Hyperosmolarity Induced Cystine Transport and Cystine-cysteine Cycle between Erythrocytes and the Plasma

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The objective of the present study was to determine if a cystine-cysteine cycle operates between the erythrocytes and the plasma. In the present study we incubated the erythrocytes in krebs ringer phosphate buffer with different osmolarity containing different amounts of cystine. Our results show that erythrocytes do not uptake cystine from the environment when the osmolarity of the buffer is 310 mOsmol/l. Erythrocytes also do not operate a cystine-cysteine cycle in this isoosmolar buffer. However, when exposed to hyperosmolar buffer in the ranges that occur in the kidney medulla which is in between 1200–1400 mOsmol/l erythrocytes start to uptake cystine from the environment and induce a cystine-cysteine cycle. The cystine uptake and cystine-cysteine cycle were characterized by measurement of changes in the free -SH concentrations in erythrocytes and in the buffer. Following incubation of erythrocytes in 1 mM cystine containing 1250 and 1300 mOsmol/l buffer, the free -SH concentrations in the buffer reached to 0.102 ± 0.002 and 0.241 ± 0.013 μmol/ml erythrocyte respectively. Our results demonstrate that erythrocytes display a cystine-cysteine cycle in hyperosmolar environment which is prevailed mainly in the kidney medulla. Our results also display that this process is biologically active and energy dependent. The observed cystine-cysteine cycle is inhibited when the erythrocytes are incubated at lower temperatures and in the absence of glucose. Our results suggest that erythrocytes uptake cystine, intracellulary reduce it to cysteine and release it back to the environment when exposed to hyperosmolar conditions. Erythrocytes may have a role in the regulation of plasma cystine and cysteine concentrations and may contribute to the regulation of plasma redox status.

Key words —— cystine-cysteine cycle, hyperosmolarity, erythrocytes

INTRODUCTION

Cystine is formed by oxidation of two cysteine molecules in the plasma. Cystine thus formed is transported and further utilized by tissues in protein and glutathione (GSH) synthesis following its intracellular reduction back to two molecules of cysteine. Cystine transport is the first step in obtaining cysteine in several tissues. Cystine is transported by sodium dependent and sodium independent systems in tissues.[1,2] Sodium dependent cystine transport is mediated by the glutamate transporters whereas in sodium independent transport systems glutamate and cystine are exchanged. Once the cystine is inside the cells it is reduced to two molecules of cysteine. Cysteine is mainly utilized in GSH synthesis in the cells.[3] Although GSH is synthesized from three amino acids, glutamic acid, cysteine and glycine, the rate of GSH synthesis is mainly determined by the amount of cysteine available.[3] GSH is a soluble antioxidant and it functions in protecting the cells against harmful free radicals.[4,5] GSH is also involved in cellular detoxification through conjugation with several xenobiotics catalyzed by GSH S-transferase.[6–13] Erythrocytes have relatively high intracellular GSH levels however it has been shown that the erythrocytes can not efflux GSH into the plasma.[14] Thus they can not contribute to the regulation of plasma redoxs status in this way. Cystine is reduced in intracellular environment and mainly exist as cysteine however in the more oxidizing environment of the plasma cystine concentrations exceeds the cysteine concentrations. It has been known that cysteine itself, as GSH, can act as an antioxidant. Studies demonstrated that *Escherichia coli* (*E. coli*) efflux cysteine to the periplasm by CydDC transporters to regulate the redoxs status of the periplasm.[15] The disturbance of the redoxs status

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in the periplasm in CydDC mutants supported this view. It has also been shown that plasma thiol levels are implicated in cardiovascular diseases. It is demonstrated that the amount of reduced homocysteine is correlated with the amount of reduced cysteine in the plasma.[16] These type of findings suggest that reduced cysteine in the plasma may significantly contribute the redox status of the plasma. Reduced cysteine in the plasma thus may effect the oxidized/reduced state of several different molecules in the plasma.

There are no in depth investigation on utilization of plasma cystine and possible existence of a cystine-cysteine cycle in the plasma. In the present study, we investigated if a cystine-cysteine cycle operates between the plasma and erythrocytes. Presence of this type of a cycle may represent a mechanism that contributes to the regulation of plasma redox status and cystine utilization. The normal osmolarity of the plasma is around 310 mOsmol/l and this is considered to be isoosmolar for the cells. However, erythrocytes are regularly exposed to plasma with higher osmolarity as they pass through the kidney medulla. Osmolarity of the plasma is reached around to 1200–1400 mOsmol/l in the kidney medulla.[17,18] Thus the presence of a cystine-cysteine cycle between the erythrocytes and plasma was investigated in 310, 1200, 1250 and 1300 mOsmol/l buffers.

MATERIALS AND METHODS

Materials —— L-cystine, L-aspartate, L-glutamate, and 5,5′-dithiobis(-nitrobenzoate) (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), buthionine sulfoximine (BSO) were obtained form Sigma Chemical Co. (St. Louis, MO, U.S.A.). Blood was obtained from the blood bank of SSK Hospital, (Antakya, Turkey) as 300 ml units derived from different individuals with no prerecorded medical conditions.

Preparation of Erythrocytes —— Plasma was separated by centrifugation at $2000 \times g$ for 5 min. The plasma and the buffy coat were then removed and discarded. The resulting erythrocyte pellet was washed three times with two volumes of phosphate buffered saline (PBS, 9 parts of 0.15 M NaCl and 1 part of 0.1 M potassium phosphate buffer, pH 7.4). PBS-glucose contained 8 mM of glucose in the PBS.[19]

L-cystine Uptake Studies —— 0.25 ml of washed erythrocytes were suspended in 1 ml of 310 mOsmol/l isoosmolar krebs ringer phosphate buffer (135 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 5 mM glucose, 10 mM NaH_2PO_4, pH 7.4) containing 0.5, and 1 mM concentrations of L-cystine and incubated for the indicated time periods at 37°C in a water bath. L-cystine uptake studies were also carried at 1300 mOsmol/l buffer whose composition is described below. At the end of incubation, erythrocytes were removed, centrifuged, and the supernatants were discarded. The free -SH concentrations in erythrocytes were then determined as described by Sedlak.[20] Briefly 100 μl of erythrocytes were lysed in 100 μl of 10% TCA prepared in sodium phosphate-EDTA buffer (0.01 M sodium phosphate/0.005 M EDTA). The erythrocyte lysates were then centrifuged at $12000 \times g$ for 5 min. At the end of centrifugation, 100 μl of the supernatant was mixed with 1.9 ml of Tris-EDTA buffer containing 0.6 μM/ml DTNB (262 mM Tris base, 13 mM EDTA, pH 8.9). Samples were allowed to stand for 5 min to develop color. The absorbencies of the samples were then measured at 412 nm and the concentrations of free-SH were calculated by using the mM extinction coefficient of 13.1.

Cystine-cysteine Cycle Studies —— Cystine-cysteine cycle was determined principally by first treating the erythrocytes with cystine for indicated time periods and then by measuring cysteine formed in the media through free -SH measurement. Any free -SH increase in the media represented the cystine uptake and the following release of reduced cysteine into the media by the erythrocytes.

Briefly 0.25 ml of washed erythrocytes were resuspended in 1 ml of krebs ringer phosphate buffer with different osmolarity in the presence of different concentrations of L-cystine. Erythrocytes were incubated at 37°C in a water bath for the indicated time periods. At the end of incubation, erythrocytes were centrifuged and the supernatants were transferred to fresh tubes. The free -SH concentrations in the supernatant was then measured as described above. Control cell supernatants without DTNB was used as blank. The osmolarity of buffers that were used in cystine-cysteine cycle studies were as the following: 310 mOsmol/l, 1200 mOsmol/l, 1250 mOsmol/l, and 1300 mOsmol/l. The composition of the 310 mOsmol/l krebs ringer phosphate buffer is described above. The other buffers with higher osmolarity were prepared by increas-

ing the NaCl concentrations. Thus 1200 mOsmol/l buffer was composed of (579.5 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 5 mM glucose, 10 mM NaH_2PO_4, pH 7.4). 1250 mOsmol/l buffer was composed of (604.5 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 5 mM glucose, 10 mM NaH_2PO_4, pH 7.4) and finally 1300 mOsmol/l buffer was composed of (629.5 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 5 mM glucose, 10 mM NaH_2PO_4, pH 7.4). Accordingly we obtained 1300 mOsmol/l sucrose buffer by addition of 990 mM of sucrose on top of 310 mOsmol/l krebs ringer phosphate buffer. Thus the ionic strength of the 310 mOsmol/l krebs ringer buffer was maintained as 1300 mOsmol/l krebs buffer was obtained.

Statistical Analysis —— One-way analysis of variance (ANOVA) and Student-Newman-Keuls multiple comparison tests were applied to process the data statistically. All tests were performed on triplicate samples. Results were expressed as mean ± S.D. $p < 0.05$ values were considered to be significant.

RESULTS

Results presented in Fig. 1 display that erythrocytes do not uptake cystine in normal plasma condition. Intracellular free -SH concentrations did not change significantly following exposure to cystine. If erythrocytes took cystine from the environment the free -SH concentrations in the erythrocytes would rise because of GSH dependent intracellular reduction of cystine to cysteine molecules. However we did not record such a rise in free -SH in erythrocytes. In the later step, we investigated if erythrocytes start to transport cystine and release cysteine back into the media. Thus, we searched for a cystine-cysteine cycle between the plasma and the erythrocytes. We incubated the erythrocytes in the presence of cystine in 310, 1200, 1250, and 1300 mOsmol/l krebs ringer phosphate buffers. If there were a cystine-cysteine cycle between the erythrocytes and buffer, the free -SH concentrations in the buffer would rise. Results as shown in Fig. 2, we did not record a rise in free -SH concentration in the media when the erythrocytes were exposed to cystine in 310 and 1200 mOsmol/l buffer. However, we found an increase in free -SH levels when the erythrocytes were exposed to cystine in 1250 and 1300 mOsmol/l buffer. These results demonstrate that erythrocytes start to take up cystine at hyperosmolar buffers, inracellularly reduce it to cystine and then efflux thus formed cysteine back into the media. We later investigated the cystine concentration dependency of this cycle. Figure 3 shows that cystine-cysteine cycle between the erythrocytes and the buffer was concentration dependent and the activity of the cycle increased as the concentration of cystine in the buffer increased.

The increase in free -SH levels in the media of erythrocytes exposed to cystine in 1300 mOsmol/l buffer indicated that erythrocytes uptake cystine under these conditions. Thus we investigated whether cystine treatment of erythrocytes in 1300 mOsmol/l buffer increases intracellular levels of free -SH. Figure 4 shows that treatment of erythrocytes this way

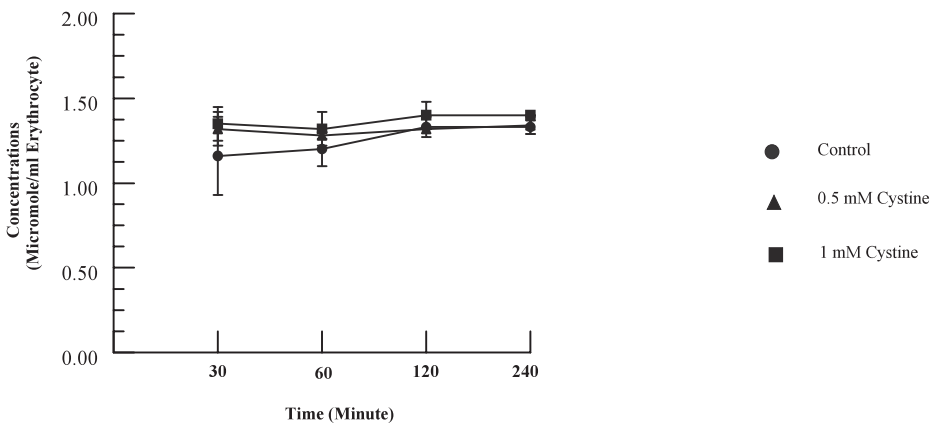

Fig. 1. Cystine Uptake by Erythrocytes

Erythrocytes were incubated in the presence of 0.5 and 1 mM of cystine for the indicated time periods in 310 mOsmol/l krebs ringer phosphate buffer. At the end of incubation erythrocytes were removed and free -SH concentrations in erythrocytes were measured. Results are the mean, S.D. of three seperate experiments. Results are not statistically different.

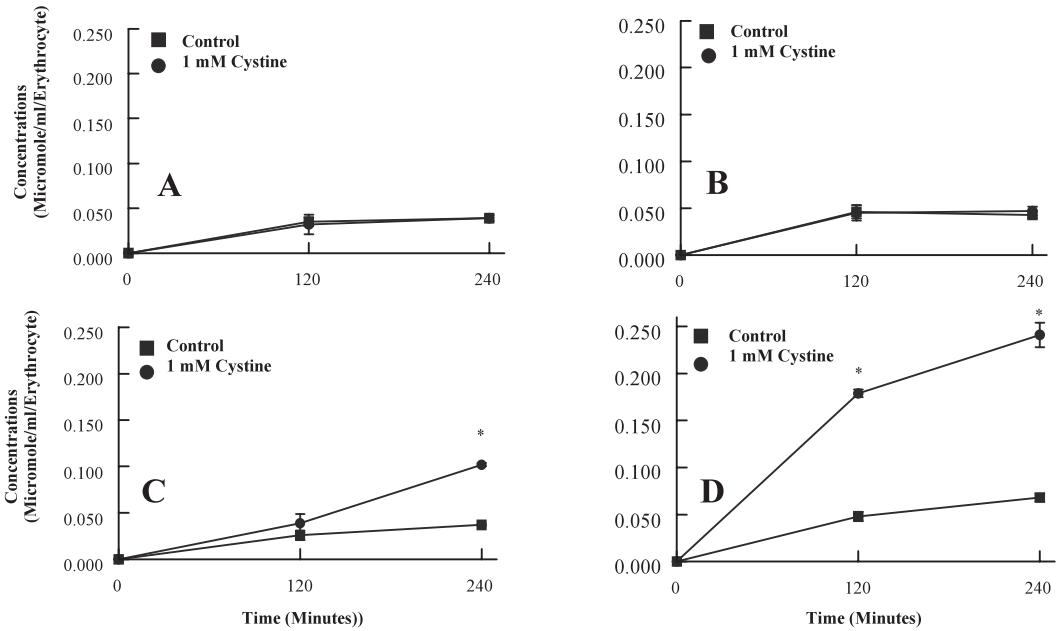

Fig. 2. Cystine-cysteine Cycle

The erythrocytes were incubated in the presence and absence of 1 mM of cystine in A: 310 mOsmol/l, B: 1200 mOsmol/l, C: 1250 mOsmol/l and D: 1300 mOsmol/l krebs ringer phosphate buffer for 0, 120, and 240 minutes. At the end of incubation the erythrocytes were removed by centrifugation and then free -SH levels in the supernatant were measured. Results are the mean and S.D. of three seperate experiments. $p < 0.05$. *Significantly different from the control.

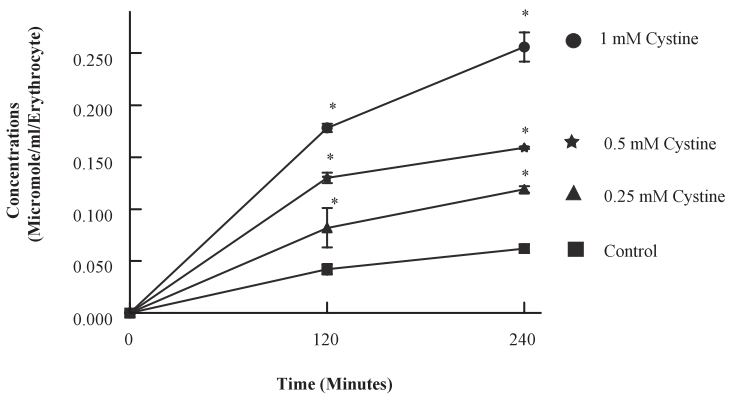

Fig. 3. Concentration Dependence of Cystine-cysteine Cycle

Erythrocytes were incubated in 1300 mOsmol/l krebs ringer phosphate buffer inthe presence of indicated concentrations of cystine for the indicated time periods. At the end of incubation the erythroctes were removed and free -SH concentrations in the supernatant were measured. Results are the mean and S.D. of three seperate experiments. $p < 0.05$. *Significantly different fron the control.

increases the free -SH levels inside the erythrocytes indicating the presence of a cystine uptake. In order to demonstrate that the cystine uptake and the cystine-cysteine cycle described between the erythrocytes and the media are biologically active, energy requiring, and carrier mediated we repeated the experiments the presence and absence of glucose and at different temperatures. As shown in Fig. 5 removal of glucose from 1300 mOsmol/l buffer resulted in the breakdown of the cystine-cysteine cycle. When glucose was omitted free -SH levels in the supernatant decreased to the control levels indicating that cystine-cysteine cycle is an energy dependent biologically active process. In similar way, when the experiments was carried out at lower temperatures the activity of the cycle is also decreased and finally became zero at 4°C, as seen in Table 1.

Cystine is most often transported into the cells by transporters that also carry glutamate and aspartate. In order to show that hyperosmolarity induced

cystine transport also share similar transport mechanism with glutamate and aspartate we repeated the experiments in the presence of equimolar concentrations of glutamate and aspartate. As described in Fig. 6 the presence of either glutamate or aspartate significantly inhibited the cystine-cysteine cycle between the erythrocytes and the media indicating that these two amino acids share similar transport pathway with cystine under hyperosmolar conditions also. In order to demonstrate that the described cystine-cysteine cycle is GSH dependent we first treated the erythrocytes with GSH depleting agent CDNB in the presence and absence of BSO a GSH synthesis inhibitor. As seen in Fig. 7, following depletion of GSH by CDNB alone and CDNB+BSO completely inhibited the cystine-cysteine cycle. This result suggest that following uptake of cystine by erythrocytes under hyperosmolar conditions, cystine is reduced to cysteine by a reaction in which GSH participates as an electron donor. In the last step of our experiments we investigated if cystine-cysteine cycle takes place in hyperosmolar buffers obtained by sucrose. Figure 8 shows that cystine-cysteine cycle also operates in sucrose hyperosmolar buffer. This result indicates that cystine-cysteine cycle is not a function of high

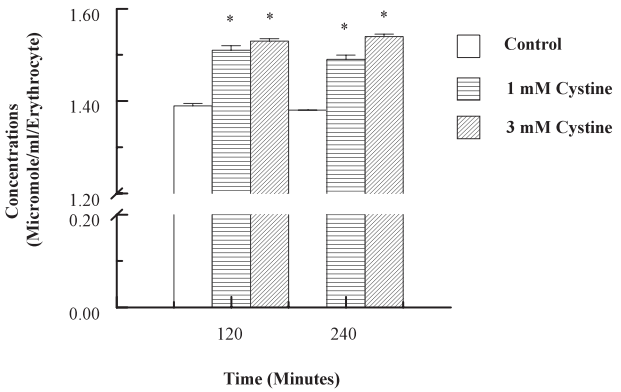

Fig. 4. Cystine Uptake by Erythrocytes in Hperosmolar Media
Erythrocytes were incubated in 1300 mOsmol/l krebs ringer phosphate buffer in the presence of different cystine concentrations for the indicated time period. At the end of incubation erythrocytes were removed and washed. The free -SH concentrations in washed erythrocytes were then determined. Results are the mean and S.D. of three seperate experiments. $p < 0.05$. *Significantly different from the control.

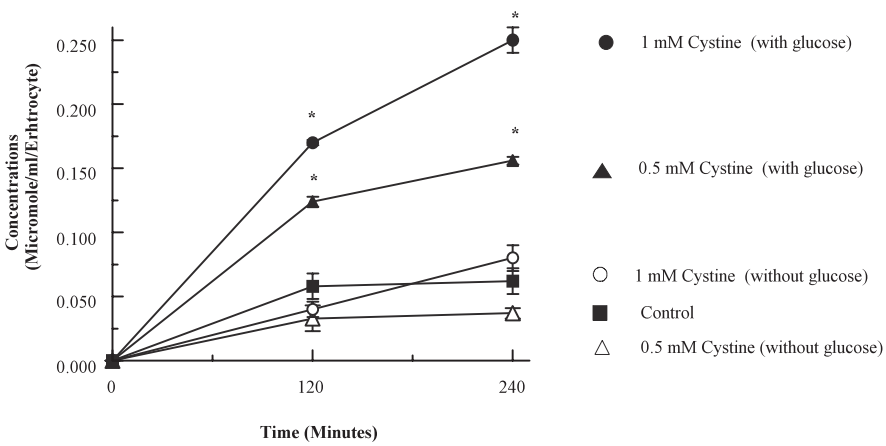

Fig. 5. Energy Dependency of Cystine-cysteine Cycle
Erythrocytes were incubated in the presence of different concentrations of cystine with glucose or without glucose in 1300 mOsmol/l krebs ringer phosphate buffer. At the end of incubation erythrocytes were removed and free -SH concentraions in the supernatants were measured. Results are the mean and S.D. of three seperate experiments. $p < 0.05$. *Significantl different from control and without glucose groups.

Table 1. Temperature Dependency of the Cystine-cysteine Cycle

	Concentrations (μmol/ml erythrocyte)					
	120 minutes			240 minutes		
	4°C	20°C	37°C	4°C	20°C	37°C
Control	0.00	0.025 ± 0.002	0.045 ± 0.003	0.00	0.033 ± 0.004	0.059 ± 0.005
0.5 mM Cystine	0.00	0.076* ± 0.005	0.120* ± 0.004	0.00	0.108* ± 0.004	0.166* ± 0.003
1 mM Cystine	0.00	0.102* ± 0.004	0.172* ± 0.004	0.00	0.176* ± 0.007	0.257* ± 0.003

Erythrocytes were incubated at different temperatures in 1300 mOsmol/l krebs ringer buffer in the presence of different concentrations of cystine for the indicated time periods. At the end of incubation erythrocytes were removed and free -SH levels in the supernatants were measured. Results are the mean and S.D. of three seperate experiments. $p < 0.05$. *Significantly different from control.

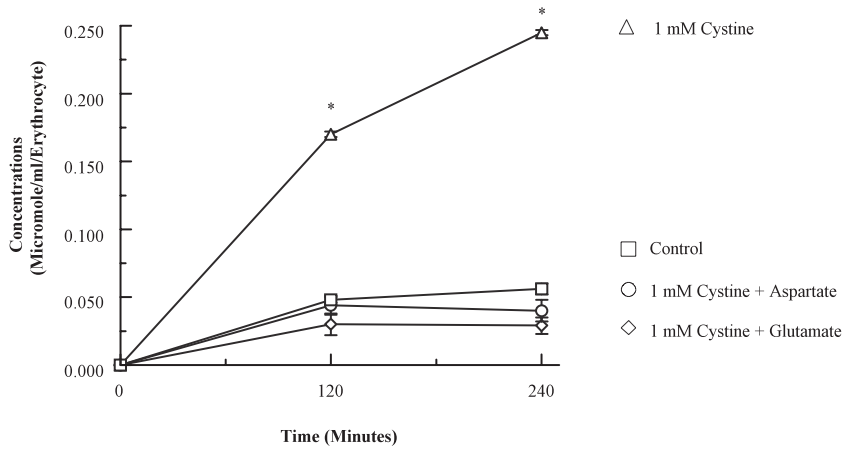

Fig. 6. The Effect of Glutamate and Aspartate on Cystine-cysteine Cycle

Erythrocytes were incubated with 1 mM of cystine in the presence and absence of 1 mM of aspartate and glutamate in 1300 mOsmol/l krebs ringer phosphate buffer for the indicated time periods. At the end of incubation, erythrocytes were removed and free -SH concentrations in the supernatants were measured. Results are the mean and S.D. of three seperate experiments. $p < 0.05$. *Significantly different from the control, glutamate and aspartate containing groups.

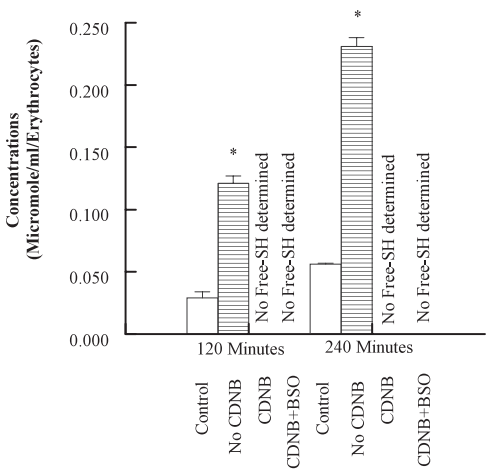

Fig. 7. The Effect of CDNB Pretreatment on Cystine-cysteine Cycle

The erytrocytes were incubated in the presence and absence of CDNB and CDNB + BSO for 40 minutes. At the end of incubation erythrocytes were removed and washed. The washed erythrocytes transferred to fresh 1300 mOsmol/l buffer in the presence of 1 mM of cystine and further incubated for the indicated time periods. At the end of incubation erythrocytes were removed and free -SH levels in the supernatants were measured. Results are the mean and S.D. of three seperate experiments. $p < 0.05$. *Significantly different from all other groups.

ionic strengths because the ionic strength of the normal krebs ringer is maintained in sucrose hyperosmolar buffer.

DISCUSSION

The objective of the present study was to investigate if a cystine-cysteine cycle operates between the plasma and the erythrocytes under any physiological condition. Operation of such a system would represent a mechanism which may be involved in regulation of plasma redox status. Uptake of cystine and subsequent reduction to cysteine and the following efflux of cysteine to the environment may contribute to the reduction of several oxidized molecules in the plasma. The efflux of cysteine from the erythrocytes has already been studied. It has been shown that cysteine pretreated erythrocytes efflux the excess intracellular cysteine to the environment.[21)]

Thus cysteine in the plasma may directly involve in the regulation of plasma redox status by either reducing the oxidized molecules or by scavenging certain free radicals in the plasma. Operation of a cystine-cysteine cycle would also function as a mechanism that would prevent the increase in plasma cystine concentrations and thereby cystine precipitation and resulting mechanical endothelial damage. Although the liver and the kidney are supposed to supply blood plasma with cysteine, the presence of additional mechanisms that especially involve a cystine-cysteine cycle in the plasma has not been investigated in depth.[22)] Fibroblast cells have been shown to display a cystine-cysteine cycle induced under hyperoxia.[23)] However, fibroblast cells do not have a direct contact with the plasma thus their cystine-cysteine cycle can not play a direct role in the regulation of plasma redox status. Erythrocytes represent a large portion of the circulating blood and thus a cystine-cysteine cycle that would operate between the plasma and the erythro-

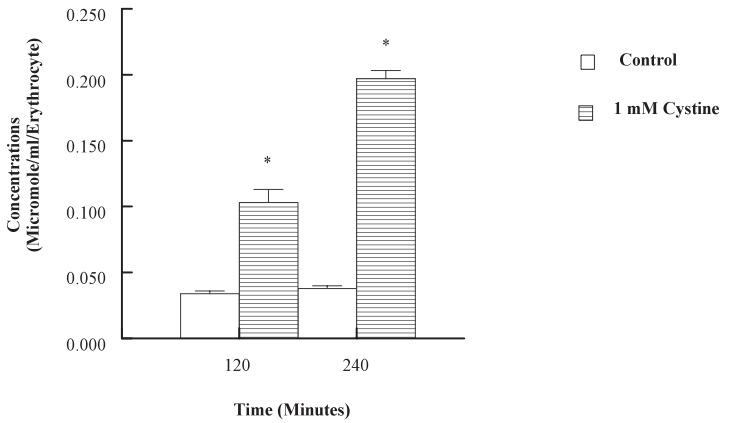

Fig. 8. Sucrose Hyperosmolarity and Cystine-cysteine Cycle

Erythrocytes were incubated in 1300 mOsmol/l sucrose hyperosmolar krebs ringer buffer in the presence of 1 mM of cystine for the indicated time periods. At the end of incubation erythrocytes were removed and free -SH levels in the supernatants were measured. Results are the mean and S.D. of three seperate experiments. $p < 0.05$. *Significantly different from the control.

cytes would have a significant importance in regulation of redox status of the plasma.

In the present study we investigated a probable cystine-cysteine cycle between the plasma and the erythrocytes in buffers with different osmolarity. As our results have shown we did not find any cystine-cysteine cycle between the plasma and the erythrocytes under isoosmolar conditions. This means that erythrocytes do not uptake cystine from the environment and utilize it under normal plasma conditions. However, we have found a cystine-cysteine cycle operating in hyperosmotic conditions that physiologically occur in the kidneys. The erythrocytes start to uptake cystine in 1250 and 1300 mOsmol/l buffers, intracellularly reduce it to cysteine and then they efflux the reduced cysteine back into the media.

We suggest that cystine uptake by erythrocytes is an inducible process. They do not normally uptake cystine but their membranes are equipped with mechanisms or transporters that are involved in cystine uptake. Evidence already exist that support this idea. Erythrocytes have been shown to uptake cystine from the environment under oxidative stress conditions.[24, 25] This function was suggested to be an emergency mechanism by which the oxidatively stressed cells can cope with deleterious effects of free radicals. Induction of cystine transport in the erythrocytes by oxidative stress and hyperosmotic conditions suggest that some membrane modifications or membrane alterations are required for the uptake process to occur. Thus we propose that hyperosmotic conditions induces certain alterations or modifications in erythrocyte membranes that results in cystine uptake. Osmotic shock and oxidative stress has been shown to open the cation channels and increase cytosolic calcium levels.[26, 27] In the end, increase in cystosolic calcium levels stimulate scramblase. Scramblase is a plasma membrane protein which is involved in transbilayer movement of phosphatidylserine and other phospholipids.[28] Calcium dependent scramblase activation leads to the breakdown of erythrocyte membrane asymmetry and results in phosphotidylserine exposure to the outer leaflet of erythrocyte membranes.[29] It has also been shown that osmotic shock of erythrocytes stimulates sphingomyelinase with the formation of ceramide which also activate scramblase that lead to the breakdown of phosphatidylserine asymmetry.[30] As a result, our findings suggest that hyperosmotic conditions induce cystine transport and start a cystine-cysteine cycle between the plasma and the erythrocytes. Such a cycle may involve in the regulation of redox status of the plasma. The cysteine made available to the erythrocytes by this route may also be utilized in GSH synthesis.

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REFERENCES

1) McBean, G. J. (2002) Cerebral cystine uptake: a tale of two transporters. *Trends Pharmacol. Sci.*, **23**, 299–302.
2) McBean, G. J. and Flyn, J. (2001) Molecular mechanisms of cystine transport. *Biochem. Soc. Trans.*, **29**,

717–722.
3) Griffith, O. W. (1992) Biologic and pharmacologic regulation of mammalian glutathione synthesis. *Free Radic. Biol. Med.*, **27**, 922–935.
4) Gille, G. and Sigler, K. (1995) Oxidative stress and living cells. *Folia Microbiol.* (*Praha*), **40**, 131–152.
5) Clemens, M. R. and Waller, H. D. (1987) Lipid peroxidation in erythrocytes. *Chem. Phys. Lipids*, **45**, 251–268.
6) Eckert, K. G. and Eyer, P. (1986) Formation and transport of xenobiotic glutathione-S-conjugates in red cells. *Biochem. Pharmacol.*, **35**, 325–329.
7) Awasthi, Y. C., Garg, H. S., Dao, D. D., Partridge, C. A. and Srivastava, S. K. (1981) Enzymatic conjugation of erythrocyte glutathione with 1-chloro-2,4-dinitrobenzene: the fate of glutathione conjugate in erythrocytes and the effect of glutathione depletion on hemoglobin. *Blood*, **58**, 733–738.
8) Ansari, G. A. S., Singh, S. V., Gan, J. C. and Awasthi, Y. C. (1987) Human erythrocyte glutathione S-transferase: a possible marker of chemical exposure. *Toxicol. Lett.*, **37**, 57–62.
9) Strange, R. C., Jones, P. W. and Fryer, A. A. (2000) Glutathione S-transferase: genetics and role in toxicology. *Toxicol. Lett.*, **112**, 357–363.
10) Olive, C. and Board, P. (1994) Glutathione S-conjugate transport by cultured human cells. *Biochim. Biophys. Acta*, **1224**, 264–268.
11) Matsuda, Y., Epstein, L. F., Gatmaitan, Z. and Arias, I. M. (1996) The role of thiols in ATP-dependent transport of S-(2,4-dinitrophenyl) glutathione by rat liver plasma membrane vesicles. *Biochim. Biophys. Acta*, **1279**, 35–42.
12) Keppler, D. (1999) Export pumps for glutathione S-conjugates. *Free Radic. Biol. Med.*, **27**, 985–991.
13) LaBelle, E. F., Singh, S. V., Srivastava, S. K. and Awasthi, Y. C. (1986) Dinitrophenyl glutathione efflux from human erythrocytes is primary active ATP-dependent transport. *Biochem. J.*, **238**, 443–449.
14) Lu, S. C., Sun, W. M., Yi, J., Ookhtens, M., Sze, G. and Kaplowitz, N. (1996) Role of two recently cloned rat liver GSH transporters in the ubiquitous transport of GSH in mammalian cells. *J. Clin. Invest.*, **97**, 1488–1496.
15) Pittman, M. S., Corker, H., Wu, G., Binet, M. B., Moir, A. J. and Poole, R. K. (2002) Cysteine is exported from the Escherichia coli cytoplasm by CydDC, an ATP-binding cassette-type transporter required for cytochrome assembly. *J. Biol. Chem.*, **277**, 49841–49849.
16) Andersson, A., Lindgren, A., Arnadottir, M., Prytz, H. and Hulfberg, B. (1999) Thiols as a measure of plasma redox status in healthy subjects and in patients with renal or liver failure. *Clin. Chem.*, **45**, 1084–1087.
17) Guyton, A. C. and Hall, J. E. (2000) The countercurrent mechanism produces a hyperosmotic renal medullary intersititum. In *Textbook of Medical Physiology*, 10th Edition, Saunders, Pennsylvania, pp. 315–316.
18) Nosek, T. M. (1999) Renal Physiology. In *Essentials of Human Physiology*, Section 7, Chapter 8, Gold Standard Multimedia Inc., Florida, USA, p. 6.
19) Awasthi, Y. C., Mısra, G., Rassın, D. K. and Srivastava, S. K. (1983) Detoxification of xenobiotics by glutathione S-transferase in erythrocytes: the transport of the conjugate of glutathione and 1-chloro-2,4-dinitrobenzene. *Br. J. Haematol.*, **55**, 419–425.
20) Sedlak, J. and Lindsay, R. H. (1963) Determination of sulfhydriyl groups in biological samples. *Anal. Biochem.*, **25**, 192–205.
21) Yıldız, D., Uslu, C., Çakır, Y. and Öztas, H. (2006) L-Cysteine influx and efflux: A possible role for red blood cells in regulation of redox status of the plasma. *Free Radic. Res.*, **40**, 507–512.
22) Rudolfs, K. Z. and Lawrence, H. L. (2006) Cystine alters the renal and hepatic disposition of inorganic mercury and plasma thiols. *Toxicol. Appl. Pharmacol.*, **214**, 88–97.
23) Bannai, S., Sato, H., Ishii, T. and Sugita, Y. (1989) Induction of cystine transport activity in human fibroblasts by oxygen. *J. Biol. Chem.*, **264**, 18480–18484.
24) Miura, K., Ishii, T., Sugita, Y. and Bannai, S. (1992) Cystine uptake and glutathione level in endothelial cells exposed to oxidative stress. *Am. J. Physiol. Cell Physiol.*, **262**, C50–C58.
25) Ohtsuka, Y., Kondo, T. and Kawakami, Y. (1988) Oxidative stress induced the cystine transport activity in human erythrocytes. *Biochem. Biophys. Res. Commun.*, **155**, 160–166.
26) Lang, K. S., Myssina, S., Lang, P. A., Tanneur, V., Kempe, D. S., Mack, A. F., Huber, S. M., Wieder, T., Lang, F. and Duranton, C. (2004) Inhibition of erythrocyte phosphatidylserine exposure by urea and Cl^-. *Am. J. Physiol. Renal Physiol.*, **286**, F1046–F1053.
27) Lang, K. S., Duranton, C., Poehlmann, H., Myssina, S., Bauer, C., Lang, F., Wieder, T. and Huber, S. M. (2003) Cation channels trigger apoptotic death of erythrocytes. *Cell Death Differ.*, **10**, 249–256.
28) Zhou, Q., Zhao, J., Wiedmer, T. and Sims, P. J. (2002) Normal hemostasis but defective hematopoietic response to growth factors in mice deficient in

phospholipid scramblase 1. *Blood*, **99**, 4030–4038.
29) Woon, L. A., Holland, J. W., Kable, E. P. and Roufogalis, B. D. (1999) Ca2+ sensitivity of phospholipid scrambling in human red cell ghosts. *Cell Calcium*, **25**, 313–320.
30) Lang, K. S., Myssina, S., Brand, V., Sandu, C., Lang, P. A., Berchtold, S., Huber, S. M., Lang, F. and Wieder, T. (2004) Involvement of ceramide in hyperosmotic shock-induced death of erythrocytes. *Cell Death Differ.*, **11**, 231–243.