2 \times 10^6$ cells per dish and the cells had reached about 50% confluency.

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[2] To whom requests should be addressed: Medizinische Klinik III, Klinikum Grosshadern, Ludwig-Maximilians-Universität, Marchioninistr. 15, D-8000 Munich 70, West Germany.

[3] The abbreviations used are: WR-2721, *S*-2-(3-aminopropylamino)-ethyl phosphorothioic acid; WR-1065, 2-(3-aminopropylamino)-ethanethiol; AP, alkaline phosphatase; CHO, Chinese hamster ovary; GSH, glutathione reduced; GSSG, glutathione oxidized; HPLC, high-pressure liquid chromatography; OvCa, Chinese hamster ovary carcinoma; PBS, phosphate buffered saline.

[^{35}S]Cystine Uptake

Incubations were performed at 37°C in a temperature-controlled water bath, the bottom of the Petri dishes being submersed in the water. The growth medium was then removed, the layer of cells rinsed with PBS, pH 7.4 (Dulbecco's solution with 0.1% glucose) and 0.5 ml of the uptake medium was added for different lengths of time as indicated. The uptake medium was the same as McCoy's 5A medium, except that glutathione and methionine were omitted and cyst(e)ine replaced by 0.1 mM [^{35}S]cystine.

In all experiments using WR-2721, the thiol compound was dissolved in N_2-gassed PBS and added in small volumes (5–50 μl) to the medium. In order to convert WR-2721 to the dephosphorylated form (WR-1065) *in vitro*, the medium also contained 20 U/ml alkaline phosphatase. Time course experiments of cystine uptake were initiated by the addition of WR-2721 to the uptake medium (time = 0 min). After continuous drug exposure (time = 0–60 min) in the presence of [^{35}S]cystine, the cell layer was rinsed with ice-cold PBS and then cells were trypsinized (0.25% trypsin) for 2 min at room temperature. The cell suspension (1 ml) was transferred into Eppendorf cups, precipitated with 1 N perchloric acid and kept on ice. The acid-insoluble pellet was solubilized for scintillation counting by a 18-h incubation period with 5 volumes of Protosol at room temperature. The acid-soluble and acid-insoluble radioactivity was counted in a xylene-based cocktail. Quench correction was achieved by the external standard channel ratio technique. If not otherwise indicated, the data for [^{35}S]cystine uptake are expressed as total uptake (acid-soluble and acid-insoluble fractions). Initial uptake rates are based on incubations of 4-min duration. The cell number was determined for each experiment from five replicate dishes by counting the trypsinized cell suspensions in a Coulter Counter. Protein content of cells was assayed by the Lowry method.

GSH Determination

Experiments designed to measure total intracellular GSH were initiated by inoculating $\simeq 10^6$ cells/T_{25} flask in two replicates containing 4.5 ml of fresh medium (total volume). After 24 h, cells were exposed to WR-2721 ± AP for different lengths of time (0–300 min) as indicated, the medium was removed, the cells were washed twice with PBS and then trypsinized (0.25% for 2 min). At the time of GSH determination, more than 90% of the cells were metabolically viable as assessed by trypan blue exclusion. After dilution in cold PBS (5 ml), the cells were counted and centrifuged at 4°C.

Total GSH. Total cellular glutathione (GSH + GSSG) was quantified following the method described by Tietze (20). The pellets were resuspended in 0.3 ml of 0.04 M EDTA and 0.6 ml of 7.5% trichloroacetic acid and centrifuged at 4°C. The cold supernatant was removed and total glutathione was determined by the glutathione reductase procedure. The total GSH content of untreated CHO cells and OvCa cells was (N = 8) 24.4 ± 4.9 (SD) nmol/mg protein and (N = 6) 15.5 + 3.6 (SD) nmol/mg protein, respectively.

GSH and GSSG. The reduced (GSH) and oxidized (GSSG) form of glutathione was determined by using the HPLC method as described by Reed (21). For this procedure, the cell pellet was extracted with 0.5 ml 1 N perchloric acid and γ-glutamylglutamate was added as an internal standard. The extract was incubated for 120 min with iodoacetic acid at pH 8.5 to form *S*-carboxymethyl derivatives of free thiol groups. Subsequently, the amino groups were derivatized with 1-fluoro-2,4-dinitrobenzene (Sanger's reagent). Aliquots (50 μl) of the reaction mixture were injected onto a μBondapak amine column (4 x 250 mm, Waters, Eschborn, West-Germany) and eluted with a sodium acetate gradient (flow rate: 1.5 ml/min) in a water-methanol-acetic acid solvent at pH 4.5. The dinitrophenyl derivatives were detected at 365 nm. GSH and GSSG derivatives were quantified in relation to the internal standard. The GSH and GSSG content of untreated CHO cells (N = 15) was 23.8 ± 4.4 (SD) nmol/mg protein and 0.31 ± 0.15 (SD) nmol/mg protein, respectively. For untreated OvCa cells (N = 8), the respective values were (GSH) 18.2 ± 3.1 and (GSSG) 0.19 ± 0.06 (SD) nmol/mg protein.

Measurement of [^{35}S]Cystine Incorporated into Glutathione

Recovery of [^{35}S]cystine incorporated into GSH after exposure of cells to WR-2721 ± AP for different lengths of time (0–200 min) was determined by the same HPLC method as described above. The eluted fractions were collected in 0.5-ml aliquots and counted in the scintillation counter. The specific radioactivity was obtained by relating the radioactivity found in the GSH- and GSSG-containing fractions to the total glutathione quantitated by HPLC.

RESULTS

Time Course of Cystine Uptake Induced by WR-2721. The influence of WR-2721 upon the uptake of [^{35}S]cystine from the McCoy's 5A medium by CHO and OvCa cells is shown in Fig. 1. For both cell lines, WR-2721 exposure alone (0–60 min) did not change the cystine uptake relative to that of control (untreated) cells. However, when AP was added to the medium (20 U/ml) prior to the addition of WR-2721, a substantial increase of [^{35}S]cystine uptake could be observed in CHO cells (Fig. 1*A*) and OvCa cells (Fig. 1*B*). For example, at 30 or 60 min after exposure to WR-2721 (0.4 mM) + AP, the uptake by both cell lines increased more than twofold compared with untreated cells. In order to approximate the initial uptake rates of cystine only incubation periods of 4 min were used in all further experiments (see below).

Dependence of Cystine Uptake Upon Concentrations of WR-2721. For untreated control cells the initial uptake rates of cystine from the medium were 0.54 ± 0.06 (SE) nmol half-cystine/min/mg (CHO) and 0.59 ± 0.04 nmol half-cystine/min/mg (OvCa), respectively (N = 3). About one-third of the total radioactivity was found in the acid-insoluble pool of both cell extracts. It should be emphasized that this latter rate, which mainly reflects incorporation of [^{35}S]cystine into protein or unspecific binding to the pellet, did not change with either WR-2721 or WR-2721 + AP treatment. Almost all of the increased radioactivity taken up after exposure to WR-2721 + AP was found in the acid soluble pool of the cell extracts. The cystine uptake was promoted in a concentration dependent manner (0.1–8 mM) and was similar for both types of cells as shown in Fig. 2. The relation between WR-2721 concentrations and initial rates of cystine uptake in repeated experiments (N = 3) allowed us to determine half-saturation constants (K_u) and maximal velocities (V_u) of the cystine transport promoted by WR-2721 + AP. By plotting the data in a Lineweaver-Burk diagram, the values obtained for CHO cells [K_u = 0.69 ± 0.14 (SE) mM and V_u = 2.27 ± 0.18 (SE) nmol half-cystine/min/mg protein] and for OvCa cells [K_u = 0.57 ± 0.05 (SE) mM and V_u = 1.95 ± 0.05 (SE) nmol half-cystine/min/mg protein] are very

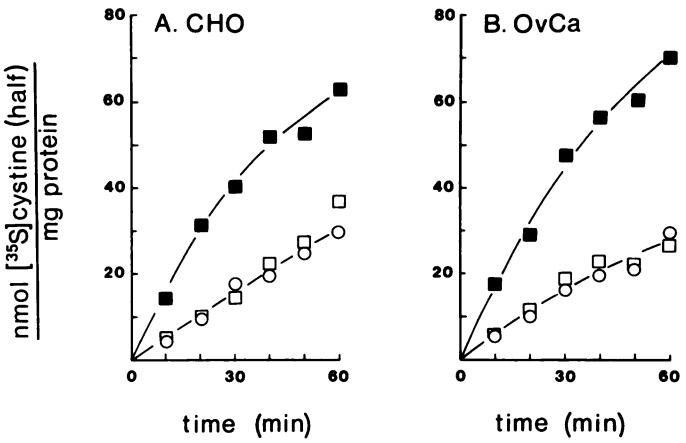

Fig. 1. Time course (0–60 min) of the uptake of 0.1 mM [^{35}S]cystine by CHO (*A*) and OvCa (*B*) cells in McCoy's 5A medium. ■, WR-2721 (0.4 mM) + AP (20 U/ml); □, WR-2721 (0.4 mM); ○, no additional thiol (control). Data are shown from a single typical experiment; qualitatively similar results have been obtained in two replicate experiments.

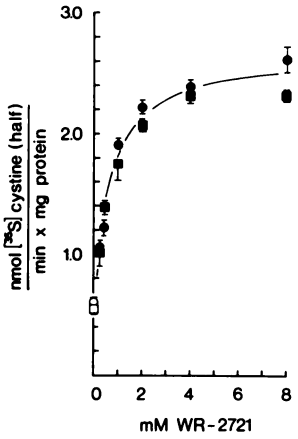

Fig. 2. Drug concentration dependence (0–8 mM) of the uptake rate of 0.1 mM [^{35}S]cystine by CHO (●) and OvCa (■) promoted by WR-2721 + AP (20 U/ml). ○ and □, uptake rate of untreated CHO and OvCa cells, respectively. Mean ± SD (N = 3).

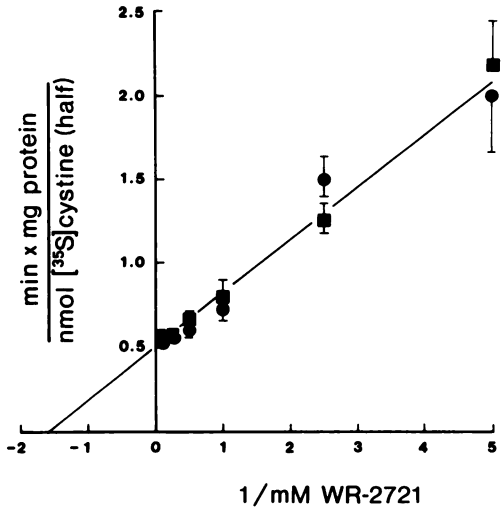

Fig. 3. Lineweaver-Burk plots of the specific uptake of [^{35}S]cystine promoted by WR-2721 + AP (20 U/ml) in CHO (●) and OvCa (■) cells. The data taken from Fig. 2 are corrected for the control uptake of untreated CHO or OvCa cells.

similar (see Fig. 3). It should be noted at this point that the clonogenic surviving fraction of CHO or OvCa cells after exposure to WR-2721 (0–8 mM) ± AP was not affected and was in the range of the plating efficiency of both cell lines, respectively (see also "Materials and Methods").

Change of the Cellular Status of Glutathione during WR-2721 Exposure. The influence of WR-2721 ± AP upon cellular GSH content during exposure of CHO and OvCa cells (0–300 min) is shown in Fig. 4. Total cellular glutathione (GSH + GSSG) of CHO cells was quantitated by the glutathione reductase procedure (20). Total glutathione content decreased to less than 10% of the control value after 150 min of WR-2721 (0.4 mM) exposure but only when AP (20 U/ml) was added to the medium prior to the addition of WR-2721 (see Fig. 4*A*). In contrast, no such glutathione depletion could be observed in OvCa cells for the same duration of WR-2721 (0.4 mM) + AP exposure. Initially, the glutathione content of these cells doubled when compared to control levels and kept constant at this level during further drug treatment (see Fig. 4*B*).

In addition to the observed depletion of the total glutathione content by WR-2721 + AP in CHO cells, we further investigated whether the redox status of glutathione was also influenced within these cells in comparison to OvCa cells. By determination of the reduced (GSH) and oxidized (GSSG) form of glutathione using the HPLC method described by Reed (21), a 10-fold reduction of GSH content was measured in CHO cells after 200-min exposure with 0.4 mM WR-2721 + AP (Table 1). However, compared to this marked depletion of GSH, the amount of GSSG/mg protein in these cells was only about twofold reduced. Therefore, the ratio of GSH/GSSG of drug-treated CHO cells was 5-fold reduced due to the exposure with WR-2721 + AP. In OvCa cells, both the reduced (GSH) and oxidized (GSSG) levels of glutathione increased. No significant change of the GSH/GSSG ratio as compared to that obtained for untreated control cells could be observed after WR-2721 + AP exposure. For comparison, exposure of both CHO and OvCa cells with the aminothiol cysteamine (0.4 mM) under the same experimental conditions increased the cellular GSH and GSSG content about threefold within 200 min.

Recovery of [^{35}S]Cystine Incorporated into Glutathione during WR-2721 Exposure. We further determined the relative amount of ^{35}S-labeled cystine incorporated into glutathione during exposure (0–200 min) of CHO and OvCa cells to WR-2721 ± AP. For this analysis, the measured amount of ^{35}S radioactivity in the glutathione (GSH + GSSG)-containing HPLC fractions of the acid-soluble cell extracts was related to the entire glutathione (GSH + GSSG) content of the same samples as quantitated by peak integration at 365 nm. As shown in Fig. 5, during WR-2721 + AP exposure a quite similar increase of [^{35}S]cystine incorporation into glutathione could be observed in both cell lines. For example, 30% of the cellular amount of glutathione was found to be ^{35}S radiolabeled after the initial drug exposure (WR-2721 + AP, 50 min) and this value increased by further drug treatment (WR-2721 + AP, 200 min) to 57 and 45% for OvCa and CHO cells, respectively. Exposure of both cell lines with WR-2721 alone showed neither an influence upon glutathione content nor a change of ^{35}S-labeled cystine incorporation into GSH (data not shown).

DISCUSSION

The agent WR-2721 is thought to be rapidly cleaved *in vivo* to its dephosphorylated metabolite WR-1065, a free thiol compound implicated as being ultimately responsible for the radio- and chemoprotective effects (22, 23). Different levels of alkaline phosphatase activity at the cell surface of mammalian tissues may be of critical importance for the enzymatic conversion of WR-2721 to WR-1065 under physiological conditions (24). In cell studies, a substantial loss of alkaline phosphatase activity after trypsinization of monolayer cells maintained in exponential growth has been observed (25). Therefore, for studying the mechanism of action of WR-2721 *in vitro*, the addition of alkaline phosphatase, along with WR-2721, to the culture me-

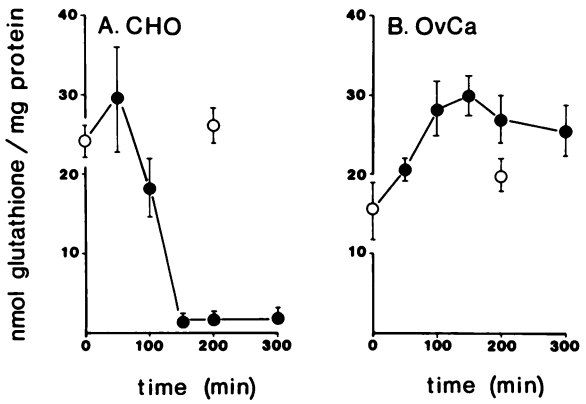

Fig. 4. Total glutathione (GSH + GSSG) as a function of time (0–300 min) for CHO (*A*) and OvCa (*B*) cells exposed to WR-2721. ●, 0.4 mM WR-2721 + AP (20 U/ml); ○, 0.4 mM WR-2721. Mean ± SD (N = 3).

Table 1 *HPLC measurement of the reduced (GSH) and oxidized (GSSG) form of intracellular glutathione after 200-min exposure*

	CHO			OvCa		
	GSH (nmol/mg)	GSSG (nmol/mg)	GSH/GSSG	GSH (nmol/mg)	GSSG (nmol/mg)	GSH/GSSG
Control	23.8 ± 4.4[a]	0.31 ± 0.15	77	18.2 ± 3.1	0.19 ± 0.06	96
0.4 mM Cysteamine	70.6 ± 24.0	0.74 ± 0.41	95	49.7 ± 4.1	0.58 ± 0.15	86
0.4 mM WR-2721	32.3 ± 3.7	0.44 ± 0.12	73	24.6 ± 5.1	0.24 ± 0.05	103
0.4 mM WR-2721 + AP	2.2 ± 1.4	0.12 ± 0.06	18[b]	22.8 ± 5.5	0.28 ± 0.12	81

[a] Mean ± SD, $N = 3$ or more independent experiments.
[b] This ratio differs significantly from the control ratio. A two-tailed t test of the logarithms of individual ratios gave $P < 0.05$.

dium as used in our experiments has been previously shown to overcome this limitation (26). It is well known that organic free thiols like WR-1065 are unstable under aerobic conditions in the medium because they react with oxygen, via a mechanism that generates superoxide and hydrogen peroxide (27). Moreover, activated oxygen species generated during the autoxidation of thiols are involved in the cytotoxic effects of such compounds (28, 29). Recently, the various factors influencing the oxidation of WR-1065 in tissue culture media have been extensively described by Tashildar *et al.* (30). Compared to their results, we used only low concentrations of WR-2721 and/or short incubation periods of cells with WR-2721 in order to avoid high rates of thiol autoxidation in the cell culture medium. This might explain why the clonogenic survival of CHO or OvCa cells after the exposure to WR-2721 + AP was not affected in our experiments.

The present study strongly suggests that WR-2721 after being dephosphorylated to its SH-metabolite (WR-1065) acts as a delivery system for cystine sulfur from the medium into the cells. In regard to the dependence of the observed increase of cystine uptake upon drug concentration or time of exposure, no significant difference could be detected between CHO and OvCa cells. The data shown in Fig. 3 fit well into a Lineweaver-Burk diagram suggesting a similar saturation type of uptake kinetics in the presence of WR-2721 + AP.

The potential of WR-2721 to promote cystine uptake is comparable with that of other aminothiols, and the mechanism seems to be a similar two-step process, as described in our previous report for cysteamine and *N*-acetylcysteine (19): firstly, the formation of the cysteine mixed disulfide with the added aminothiol (Cys-SS-WR-1065) and the simultaneous generation of equimolar amounts of cysteine in the medium; secondly, the uptake of cysteine and/or the mixed disulfide across the cell membrane. In accordance with the ability to form mixed disulfides, WR-2721 after being dephosphorylated has been shown to bind preferentially to cystine-rich proteins via mixed disulfide formation (31). In more detail, Blackburn and Peterson have demonstrated the nonenzymatic formation of the mixed disulfide of WR-1065 and cysteine *in vitro* (32).

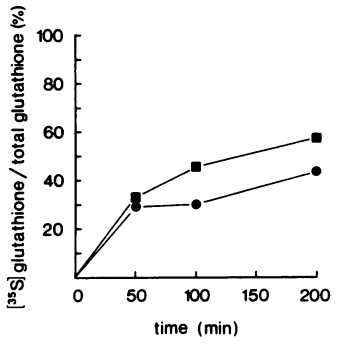

Fig. 5. Relative amount (%) of [^{35}S]cystine labeled glutathione in total glutathione (GSH + GSSG) as a function of time (0–200 min) for CHO (●) and OvCa (■) cells exposed to WR-2721 (0.4 mM) + AP (20 U/ml). Data are shown from a typical experiment ($N = 2$).

The promotion of cystine uptake by WR-2721 was completely dependent upon the addition of alkaline phosphatase to the medium as shown by our results in Figs. 1 and 2. This also fits in the mechanism of cysteine-mixed disulfide formation between WR-2721 and cystine in the medium because the reaction requires a free SH-group. Since drug hydrophilicity seems to be a major factor for the uptake of WR-2721 in cells and tissues (33), less hydrophilic thiol derivatives like the cysteine-mixed disulfide form may also more readily cross cell membranes.

Several previous studies have shown that exposure to aminothiols or cyst(e)ine derivatives leads to a strong modulation of the cellular GSH content (13–15) depending upon the potential of cells to utilize the added aminothiols per se or the transported cyst(e)ine as a precursor for GSH biosynthesis (19). With regard to the further utilization of cystine sulfur taken up from CHO and OvCa cells in the presence of WR-2721 + AP, our results clearly demonstrate that the cysteine moiety of newly synthesized GSH is derived from the extracellular pool of cystine. Moreover, GSH biosynthesis was increased by WR-2721 + AP in both CHO and OvCa cells and the increase of the ratio of ^{35}S-labeled glutathione to total glutathione was similar during the time of drug exposure. Therefore, the intrinsic γ-glutamylcysteine synthetase activity of cells, which is the first enzymatic step of GSH biosynthesis, seems not to be impaired by WR-2721 + AP exposure *in vitro*, although inactivation of the purified enzyme has been demonstrated by the disulfide cystamine (34) and more recently by the disulfide analogue of WR-2721 (35). In regard to the cellular status of glutathione, the most striking difference between the cell lines observed after WR-2721 + AP exposure is the strong depletion of GSH content in CHO cells. However, from the analysis of our data, availability of cysteine as a precursor and GSH biosynthesis itself are promoted by WR-2721 + AP in CHO cells. In addition, we found no significant effect on either total protein sulfhydryl levels or the amount of protein-mixed disulfides with glutathione (protein-S-S-G) in both cell lines exposed to WR-2721 + AP (data not shown). Therefore, the observed glutathione depletion by WR-2721 in these cells must be explained by other mechanisms than a decrease in GSH biosynthesis or an increase of protein-bound GSH. In accordance with our results, depletion of hepatic glutathione content by WR-2721 *in vivo*, as previously reported by Schor (36) was found by the same author more recently to be not simply the result of decreased precursor availability (35). Different from other aminothiols (*e.g.*, cysteamine, *N*-acetylcysteine), WR-2721 can act as a substrate for polyamine oxidase because of its spermidine-like polyamine structure (37). In addition, depletion of cellular glutathione has been shown *in vitro* after the exposure to exogenous polyamines (*e.g.*, spermine, spermidine) and this effect was explained by glutathione adduct formation with reactive intermediates formed during the enzymatic degradation of such polyamines by polyamine oxidase (38). Similar reactive intermediates of WR-2721 catabolism by polyamine oxidase might be involved in the mechanism of the observed GSH

depletion by WR-2721. Whether the mechanism of glutathione modulation by WR-2721 in different cell lines is based upon a cell type specific metabolism is the subject of currently ongoing studies in our laboratory.

In conclusion, our data clearly demonstrate the potential of WR-2721 to modulate cellular glutathione status and indicate the need for prudence in the clinical adjunctive use of WR-2721 as a free radical scavenger. Chemotherapeutic agents which themselves deplete glutathione (16) or generate oxygen radicals (8) could lead to synergistic toxicity rather than to chemoprotective effects if combined with WR-2721 as shown for the drug 6-hydroxydopamine (35). Also, WR-2721 could inhibit DNA single-strand break repair of irradiated cells, because such inhibition was found to correlate with a cellular loss of glutathione and a reduction of the GSH/GSSG ratio (39), as observed in our WR-2721 + AP treated CHO cells (see Table 1). Finally, because of the influence of glutathione on cellular response against hyperthermic stress (18), thermosensitizing effects of WR-2721 *in vitro*, as shown by our preliminary results (40), might also have implications for the clinical use of aminothiols like WR-2721 combined with hyperthermia.

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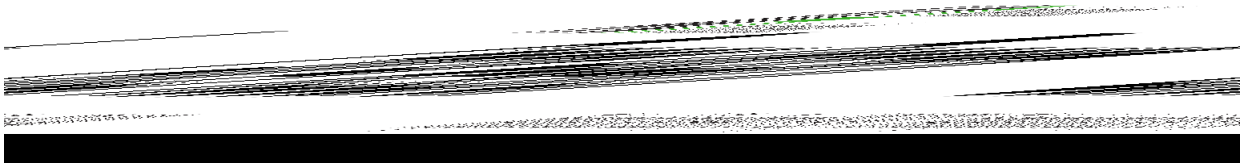

Promotion of Cystine Uptake, Increase of Glutathione Biosynthesis, and Modulation of Glutathione Status by *S*-2-(3-Aminopropylamino)ethyl Phosphorothioic Acid (WR-2721) in Chinese Hamster Cells

Rolf D. Issels and Arno Nagele

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