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Mechanisms of glutathione disulfide efflux from erythrocytes

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ABSTRACT

Glutathione (GSH) plays numerous critical protective roles in the erythrocyte and GSH turnover is likely an important factor in regulating susceptibility to oxidative stress and toxins. Efflux of glutathione disulfide (GSSG) from erythrocytes is an important component in the regulation of GSH levels; however, little is known of the mechanisms involved. We hypothesize that multidrug resistance associated protein 1 (MRP1) is responsible, in part, for GSSG transport from erythrocytes. To test this, we determined the levels of MRP1 protein in erythrocyte membranes from healthy adults and compared them with intracellular levels of GSH. MRP1 levels varied substantially from person to person and were inversely correlated with levels of GSH ($r = -0.39$, $P < 0.05$). In contrast, activity levels of glutamyl cysteine ligase, the rate limiting GSH biosynthetic enzyme, were unrelated to GSH levels. To directly determine the role of MRP1 in GSSG transport, *in vitro* studies were conducted examining the effects of MRP1 inhibitors MK571 and verapamil on GSSG efflux. Both compounds resulted in significant but not complete inhibition (20–53%) of GSSG efflux from cells. Overall, these findings support a role for MPR1 in the regulation of erythrocyte GSH levels through the transport and elimination of GSSG from cells.

1. Introduction

Glutathione (GSH) is the most abundant thiol and antioxidant present in animal tissues (1–15 mM) [1] and, together with its associated biosynthetic, redox and detoxification pathways represents the key defense system against oxidative stress and free radical damage in the cell [2–4]. In addition, GSH plays numerous protective roles including the detoxification of a variety of endogenous and exogenous compounds such as xenobiotics and carcinogens, preservation of protein structure and function, regulation of protein synthesis and degradation and modulation of immune function [5–7].

In erythrocytes, glutathione functions as the most abundant redox buffer, regulating the activity of redox-sensitive enzymes, limiting lipid peroxidation of the plasma membrane *via* glutathione peroxidases, and preventing oxidative denaturation of hemoglobin [8,9]. Erythrocyte glutathione also plays a key role in detoxification of endogenous and exogenous toxins in blood *via* the glutathione S-transferase family of enzymes.

Glutathione in erythrocytes from healthy individuals is normally found in high concentrations (~2 mM), but these levels can vary substantially from person to person [10,11]. Low GSH levels may be indicative of many diseases and disorders, including Diabetes, HIV infection, alcoholic liver disease and aging, and possibly play an instrumental role in their pathogenesis [12–14]. Low erythrocyte GSH levels are associated with increased oxidative stress [15] and consequences of severe erythrocyte GSH deficiency include hemolytic anemia, jaundice and central nervous disorders [16]. In conditions associated with GSH depletion, the restoration of normal erythrocyte GSH levels has been shown to have positive therapeutic effects [17,18].

In defending against oxidative stress, GSH is oxidized to glutathione disulfide (GSSG), which can in turn be reverted back to GSH by glutathione reductase. Alternatively, GSSG can be cleared from erythrocytes in an ATP-dependent manner [19–21]. Therefore, in addition to GSSG reduction by glutathione reductase, export of GSSG represents an important mechanism for preventing accumulation of GSSG in the erythrocyte and protecting against oxidative stress. Further, GSSG efflux is likely an important regulator of glutathione turnover in erythrocytes [22] since GSH itself is not transported from erythrocytes as it is in other cell types.

Abbreviations: MRP1, multidrug resistance associated protein 1; GSH, glutathione; GSSG, glutathione disulfide; GSX, glutathione S-conjugate; GCL, glutamine cysteine ligase; BSO, buthionine sulfoxamine; MK571, (E)-3-[[[3-[2-(7-Chloro-2-quinolinyl)ethenyl]phenyl]-[[3-dimethylamino)-3-oxopropyl]thio]methyl]-thio]-propanoic acid; GSSP, protein bound glutathione; γGC, γ-glutamyl cysteine.

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While the mechanism of GSSG efflux from erythrocytes is not clear, both high and low affinity components ($K_{m\ GSSG}$ = 0.023–0.1 mM; $K_{m\ GSSG}$ = 5.0–7.3 mM) have been reported, similar to that observed for GSH S-conjugate (GSX) transport [23–25]. In addition, the transport of both GSSG and GSX are inhibited competitively by GSSG ($K_{i\ GSSG}$ = 0.088 mM) and GSX ($K_{i\ GSX}$ = 0.003 mM), respectively [23,25]. Thus, we hypothesize that multidrug resistance associated protein 1 (MRP1), which plays a major role in the transport of GSX is also responsible for GSSG efflux in the erythrocyte. Indeed, this ABC transporter has been identified as a GSSG pump in both HeLa and HL60 cells [26]. To test our hypothesis, we utilized inhibitors of MRP-1 (MK571 and Verapamil) and glutamate cysteine ligase, GCL, (buthionine sulfoximine, BSO) in incubation studies with erythrocytes and determined their effects on glutathione export and also examined relationships between erythrocyte membrane MRP-1 levels and intracellular GSH levels *in vivo*.

2. Materials and methods

2.1. Materials

Benzenacetonitrile (Verapamil) and (E)-3-[[[3-[2-(7-Chloro-2-quinolinyl)ethenyl]phenyl]-[[3-dimethylamino)-3-oxopropyl]thio]methyl]thio]-propanoic acid (MK571) were obtained from Alexis Biochemicals (Axxora, LLC, San Diego, CA). MRP1-A23 polyclonal antibody was obtained from Alexis Biochemicals (Axxora, LLC, San Diego, CA). Goat anti-rabbit antibody was obtained from Santa Cruz (Santa Cruz, CA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Study subjects

Blood was obtained from healthy volunteers by venipuncture and collected into tubes containing EDTA as an anticoagulant. All subjects were employees of the Institute for Cancer Prevention and had signed a consent form before participating. All protocols and consent procedures were approved by the Institute for Cancer Prevention institutional Review Board for the protection of human subjects. Subjects were 22 healthy adult non-smokers, 5 males and 17 females, with a mean ± SD age of 36.8 ± 11.4 years. Prior to obtaining blood, subjects underwent an 8 h (overnight) fast.

2.3. Analysis of erythrocyte membrane MRP-1 levels

Erythrocytes were isolated by centrifugation (2100 × *g*) of whole blood for 15 min at 4 °C. Hemolysates (1:90, v/v) were prepared fresh in 2 mM HEPES/0.1 mM EDTA (pH 7.5) and centrifuged (28,000 × *g*) in polypropylene conical tubes for 20 min. Erythrocyte membrane-containing pellets were washed three times in HEPES. Washed pellets were resuspended in 10 mM Tris–HCl (pH 7.4). Protein concentration was determined using the Bio-Rad method (Bio-Rad Laboratories, Hercules, CA) and samples were frozen at −80 °C until use.

Erythrocyte membranes were suspended in 10 mM Tris–HCl and samples containing 25 μg of protein were loaded onto 7% SDS-Polyacrilimide gels and electrophoresis was conducted at 120 V. Proteins were transferred onto nitro-cellulose membranes using a semi-dry transfer blot system (Bio-Rad) at 20 V for 30 min. Membranes were incubated (4 °C) with the polyclonal antibody, MRP1-A23 (1:750), overnight, followed by incubation with goat anti-rabbit antibody (1:10,000) for 60 min. Visualization was achieved by enhanced chemiluminescence (Amersham Biosciences, GE Healthcare, Piscataway, NJ). Membranes were stained with Ponceau Solution to detect equal loading of lanes. Density of the bands was analyzed using ImageJ software. Mean optical density was normalized to a control sample (25 μg of erythrocyte hemolysate) run on each gel.

2.4. Erythrocyte incubations

Erythrocytes obtained from randomly selected study subjects described above were washed three times in 154 mM NaCl/8 mM glucose/10 mM NaH_2PO_4 (pH 7.4) and resuspended to 25% hematocrit in the buffer as above and incubated at 37 °C for 0–24 h in the presence or absence of 25–50 μM BSO, 25–50 μM MK571 or 100–250 μM verapamil. At various times thereafter, 200 μL aliquots were removed for GSH and GSSG measurement. Aliquots were centrifuged (3000 × *g*) for 10 min to yield erythrocyte and buffer fractions. Erythrocyte fractions were treated with 5% MPA (1:5, v/v) for protein precipitation. Acid soluble supernatants and acid insoluble pellets were isolated by centrifugation at 14,000 × *g* for 3 min). The resulting buffer fraction was treated with MPA as above.

2.5. Analysis of glutathione and its metabolites

Intracellular levels of GSH and GSSG were analyzed in MPA extracts of erythrocytes by HPLC with electrochemical detection as described previously [27]. Levels of protein bound GSH (GSSP) were analyzed in potassium borohydride-reduced MPA-insoluble pellets as described previously [28]. GSSG efflux was determined by measuring GSSG levels in MPA treated buffer fractions after erythrocyte incubations by a previously described enzymatic recycling method [10].

2.6. Analysis of GCL activity

On the day of analysis, hemolysates in dH_2O (1:10, v/v) were prepared and exposed to three cycles of freeze/thawing and centrifugation at 10,000 × g for 20 min. Proteins were concentrated ~10-fold by centrifugal ultrafiltration in microcon-10 tubes (Millipore, Bedford, MA) to remove small molecules. Protein concentration was measured using the Bradford method and samples were diluted 20-fold in 100 mM Tris buffer (150 mM KCl, 20 mM $MgCl_2$, 2 mM EGTA; pH 8.2) containing substrates Glu (20 mM), Cys (5 mM) and ATP (10 mM). Following incubation (15–30 min, 37 °c), reactions were stopped by addition of 1 volume of 5% MPA. Precipitated proteins were removed by centrifugation and, after passing through 0.2 μm filters, γ-glutamyl cysteine (γGC) levels were measured using a PerkinElmer Liquid Chromatograph equipped with an 8-channel coulometric array (CoulArray) detector (ESA, Inc., Chelmsford, MA) [29]. A Bio-Sil ODS-5S 5-μm, 4.0 × 250 mm, C18 column (Bio-Rad, Life Science Research Group, Hercules, CA) was used with a mobile phase consisting of 50 mM NaH_2PO_4, 0.05 mM octane sulfonic acid, 1% (v/v) acetonitrile and 0.5% *N*,*N* dimethylformamide (v/v) (pH 2.52) at a flow rate of 1 mL/min and a sample volume of 5 μL. The 8-channel CoulArray detector channels were set at potentials of 250, 400, 450, 500, 550, 600, 650, and 700 mV, respectively. Concentrations were obtained from standard curves based on peak areas.

2.7. Biostatistics

Data represent the mean of three separate experiments. Data were analyzed using NCSS statistical software (Kaysville, UT). Analysis of variance (ANOVA) with Bonferroni post hoc test was utilized to determine if the effects of BSO, MK571 and verapamil were significantly different from untreated cells and from each other. Tests were determined to be significant at $P < 0.05$.

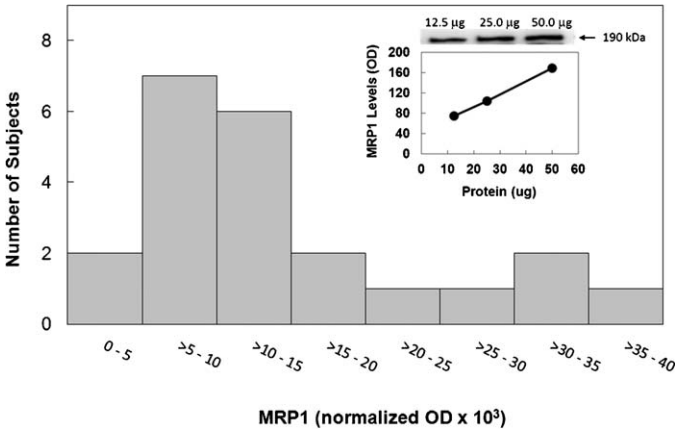

Fig. 1. MRP1 protein expression in erythrocyte membranes. Linearity and proportionality of MRP1 protein levels were examined in a representative sample (inner panel). MRP1 protein levels were identified using Western Blot analysis of 12, 25 and 50 µg of protein and compared to a molecular weight standard (190 kDa). Band densities were analyzed by ImageJ software. Frequency distribution of MRP1 protein levels in 22 healthy adults is shown in the outer panel. MRP1 levels ranged from 0 to 128 absorbance units with a mean ± standard deviation of 19.6 ± 23.9.

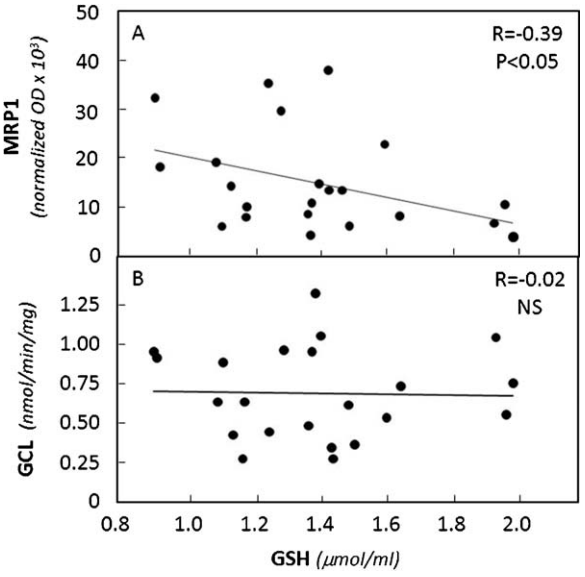

Fig. 2. Correlation of erythrocyte glutathione levels with membrane MRP1 levels and intracellular GCL activity. Red blood cells were obtained from 22 healthy adult non-smokers and intracellular GSH levels were correlated with membrane MRP1 levels (A) and GCL activity (B).

3. Results

3.1. MRP1 expression in erythrocytes

MRP1 was expressed in all but one of the erythrocyte samples examined and appeared as a 190 kDa band (Fig. 1, inner panel). Upon densitometric analysis of the bands, response was proportional to amount of erythrocyte membrane protein loaded between 10 and 50 mg. Analysis of relative MRP1 levels in erythrocyte membranes from 22 healthy adults demonstrated substantial interindividual variation (%CV = 70) and a mean ± SD absorbance of 0.015 ± 0.011 (Fig. 1). When MRP-1 levels were compared with intracellular GSH levels, a significant negative correlation was observed ($r = -0.39$, $r^2 = 0.15$) suggesting that 15% of the variation in GSH levels could be explained by differences MRP1 levels (Fig. 2A).

3.2. GCL activity in erythrocytes

The activity of GCL in erythrocytes was assessed directly by incubating erythrocyte hemolysates with precursor amino acids Glu and Cys and measuring the production of the dipeptide γGC over time by HPLC with coulometric detection as described in Methods. A typical chromatogram of a γGC standard (100 µM) is shown in Fig. 3A. γGC was detected primarily at 400, 450 and 500 mV channels and eluted at 3.85 min, well after peaks corresponding to MPA and EDTA. The linearity of γGC synthesis over time was assessed in erythrocyte hemolysates containing 2 and 4 mg protein/mL (Fig. 3B). At both concentrations, γGC production was linear up to at least 30 min, thus, this time point was used for all further assays. The proportionality of γGC synthesis with protein concentration was also assessed (Fig. 3B,

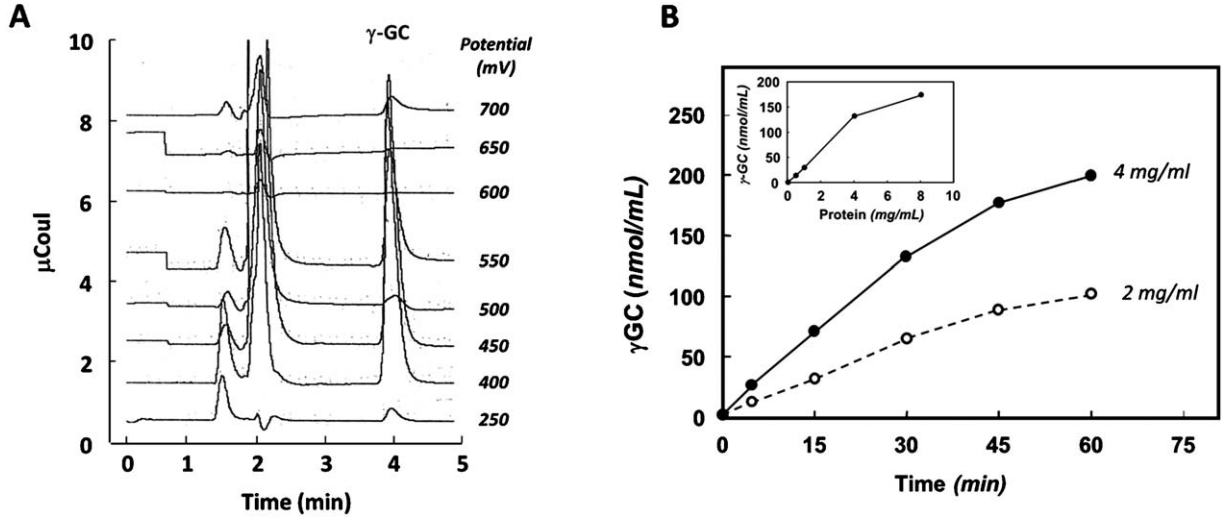

Fig. 3. Determination of GCL activity. (A) A standard containing 100 µM γGC in 5% (w/v) MPA and 2 mM EDTA was injected (5 µL) on to the HPLC and resulting currents were monitored on the Coularray detector at 8 different potentials ranging from 250 to 700 mV. γGC was detected primarily at 400, 450 and 500 mV channels and eluted at 3.85 min. (B) To assess the linearity of γGC synthesis over time, sample preparations of 2 and 4 mg protein/mL were incubated for different times from 5 to 60 min and γGC was measured as described in the Methods. Proportionality of γGC synthesis was assessed by incubation of samples containing 0.5, 1, 4 and 8 mg protein/mL for 30 min and measuring γGC (inner panel).

inner panel). A proportional response was observed at protein concentrations of 0.5–4 mg/mL. Thus, all further analyses were performed at protein concentrations within this range.

GCL activity was assessed in erythrocyte samples from the same 22 healthy adults as for MRP1. Values ranged from 0.27 to 1.32 nmol/min/mg with a mean ± SD of 0.69 ± 0.29 nmol/min/mg and %CV of 42.4. No significant association was observed when GCL activity was compared with intracellular GSH levels ($r = -0.02$) (Fig. 3B).

3.3. Time course of changes in erythrocyte GSH, GSSG and GSSP levels at 37 °C

In order to determine the time course of changes of GSH and its metabolites, erythrocytes were incubated at 37 °C for up to 24 h (Fig. 4). GSH levels were stable for the initial 4 h and thereafter decreased at a rate of 65 ± 29 nmol/mL/h, reaching a maximum decrease of 71% by 24 h ($P < 0.05$). This profile of GSH was paralleled by an increase in intracellular GSSG levels beginning at 4 h and progressively increasing at a rate of 23 ± 21 nequivalents GSH/mL/h, reaching a maximum increase of 8.5-fold by 24 h ($P < 0.05$). No changes in the levels of GSSP were observed over the incubation period. The loss of GSH was not completely balanced by the increase in GSSG such that the total pool of glutathione (GSH + GSSG) decreased progressively after 4 h at a rate of 42 ± 15 nmol/mL/h, reaching a minimum (50% of baseline) at 24 h. Based on these results, subsequent incubation studies to assess GSSG export were conducted for 24 h.

3.4. Effects of MRP1 and GCL inhibition on erythrocyte GSSG efflux

Studies were conducted to determine the impact of MRP1 inhibition by incubation with MPR1 inhibitors MK571 and Verapamil on the efflux of GSSG from erythrocytes (Fig. 5). Incubation of erythrocytes without treatment resulted in an efflux of GSSG, as measured in the media, of 272 ± 18 nmol/mL erythrocytes after 24 h and an average 24 h rate of GSSG efflux of 11.4 ± 0.7 nmol/h/mL of erythrocytes. Incubation of erythrocytes with MK571 resulted in a dose dependent inhibition of GSSG efflux from erythrocytes. A high dose (50 μM) of MK571 resulted in a 20% inhibition of GSSG efflux as compared to untreated cells ($P < 0.05$), with a total efflux of 220 ± 2.0 nmol/mL erythrocytes of GSSG and a rate of efflux of 9.2 ± 0.1 nmol/mL/h of erythrocytes. At a lower dose of MK571 (20 μM) the rate of efflux (11.2 ± 0.1 nmol/mL/h) was not significantly lower that the untreated control. Incubation of erythrocytes with verapamil also resulted in a dose dependent inhibition of GSSG efflux from erythrocytes. At a high dose (250 μM) verapamil resulted in a 53% inhibition of GSSG efflux as compared to untreated cells ($P < 0.05$), with a total efflux of 128 ± 6.0 nmol/mL erythrocytes of GSSG and rate of efflux of 5.3 ± 0.3 nmol/h/mL. Likewise, at a lower dose (100 μM) verapamil also resulted in a 30% inhibition of GSSG efflux (8.0 ± 0.2 nequivalents GSH/h/mL) compared to untreated erythrocytes ($P < 0.05$).

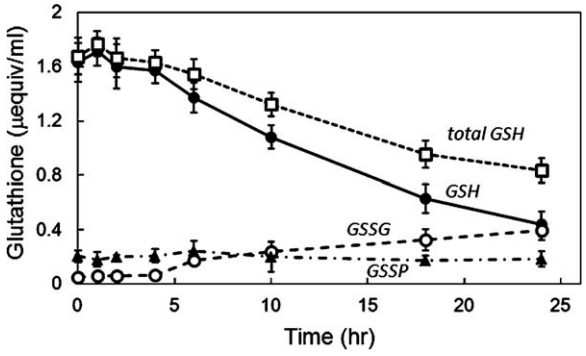

Fig. 4. Time course of intracellular glutathione depletion in erythrocytes. Erythrocytes were incubated for 0, 4, 8 and 24 h at 37 °C and intracellular GSH, GSSG, and GSSP were analyzed. Values expressed are mean ± standard deviation of three experiments.

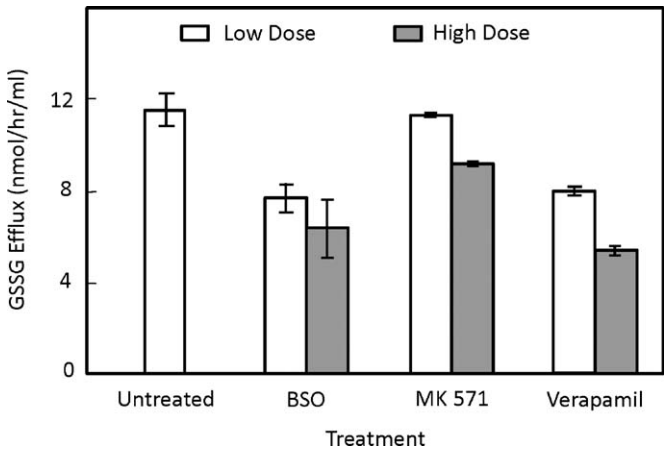

Fig. 5. Inhibition of GSSG Efflux by BSO, MK571 and Verapamil. Erythrocytes were incubated either alone (untreated) or with low and high concentrations of BSO (low = 20 μM; high = 50 μM), MK571 (low = 20 μM; high = 50 μM) or verapamil (low = 100 μM; high = 250 μM) at 37 °C for 24 h. Rate of GSSG efflux is expressed as nmol GSSG transported per hour per mL of erythrocytes. Rates are mean ± standard deviation of three experiments.

The effect of inhibition of GSH synthesis, *via* inhibition of GCL by BSO, on GSSG efflux was also measured. To determine the concentrations of BSO required for GCL inhibition, the effect of BSO on the specific activity of partially purified erythrocyte GCL was determined. GCL activity decreased with increasing concentration of BSO with a maximum inhibition of 83% being observed with 20 μM and 80 μM BSO (data not shown) ($P < 0.05$). Incubation of erythrocytes with 50 μM BSO resulted in a 48% depletion of free glutathione over 24 h with a total loss of 800 ± 114 nmol/mL GSH and estimated apparent half-life of 49.8 h ($P < 0.05$) (data not shown). Incubation of erythrocytes with 20 and 50 μM BSO resulted in decreases in GSSG efflux compared to untreated controls of 33% and 45%, respectively ($P < 0.05$) (Fig. 5).

4. Discussion

As the first line of defense against oxidative stress in an environment whose function is to transport oxygen, erythrocyte GSH plays a critical role. Its regulation is achieved through a complex system of synthesis, oxidation, redox cycling and export (in the form of glutathione disulfide). Understanding the regulation of GSH in erythrocytes from healthy individuals is critical in determining the mechanism by which glutathione levels are altered in unhealthy individuals and the pathogenesis of the myriad of diseases in which glutathione levels are altered.

GSSG is effluxed from erythrocytes in an ATP-dependent manner [20,21]. However the mechanism by which this transport occurs is unknown. Although *in vivo* turnover of erythrocyte GSH is slow (approximately 4 days), it has previously been determined that efflux of GSSG can account for turnover [22]. MRP1, an ATP-binding cassette transporter protein which is present in erythrocytes, has previously been identified as the GSX transporter in erythrocytes and the GSSG efflux pump in other cell types [30]. Based upon this data, it was hypothesized that MRP1 is the GSSG efflux pump in erythrocytes.

In order to determine the role MRP1 is playing in regulating GSH levels in erythrocytes *in vivo*, we examined the expression of

MRP1 in erythrocyte membranes obtained from healthy adults and compared them with intracellular GSH. To our knowledge, this is the first study of erythrocyte MRP1 levels in a healthy human population. We observed substantial intraindividual variation in MRP1 levels which may be indicative of differences between individuals in the capacity for transporting GSSG from erythrocytes. Our finding that the levels of MRP1 were significantly correlated with intracellular GSH levels indicates that MRP1 may be playing a role in regulating GSH in red cells by regulating its efflux through GSSG. While only 15% of the variation in GSH was explained by variation in MRP1 levels, it is highly likely that other factors are also involved. The variation observed in MRP1 levels may also be an important factor regulating susceptibility of individuals to toxic agents detoxified by erythrocytes. Those individuals with lower levels of MRP1 may be at greater risk due to an impaired ability to remove GSH conjugates from the cell.

GSH biosynthesis *via* GCL is commonly considered a key regulatory step in GSH homeostasis. However, the present findings that GCL activity is unrelated to intracellular GSH levels, indicates that interindividual variation in GSH cannot be accounted for by differences in GCL activity. Other pathways, such as the transport of precursor amino acids into cell, may also impact GSH biosynthesis and should be examined in future studies.

The mechanisms responsible for MRP1 variation among subjects are not known, but may be genetic in nature. Numerous polymorphisms in the gene for MRP1 and related transporters have been identified and, in some cases, linked to altered transport function and specific disease outcomes [31–33]. Future studies comparing erythrocyte MPR1 expression with specific and relevant polymorphisms may help elucidate the mechanisms involved.

In order to determine the direct effect of MRP1 activity on GSSG efflux, we conducted *in vitro* experiments in which the effect of two specific MRP1 inhibitors, MK571 and verapamil, on the rate of erythrocyte GSSG efflux into the media was measured. The concentrations of MK571 (50 μM) and verapamil (250 μM) used here were previously shown to be noncytotoxic and effective at inhibiting transport of other MRP1 substrates in erythrocytes and GSSG in other cell types [26,34,35]. Treatment with MRP1 inhibitors, MK571 and verapamil, resulted in 19% and 53% inhibition, respectively, of GSSG efflux into the media. In addition, there was an apparent dose response whereby lower doses of both MK571 and verapamil inhibited the efflux of GSSG, but to a lesser extent. These results indicate that MRP1 is responsible, at least in part, for the transport of GSSG from erythrocytes.

The extent to which GSSG is transported by MRP1 could not be determined since there was no effective means of determining, *in vivo*, the extent of MRP1 inhibition by either MK571 or verapamil. While it is possible that higher concentrations of both MK571 and verapamil might be more effective, higher concentrations are known to result in cytotoxicity in certain cell types. Also, the concentrations used for both MK571 (50 μM) and verapamil (250 μM) were found to provide the most effective inhibition of GSSG export in preliminary dose response experiments. It is not known whether complete inhibition of MRP1 was achieved by these inhibitors as the use of a positive control (e.g. calcein) to confirm inhibition of MRP1 activity by MK571 and verapamil was not feasible in intact erythrocytes. Thus, it is possible that the impact of MRP1 on GSSG transport may be underestimated in these studies due to incomplete inhibition. However, our data in human subjects showing that variation in erythrocyte GSH levels was only partially explained by variation in MRP1 is consistent with the notion that there may be other factors involved. As for potential alternative pathways for GSSG efflux, two other isoforms of MRP, MRP4 and MRP5 have been identified in erythrocytes [36]. However, their role in the transport of GSSG is unknown. MRP2 is thought to be an important transporter in other cell types but is not expressed in erythrocytes.

In order to determine if the impact of GSH biosynthesis on the rate of GSSG export, BSO was used to inhibit *de novo* synthesis. BSO was previously shown to pass freely into cells and effectively and specifically inhibit GSH synthesis [37]. We observed that inhibition of GSH biosynthesis by BSO resulted in similar decreases in GSSG efflux as observed for MRP1 inhibitors. These results indicate that GSH biosynthesis, in addition to MRP1, is an important regulator of GSH turnover. However, based upon our *in vivo* findings above, interindividual variation in GCL does not appear to GSH levels.

Previous research on GSSG transport has focused primarily on the metabolism of GSH under conditions of acute oxidative stress, modeled by treatment of erythrocytes with either hydrogen peroxide or tert-butyl hydrogen peroxide [38]. While these models of oxidative stress may be relevant to conditions involving acute exposure to environmental toxins and oxidants, it is quite unlikely that a cell would be exposed to this great of an oxidative insult under "normal" conditions. Since the aim here was to examine those factors responsible for the regulation of steady state GSH levels, non-stressed red cells incubated under physiological conditions were utilized. Indeed, few have examined the metabolism of GSH under non-stressed conditions and none have previously examined the intracellular GSH pool and GSSG efflux simultaneously, under "normal" *in vitro* conditions.

Elucidation of the mechanism by which GSSG is effluxed from erythrocytes is integral in understanding what regulates intracellular GSH levels, since GSSG is the only unconjugated form of glutathione that can leave the cell. Identification of MRP1 as a GSSG efflux pump in erythrocytes provides a potential marker to identify variations in steady-state GSH levels in individuals and may reveal insights as to how glutathione levels are altered in unhealthy individuals.

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