Diminution of Hg(II)-induced cellular and DNA damage in human blood cells by uric acid

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Abstract

Mercury (Hg) is a widespread environmental pollutant and toxicant which induces multiple organ damage in humans and animals. Hg toxicity is mediated by the induction of oxidative stress in target cells. We have used uric acid (UA), a potent antioxidant found in biological fluids, to protect human red blood cells (RBC) and lymphocytes against Hg-mediated cell, organelle and geno-toxicity. RBC were incubated with HgCl_2 , an Hg(II) compound, either alone or in presence of UA. Incubation of RBC with only HgCl_2 increased production of nitrogen and oxygen radical species, enhanced methemoglobin levels, heme degradation, free ferrous iron, oxidation of proteins and membrane lipids and reduced antioxidant capacity of cells. Prior incubation of RBC with UA led to considerable UA concentration-dependent decline in formation of reactive oxygen and nitrogen species and also attenuated alterations in oxidative stress and biochemical parameters. UA enhanced the antioxidant capacity of RBC and restored metabolic, plasma membrane-bound and antioxidant enzyme activities. Scanning electron microscopy showed that UA prevented HgCl_2 -mediated morphological changes in RBC. HgCl_2 dissipated the mitochondrial membrane potential and increased lysosomal membrane damage in lymphocytes, but UA pre-treatment attenuated these effects. Genotoxicity analysis by comet method showed that UA protected lymphocyte DNA from HgCl_2 -induced damage. Importantly, UA itself did not exhibit any deleterious effects in either RBC or lymphocytes. Thus, UA protects human blood cells from Hg(II) -mediated oxidative damage reducing the harmful effects of this extremely toxic metal. We suggest that UA performs a similar protective role in the plasma against heavy metal toxicity.

Introduction

Mercury (Hg) is a toxic heavy metal that is naturally present in the environment and also as an anthropogenic contaminant (Pirrone et al. 2009). It is widely dispersed in nature; the sources of Hg pollution are mainly the burning of garbage and fossil fuels, chloride-alkaline industry and mining (Kim et al. 2016). Hg and its compounds have applications in chemical laboratories, hospitals, dental clinics and in industries producing fluorescent light bulbs, explosives and batteries (Raj and Maiti 2019). Hg is extremely toxic and exposure to even small amounts causes serious health problems. Poisoning caused by Hg, or its compounds, is a major worldwide concern. WHO considers Hg among the ten chemicals most dangerous to public health (Bjorklund et al. 2017). Hg affects the digestive, renal and immune systems and causes neurological diseases such as Alzheimer's and Parkinson's (Miller et al. 2013; Carocci et al. 2014; Maqbool et al. 2017). Hg exists in inorganic, elemental and organic forms that differ widely in their reactivity and toxicity (Clarkson and Magos 2006). Elemental and methylmercury are harmful to the nervous, digestive and immune systems, lungs and kidneys. Inorganic mercury is highly toxic to the skin, eyes, damages the gastrointestinal tract and kidney.

Mercuric chloride (HgCl_2), an inorganic salt of divalent mercury [Hg(II)], is the major form of inorganic mercury in soil. It is easily absorbed by plants and deposits in various parts, affecting their growth and development (Azavedo and Rodriguez 2012). HgCl_2 was earlier used for photographic film development and in some skin lightening creams. HgCl_2 enters the cell where it enhances reactive oxygen species (ROS) production, which can modify the structure and function of several cell components. Studies on molecular mechanism of Hg toxicity suggest that reactive oxygen species (ROS) play a major role in it (Crespo-López et al. 2009; Park and Park 2007; Yang et al. 2020). Hg disrupts functioning of mitochondria resulting in ROS production. This is accompanied by lowering of thiol content and activation of caspases which can result in apoptosis (Shenker et al. 2002). HgCl_2 -induced oxidative damage in rat tissues, under both in vivo and in vitro conditions, has been previously reported (Lund 1991). Hg-induced free radical generation in kidney results in oxidation of lipids, proteins and DNA (Nath et al. 1996). Due to its high affinity for sulfhydryl groups, Hg(II) easily binds to enzymes and proteins (Ynalvez et al. 2016). HgCl_2 damages human brain tissue, at 100 μM concentration (Lohren et al. 2015). Submicromolar concentrations of HgCl_2 were shown to be neurotoxic, inhibiting many metabolic events in the brain (Fox et al. 1975). They also depress the uptake of the excitatory neurotransmitters such as glutamate leading to neuronal damage (Albrecht and Matyja 1996).

Uric acid (UA) is a strong antioxidant (AO) found in all biological fluids. It is the major AO in blood plasma (Song et al. 2019) and in secretions of the human nasal airway (Peden 1990). UA possesses radical chain breaking activity and is highly effective in scavenging various forms of free radicals like hydroxyl, superoxide and peroxynitrite (Becker et al. 1991). UA also reduces free radical generation by increasing the synthesis of glutathione (GSH), a non-enzymatic AO (Aoyama et al. 2011). In presence of free radicals UA forms anion, urate radical, which is efficiently quenched by vitamin C (Cutler 1984). UA also forms complex with free iron and suppresses its reaction with hydrogen peroxide (Fenton reaction), a reaction that generates hydroxyl radical, the most reactive and damaging ROS (Davies et al. 1986). UA also scavenges peroxynitrite, formed when superoxide radical reacts with nitric oxide; peroxynitrite induces neuronal damage in amyotrophic lateral sclerosis, Alzheimer's and Parkinson's diseases (Squadrito et al. 2000). UA has beneficial role in allergic encephalomyelitis in whose pathogenesis peroxynitrite is involved (Hooper et al. 1998). UA was shown to have protective role in ischemic renal and brain injury in mice since the damage was considerably lowered in UA administered animals (Yu et al. 1998). Diminished UA level is linked to type I diabetes (Nan et al. 2007) and multiple sclerosis (Hooper et al. 2000).

Plasma concentration of UA (0.15-0.45 mM) is much higher than that of other plasma AOs like vitamin C (Sevanian et al. 1985). UA enters the red blood cells (RBC) by active transport (Lucas-Heron and Fontenaille 1979) and can protect them against peroxidative damage and cell lysis (Muraoka and Miura 2003). We have earlier reported that HgCl_2 enhances formation of ROS and reactive nitrogen species (RNS) and increases hemoglobin (Hb) oxidation in human RBC. It lowers the AO capacity and inactivates plasma membrane redox system (PMRS) (Ahmad and Mahmood 2019). Here, we have used UA to protect human RBC and lymphocytes against HgCl_2 -mediated toxicity under in vitro conditions. This was done since UA can possibly attenuate HgCl_2 induced oxidative damage using its AO property. We show for the first time that UA, at physiological concentrations, lowers the hemotoxicity of HgCl_2 . The implications of this protective effect are discussed.

Methods

Preparation of RBC and treatment with uric acid and HgCl_2

Young (20–29 year-old), non-smoking and healthy volunteers were used as donors and informed consent was taken from all of them. Venous blood was taken in heparin coated glass tubes and RBC prepared as previously described (Qasim and Mahmood, 2015). Briefly, blood was spun at 1,200 rpm for 10-12 min at 4 °C, supernatant removed, packed RBC washed thrice with phosphate buffered saline (PBS; 0.9% NaCl in 10 mM sodium phosphate buffer, pH 7.4) and suspended in PBS to give a 10% (v/v) cell suspension or hematocrit.

UA and HgCl_2 were purchased from Sigma Aldrich, USA. The 10% hematocrit was first incubated with UA (0.3, 0.4 and 0.6 mM) for 30 min at 37 °C, then 0.05 mM HgCl_2 was added and cell suspensions kept for another 60 min at 37 °C. Control RBC were not incubated with UA and HgCl_2 and kept at 37 °C for 90 min. RBC incubated with 0.05 mM HgCl_2 for 60 min at 37°C served as positive control while those treated with 0.6 mM UA for 90 min at 37 °C showed UA alone effects. After the 37 °C incubations, cell suspensions were spun for 10-12 min at 2600 rpm and the pellets carefully washed thrice with PBS. RBC pellets were resuspended in 10x volume of 5 mM sodium phosphate buffer, pH 7.2, and kept for 2 h at 4 °C to lyse the cells. The samples were spun at 3000 rpm for 12 min, and the hemolysates (supernatants) were either used at once or aliquoted and kept at -20 °C for later use.

Hemoglobin and methemoglobin (MetHb) levels and MetHb reductase

The cyanomethemoglobin method was employed to determine hemoglobin concentration in hemolysates (Drabkin and Austin 1935). Hemolysate absorbance at 630, 576 and 540 nm was taken and MetHb level determined from the equation given below (Benesch et al. 1973):

$$[\text{MetHb}] = [2.985 A_{630} + 0.194 A_{576} - 4.023 A_{540}] \times 10^{-4} \text{ moles per liter}$$

MetHb reductase activity was determined by adding 0.05 ml hemolysate to 0.1 mM of 2,6-dichlorophenolindophenol and 0.1 mM reduced nicotinamide adenine dinucleotide (NADH) and monitoring the enhancement in absorbance of solution at 600 nm (Kuma et al., 1972).

Heme degradation and free iron release

Degradation of heme was assayed in 50x diluted hemolysates by monitoring fluorescence emission at 480 nm, after excitation at 321 nm (Nagababu et al. 2008). The release of iron (Fe^{2+}) was quantified in hemolysates using ferrozine that chelates free Fe^{2+} ions to form an adduct that absorbs at 550 nm (Panter, 1994).

Reactive oxygen species (ROS)

Intracellular generation of ROS was monitored by dichlorodihydrofluorescein diacetate (DCFH-DA) while DHE assay was used to detect superoxide radicals (Keller et al., 2004; Wojtala et al. 2014). The 10% RBC suspension was mixed with 10 μM DCFH-DA or DHE, kept in 37 °C water bath for 60 min and then centrifuged. Supernatants were discarded, cell pellets rinsed thrice with PBS and RBC suspended in PBS to again give 10% hematocrit. Cell suspensions were incubated with UA (0.3, 0.4, 0.6 mM) for 30 min, then 0.05 mM HgCl_2 was added and RBC left for 15 min at 37 °C. Fluorescence of samples was recorded, setting the excitation and emission wavelengths at 485 and 530 nm (DCFH-DA) or 508 and 605 nm (DHE), respectively.

Reactive nitrogen species (RNS)

Peroxynitrite formation was monitored by adding 15 μM folic acid (final concentration) to hemolysates and after 5 min precipitating proteins with NaOH-ZnSO_4 . The samples were microfuged at top speed, the supernatants removed and their fluorescence noted at 460 nm after excitation at 380 nm (Huang et al., 2007). Nitric oxide (NO) was quantified from the total nitrite and nitrate content. Proteins were first precipitated by $\text{ZnSO}_4\text{-NaOH}$ and removed by centrifugation.

Vanadium(III) chloride was added to supernatants to convert nitrate to nitrite. This was followed by addition of Greiss reagent which reacts with nitrite to give chromophoric azo-derivative that absorbs light at 540 nm (Miranda et al., 2001). A calibration curve was simultaneously constructed using sodium nitrite.

Oxidative stress markers

Oxidative stress parameters were measured in hemolysates. Carbonyl groups, introduced during protein oxidation, give hydrazone adducts with 2,4-dinitrophenylhydrazine that absorb at 360 nm (Levine et al.1990). Advanced oxidation protein products (AOPP) were quantified by adding 0.2 M citric acid and 1.16 M potassium iodide to hemolysates and recording absorbance at 340 nm. A calibration curve was constructed using different concentrations of Chloramine T (Hanasand et al. 2012). Malondialdehyde, a marker and end product of lipid peroxidation (LPO), was quantified from its reaction with thiobarbituric acid. The absorbance of pink chromophore formed was recorded at 531 nm (Buege and Aust 1978).

Sulfhydryl groups react with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and form yellow thionitrobenzoate anion (TNB) that absorbs at 410 nm (Sedlak and Lindsay, 1968). Glutathione (GSH) concentration was measured by reaction of its sulfhydryl group with DTNB. Hemolysate proteins were first precipitated by metaphosphoric acid containing reagent and removed by centrifugation. DTNB was added to supernatants and the absorbance of TNB anion was noted at 410 nm (Beutler, 1984).

Total antioxidant capacity

Methods based on metal ion reduction and free radical quenching by sample AOs were employed. Hemolysates were used in the assays.

a) Metal reduction

In the ferric reducing ability of plasma (FRAP) method, sample AOs convert Fe^{3+} to Fe^{2+} and the Fe^{2+} ions give a colored complex with 2,4,6-tris(2-pyridyl)-s-triazine (Benzie and Strain 1996). In the cupric reducing antioxidant capacity (CUPRAC) assay, Cu^{2+} ions are reduced to Cu^{+} ; neocuproine then forms a colored complex with Cu^{+} (Çekiç et al., 2012). In the phosphomolybdenum method, sample AOs reduce Mo^{6+} to Mo^{5+} and absorbance of the green phosphate/ Mo^{5+} product formed was recorded at 695 nm (Prieto et al., 1999).

b) Free radical quenching

In the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, the purple DPP• radical is converted to light yellow non-radical form by H atom donated by sample AOs. The absorbance of solutions was noted at 517 nm (Mishra et al. 2012). In the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method, potassium persulfate converts ABTS to its colored radical form. Sample AOs donate electron to change $\text{ABTS}^{\cdot+}$ to the non-radical colorless ABTS which was monitored from the decrease in absorbance of solution at 734 nm (Re et al., 1999). Trolox was used as standard and results expressed as Trolox equivalents.

Antioxidant enzymes

The specific activities of RBC AO enzymes were determined in hemolysates. Cu–Zn superoxide dismutase (SOD) inhibits the auto-oxidation of pyrogallol which decreases the formation of colored product (Marklund and Marklund, 1974). The enzyme activity was determined by following the change in absorbance of pyrogallol solution at 420 nm in presence and absence of the enzyme. Catalase was assayed using H_2O_2 as substrate. The enzyme catalyzes breakdown of H_2O_2 to water and molecular oxygen which decreases the absorbance of solution at 240 nm (Aebi, 1984). Glutathione reductase cleaves the disulfide bond of oxidized glutathione to give GSH, using NADPH as the reductant. The resulting decrease in absorbance at 340 nm was followed for 3 min (Mannervik and Carlberg, 1985). Thioredoxin reductase activity was monitored from the increase in absorbance at 410 nm upon reduction of disulfide bond of DTNB, in presence of NADPH, to give TNB (Tamura and Stadtman, 1996). Glutathione peroxidase converts peroxides to water or alcohol using NADPH as reductant. The resulting decrease in 340 nm absorbance of solution was monitored for 3 min (Flohé and Günzler, 1984). Glutathione-S-transferase (GST) was assayed as described earlier by Habig et al. (1974); GSH and 1-chloro-2,4-dinitrobenzene were used as substrates.

Metabolic enzymes

All enzymes were assayed in hemolysates. The activity of hexokinase was measured by a two enzyme reaction using glucose 6-phosphate dehydrogenase (G6PD) (Bergmayer et al. 1983). Pyruvate kinase was also assayed by a coupled enzymatic reaction involving lactate dehydrogenase. The reaction converts NADH to NAD^{+} which leads to decrease in the absorbance of solution at 340 nm; this was monitored spectrophotometrically for 5 min (Bergmeyer, 1974). Lactate dehydrogenase, in presence of sodium pyruvate, converts NADH to NAD^{+} decreasing absorbance at 340 nm (Khundmiri et al., 2004). Activity of G6PD was determined from the enzymatic conversion of NADP^{+} to NADPH, enhancing the absorbance at 340 nm (Shonk and Boxer 1964). AMP-deaminase liberates ammonia from AMP. The ammonia reacts with phenol-sodium nitroprusside and alkaline hypochlorite to produce a blue indophenol (Pederson and Berry, 1977). The 5'-nucleotidase activity was assayed by quantifying inorganic phosphate released from AMP using arsenomolybdic acid reagent (Heppel and Hilmoe, 1951). Assay of glyoxalase-I involves mixing methylglyoxal and glutathione to form hemithioacetal. When enzyme (hemolysate) is added, it converts hemithioacetal to S-lactoyl glutathione which absorbs at 240 nm (Arai et al. 2014).

Membrane bound enzymes

Acetylcholinesterase was assayed in hemolysates using S-acetylthiocholine iodide and DTNB as substrates and recording absorbance of TNB anion formed at 410 nm (Ellman et al. 1961). Na,K-ATPase was assayed by quantifying the inorganic phosphate, formed upon ATP hydrolysis, in absence and presence of 1 mM ouabain (Bonting et al, 1961). The difference between the two gives Na,K-ATPase activity and inorganic phosphate concentration in absence of ouabain yields total ATPase activity.

PMRS and AFR reductase

In the plasma membrane redox system (PMRS) assay, 1 volume of packed RBC was suspended in 9 volumes of PBS containing 5 mM glucose and 1 mM potassium ferricyanide. The cell suspensions were left for 30 min at 37 °C (Rizvi and Srivastava, 2010) and then spun for 10 min at 2000 rpm. The supernatants were analyzed for sodium ferrocyanide content by adding 1,10-phenanthroline and reading sample absorbance at 510 nm (Avron and Shavit, 1963). The method of May et al. (2004) was used to assay ascorbate free radical (AFR) reductase in hemolysates.

Scanning electron microscopy

Pellets of control and treated RBC were carefully rinsed thrice with PBS. Then 0.05 ml cells were suspended in 2.5% glutaraldehyde (fixing agent) and left at room temperature for 1 h. After another wash with PBS, the cell suspensions in PBS were carefully layered on glass slides, air dried and treated with increasing concentrations of ethanol (50%-70%-90%-100%). The dehydrated cells were dried, covered with a 10 nm thick gold-palladium layer and RBC visualized under a scanning electron microscope at 1500 fold magnification (Wang et al., 2009).

Lymphocyte isolation and comet assay

Blood (3 ml) was diluted with 3 ml of 0.9% NaCl (saline), carefully layered over 2 ml Histopaque 1077 and spun for 20 min at 4 °C and 2600 rpm. Lymphocytes present in buffy white layer in the middle were carefully removed and recentrifuged at 2600 rpm for 10 min. The lymphocyte pellets were washed and a 10% suspension prepared in saline. This was then incubated with HgCl₂ and UA at 37 °C as mentioned above for RBC.

The comet assay of Singh et al. (1988) was used to detect DNA strand scission but with slight alterations. Briefly, 0.5% low melting point agarose and lymphocyte suspensions were mixed and spread on 1% agarose coated frosted glass slides. After a coat of low melting point agarose, the slides were left on ice for 10 min. Cells were lysed by adding solution containing 2.5 M NaCl, 100 mM EDTA, 10 mM Tris-HCl, 1% Triton-X-100, pH 10.0, and left on ice for 3 h. Slides were immersed for 20 min in ice cold electrophoresis solution (1.5 mM EDTA, 0.42 M NaOH, 0.9% NaCl); the high pH of this solution allows scission of alkali-labile sites and DNA uncoiling. Gel electrophoresis was performed for 20 min at 4 °C (300 mA, 25 V at 0.8 V/cm). Later, slides were dipped in 0.4 M Tris-HCl, pH 7.5, the DNA stained by ethidium bromide and visualized under a fluorescence microscope (CX41, Olympus, Japan) at 100x magnification. Images of 50 cells from every slide were scored. An automated image analysis system (Komet 5.5, Kinetic Imaging, Liverpool, UK) was used to determine comet tail lengths.

Lysosomal membrane destabilization and mitochondrial membrane potential (MMP) in lymphocytes

Equal volumes of 10% lymphocyte suspension and 5 µM acridine orange were mixed and samples left for 10 min at 37 °C. After centrifugation, the cell pellets were rinsed with saline and fluorescence of diffused acridine orange dye, upon lysosomal membrane damage, was recorded at 540 nm after excitation at 470 nm (Pourahmad et al., 2011).

The MMP was also determined fluorometrically in lymphocytes. Control, HgCl₂ alone, UA alone and UA + HgCl₂ treated lymphocytes (0.2 ml) were mixed with 0.1 ml of 5 µM Rhodamine 123 and left at room temperature for 15 min in the dark. Dye fluorescence was monitored at 525 nm after excitation at 505 nm (Pourahmad et al., 2009).

Results

The cyto- and geno-toxic effects of HgCl_2 were studied in presence and absence of UA. Human RBC and lymphocytes were first incubated with 0.3, 0.4 and 0.6 mM UA at 37°C for 30 min, then 0.05 mM HgCl_2 was added and samples kept at 37°C for 1 h. HgCl_2 at 0.05 mM was selected since our previous results (Ahmad and Mahmood, 2019) showed that HgCl_2 at this concentration produces quantifiable and measurable changes in the parameters to be determined so that the protective effect of UA could be analyzed. UA concentrations used are similar to those present in blood plasma. There were 4 groups of cells: HgCl_2 untreated (control), UA alone, HgCl_2 alone and UA+ HgCl_2 . After centrifugation, excess HgCl_2 and UA in supernatants were discarded and cells lysed by low salt buffer. Different parameters were studied in whole cells or cell lysates from the four groups.

MethHb and MethHb reductase

The ferrous ion bound to heme of Hb is easily converted to ferric form to give MetHb that cannot transport oxygen. The enzyme NADH-dependent MetHb reductase maintains low MetHb level in RBC by converting Fe^{3+} back to Fe^{2+} . MetHb level and MetHb reductase activity were increased in RBC incubated with HgCl_2 alone. These changes were significantly restored in the UA+ HgCl_2 group in a UA dose-dependent manner (Fig. 1). Incubation of RBC with only UA did not change MetHb content or alter MetHb reductase activity.

Heme degradation and free iron release

Heme degradation was enhanced in 0.05 mM HgCl_2 treated RBC as shown by increased fluorescence of hemolysate, which was 2.5 fold of the control value (Fig. 2A). Heme degradation is due to heme protein damage and results in release of bound iron (Fe^{2+}). The free iron is chelatable and forms coloured complex with ferrozine. Free iron concentration was also elevated in HgCl_2 treated cells. UA, in a concentration-dependent manner, protected heme proteins from this damage and free iron level in hemolysates was lowered in the UA+ HgCl_2 group, compared to only HgCl_2 treated RBC (Fig. 2B). Heme degradation and free iron in UA alone samples were almost similar to the control values.

ROS and RNS

The intracellular formation of ROS was examined using DCFH-DA and DHE. Both compounds enter the cell by passive diffusion where they are oxidized by ROS to yield fluorescent products. DCFH-DA represents a general probe for ROS (Keller et al., 2004) but DHE is specific for only superoxide radical (Wojtala et al., 2014). In both assays, higher fluorescence was seen in HgCl_2 alone treated RBC indicating increased formation of ROS. UA, in a concentration dependent manner, lowered ROS generation since fluorescence intensity was less in the UA+ HgCl_2 group, compared to HgCl_2 alone treated cells (Fig. 3A and 3B).

Peroxynitrite (ONOO^-) and NO were used to monitor RNS generation. At 0.05 mM HgCl_2 , the NO level was 5.2 fold of the control value. Reaction of folic acid and peroxynitrite generates a highly fluorescent complex which is used to follow peroxynitrite generation. The HgCl_2 alone incubated RBC exhibited three times higher fluorescence than control showing increased levels of peroxynitrite. HgCl_2 -induced increase in the production of both NO and peroxynitrite was considerably reduced by UA in a concentration-dependent manner (Fig. 4A and 4B). The fluorescence of 0.6 mM UA alone sample was similar to control showing that UA does not affect intracellular generation of ROS and RNS.

Oxidative stress parameters

Hemolysates were assayed for non-enzymatic indicators of oxidative stress condition and modification of cellular components. These include oxidation of proteins, lipids and thiols (Table 1). Exposure of RBC to HgCl_2 alone enhanced

protein oxidation shown by increased level of carbonyl groups. AOPP consist of numerous chromophoric products of protein oxidation and their level was also increased. An increase in LPO, as determined from the concentration of its marker malondialdehyde, was also seen in HgCl_2 alone treated RBC. Content of total free sulfhydryl groups and GSH in HgCl_2 incubated RBC was dramatically lowered to less than half of control values but both were significantly restored in the UA + HgCl_2 group. Protein oxidation, AOPP, LPO and total SH content in the 0.6 mM UA+0.05 mM HgCl_2 samples were very similar to control values. In fact, LPO, AOPP and protein oxidation were even less than the control values in the 0.6 mM UA alone RBC (Table 1). Importantly, UA exerted no harmful or toxic effects even at 0.6 mM, highest concentration used, while significantly attenuating HgCl_2 -mediated oxidation of biomolecules.

Antioxidant capacity

AOs in samples (hemolysates) can donate electron and/or H atom which can convert redox active metal ions to their corresponding lower oxidation states and also scavenge free radicals. The metal reducing as well as free radical quenching power of the cell is an indicator of its AO capacity. The reduction of metal ions by AOs in hemolysates was examined by FRAP, CUPRAC and phosphomolybdenum methods in which ferric ions are reduced to ferrous form, cupric to cuprous and Mo^{6+} to Mo^{5+} , respectively. Treatment of human RBC with HgCl_2 significantly reduced their ability to reduce these three metal ions (Table 2). The scavenging of free radicals, determined by DPPH and ABTS methods, also declined significantly in HgCl_2 exposed cells. Prior incubation with UA mitigated this lowering of metal reducing and radical scavenging capacity of RBC (Table 2). These assays also show that AO capacity in UA alone samples was insignificantly different from the corresponding control values.

Antioxidant enzymes

All aerobic cells are protected from damage by a robust enzymatic AO defense system comprised of several enzymes. Treatment of RBC with HgCl_2 alone inhibited the activities of SOD, thioredoxin reductase, glutathione reductase, catalase and glutathione peroxidase (Table 3). These alterations were significantly mitigated by UA. In fact, the presence of 0.6 mM UA restored the HgCl_2 -induced changes in enzyme activities to almost control values. UA itself did not alter the specific activity of any AO enzyme.

Membrane bound enzymes

Damage to RBC membrane was monitored from spectrophotometric assay of bound enzymes ($\text{Na}^+\text{K}^+\text{ATPase}$ and acetylcholinesterase), PMRS and AFR reductase. The activity of acetylcholinesterase greatly decreased in presence of 0.05 mM HgCl_2 and was one third of control value. Total ATPase and $\text{Na}^+\text{K}^+\text{ATPase}$ were also significantly inhibited and were less than 50% of control (Table 4). Inhibition of these enzymes was greatly overcome by pre-incubation of RBC with UA (Table 4). Similarly, the activity of PMRS declined in RBC treated with HgCl_2 alone, as compared to control values. Pre-treatment of RBC with UA significantly attenuated this inhibition (Fig. 5A). Treatment of RBC with HgCl_2 led to 50% inhibition of AFR reductase activity but this decline was also mitigated in presence of UA (Fig. 5B).

Metabolic enzymes

Specific activities of metabolic enzymes, especially of glucose metabolism, were determined next. RBC exposure to HgCl_2 reduced the activity of glycolytic enzymes hexokinase and pyruvate kinase by 1.75 and 3.0 fold, respectively, while activity of lactate dehydrogenase decreased 2.5 fold. G6PD activity was inhibited and was almost half of control (Table 5). The activity of 5'-nucleotidase was similarly inhibited to 50% of control. In contrast, the activities of carbonyl stress marker glyoxylase-I and RBC oxidative stress indicator AMP deaminase were elevated 5.3 and 3.1 times respectively. UA significantly attenuated the Hg-mediated alterations in the specific activities of these metabolic enzymes, restoring them close to control values (Table 5).

Scanning electron microscopy

Scanning electron microscopy was used to visualize the protective effect of UA against HgCl_2 -mediated changes in cell morphology. The control group had the normal biconcave shape of RBC (Fig. 6A) which was not altered upon incubation of cells with 0.6 mM UA alone (Fig. 6B). In RBC exposed to 0.05 mM HgCl_2 , large number of echinocytes were seen (Figure 6C). However, in RBC treated with 0.05 mM HgCl_2 and 0.3 UA, more RBC showed normal shape and at 0.6 mM UA only few echinocytes were present (Fig. 6D and 6E).

Lymphocyte DNA damage

Nuclear DNA damage by HgCl_2 in lymphocytes and the protective effect of UA were analyzed by the comet assay. This is a sensitive method and detects even single strand scission in DNA which, upon electrophoresis of whole cells, gives rise to a 'comet' whose tail length is proportional to the extent of DNA damage. Due to increased degradation and strand scission, more DNA was present in comet tail in HgCl_2 treated RBC; the tail length was 3.7 times the control. Much smaller comet tail lengths were seen in the UA + HgCl_2 group indicating UA protects lymphocytes from the genotoxic effects of HgCl_2 (Fig. 7). UA itself did not damage lymphocyte nuclear DNA and the comet tail length was slightly smaller than control.

Lysosomal membrane destabilization and mitochondrial membrane potential

Acridine orange fluoresces at acidic pH caused by lysosomal membrane damage. Lymphocytes in the control, UA alone, HgCl_2 alone and UA+ HgCl_2 groups were first loaded with the fluorescent dye acridine orange, washed to remove excess dye and their fluorescence noted. Treatment with HgCl_2 alone led to significant damage to lysosome membrane. In 0.05 mM HgCl_2 treated lymphocytes, the dye fluorescence was 3.8 fold of control. The presence of UA led to considerable decline in acridine orange fluorescence suggesting less lysosomal damage in the UA+ HgCl_2 group when compared to HgCl_2 alone (Fig. 8).

The effect of HgCl_2 on MMP was studied using the fluorescent dye Rhodamine 123 which concentrates in mitochondria of cells. Incubation with HgCl_2 alone led to considerable decline in MMP of lymphocytes and it decreased to half the control value (Fig. 9). However, in presence of 0.6 mM UA, the MMP was significantly restored in lymphocytes.

Discussion

Mercury is a highly toxic and environmentally widespread heavy metal which greatly affects both human health and the ecosystem (Driscoll et al. 2013). HgCl_2 is an inorganic form of mercury which is used in production of batteries and fluorescent light bulbs. HgCl_2 is an environmental toxicant that damages multiple organs in humans, especially intestine, kidney, liver and nervous system (Zalups 2000; Stacchiotti et al. 2003). Blood is also an important target of Hg that enters the human body via any route and RBC, the most abundant human cells, are quite susceptible to its adverse effects. We have previously reported the cytotoxic effect of HgCl_2 on RBC (Ahmad and Mahmood, 2019). Here the protective role of UA on HgCl_2 -mediated cyto-, organelle- and geno-toxicity was studied. Studies on the prevention and diminution of HgCl_2 -induced toxicity are important given the wide use of HgCl_2 in various industries and risk of human occupational exposure.

Hemoglobin is a primary target of ROS and toxicants like heavy metals because of its high concentration in RBC (≥ 95 percent of total cellular protein). Treatment of RBC with HgCl_2 significantly enhanced Hb oxidation thereby increasing MetHb levels. The formation of MetHb, which generates superoxide radical, is considered a marker of oxidative stress in RBC (Ribarov 1984; Winterbourn 1990). Under normal conditions, low MetHb level is maintained in RBC by MetHb reductase which converts Fe^{3+} to Fe^{2+} thereby regenerating Hb, with NADH being the reducing agent. Surprisingly, specific activity of MetHb reductase was enhanced in HgCl_2 -incubated RBC, probably as an adaptive response to high MetHb

levels. Pre-treatment of RBC with UA greatly restored MetHb reductase activity and MetHb level in a concentration-dependent manner. ROS mediated damage to Hb can degrade heme, which serves as an indicator of oxidative stress in RBC (Nagababu et al. 2008). Incubation of RBC with HgCl_2 enhanced degradation of heme, accompanied by the release of free chelatable iron. The free ferrous iron reacts with H_2O_2 to produce hydroxyl radical, the most damaging ROS. Hb instability, with heme degradation and increase in free iron, causes membrane damage (Comporti et al. 2002). UA prevented Hb oxidation, heme degradation and free iron level also declined.

ROS readily react with sulfhydryl groups and, therefore, total SH group content declined upon HgCl_2 treatment. GSH is an abundant tripeptide (glutamate-cysteine-glycine) AO which provides reducing environment inside the RBC and protects them from oxidative damage. GSH level also declined significantly in HgCl_2 treated cells, probably upon oxidation of its cysteine sulfhydryl group. ROS oxidize histidine, proline, arginine and lysine side chains in proteins. They also generate cross-linked protein oxidation products called AOPP, recent markers of oxidative damage in cellular proteins. ROS oxidize unsaturated membrane lipids also, giving lipid peroxides. LPO alters integrity and function of biomembranes (Yonaha et al. 1980). Treatment of RBC with HgCl_2 alone increased carbonyl groups, AOPP, LPO and lowered thiol content. These alterations declined greatly upon pre-incubation of RBC with UA. These results show that HgCl_2 increased production of ROS, leading to oxidative stress and oxidative modification of cell components. The presence of UA in reaction significantly protected RBC against these changes.

Major AO enzymes such as SOD, thioredoxin reductase, glutathione reductase, catalase and glutathione peroxidase function as important front line defense system against ROS in aerobic cells. Treatment of RBC with HgCl_2 reduced the specific activities of all AO enzymes. Many of these enzymes are themselves inactivated by RNS and ROS (Bas et al. 2015). UA, probably by its radical scavenging activity, prevented the inactivation of all AO enzymes. Lowering of GSH level and inhibition of AO enzymes can impair the AO capacity of RBC. This was analyzed by numerous methods due to the diversity of AOs present in the cell. The methods employed analyze the ability of AOs to donate electron and H atom that reduce metal ions and scavenge free radicals. These methods showed that HgCl_2 reduced the AO capacity of RBC which was prevented by UA. This increase in AO capacity towards control values by UA will enhance the ability of RBC to neutralize and quench ROS and protect cell components from harmful effects of toxicant-mediated oxidative stress (Waring et al. 2001).

HgCl_2 treatment led to a decline in the activities of several enzymes of glycolysis (hexokinase, pyruvate kinase and lactate dehydrogenase) and pentose phosphate pathway (G6PD). This will lower ATP and NADPH levels. Reduced ATP concentration makes the RBC more vulnerable to oxidative damage. NADPH is the major cellular reducing agent. Low NADPH content will also affect thioredoxin reductase and glutathione peroxidase which use it as cofactor. UA incubation greatly prevented the inhibition of glucose metabolic pathways which will restore the cellular content of both ATP and NADPH. Oxidative stress also enhances the level of endogenous toxins like methylglyoxal that impair the glyoxalase system which protects RBC against carbonyl stress. Increase in AMP deaminase activity is an oxidative stress indicator in RBC (Tavazzi et al. 2001). Expectedly, specific activities of both AMP deaminase and glyoxylase-1 were enhanced in HgCl_2 treated RBC but UA pretreatment significantly brought both enzyme activities towards control levels.

Oxidation of membrane proteins and unsaturated lipids can inactivate membrane bound enzymes. HgCl_2 treatment inhibited acetylcholinesterase and $\text{Na}^+\text{K}^+\text{ATPase}$, well known markers of membrane damage in RBC. This might be caused by direct chemical modification of essential sulfhydryl group(s) by Hg(II) or enzyme inactivation by free radicals (Yoshida 1983; Aijafari 1995). RBC have a membrane bound PMRS that scavenges extracellular oxidants and is important in overcoming oxidative stress (Hyun et al., 2006). This system is required for normal energy homeostasis and changes in PMRS activity are used to follow oxidative stress in cells (Rizvi and Srivastava 2010). The PMRS and its associated AFR reductase activity were inhibited in HgCl_2 treated RBC. PMRS also regenerates plasma ascorbate, a major plasma AO. PMRS inactivation will lower the ROS quenching ability of not only RBC but also of blood plasma due to decreased

formation of extracellular ascorbate from AFR. Both PMRS and AFR reductase are inhibited by oxidation of their essential sulfhydryl groups by ROS (Pandey and Rizvi 2010). Another reason can be ROS-mediated plasma membrane damage which can potentially inhibit bound enzymes (Hazarika et al. 2001). UA strongly protected PMRS and AFR reductase activity against the inhibitory effect of HgCl_2 .

Scanning electron microscopy showed that HgCl_2 -treated RBC had numerous blebs on the surface and the cell changed from discoidal shape to echinocytes (spike cells). Membrane damage by LPO and oxidation of cytoskeleton proteins causes bleb formation (Shaw et al. 1991). Only few blebs and echinocytes were seen in the UA+ HgCl_2 group. Importantly, the UA alone incubated cells had the discoidal shape of control RBC. These SEM results agree and confirm those obtained in biochemical experiments i.e. UA protects RBC from damage by HgCl_2 but itself was not harmful to RBC.

Since RBC are devoid of nucleus and other organelles, DNA and organelle damage was studied in the nucleated lymphocytes. HgCl_2 alone caused lysosomal membrane damage which can cause cytosolic acidification and reduce secretion of damaged macromolecules. Mitochondria are unique sites of ROS production but themselves can be target of toxicants. The collapse of MMP due to HgCl_2 exposure can be the result of membrane damage in mitochondria by ROS-induced LPO, altering membrane stability. Dissipation of MMP causes mitochondrial dysfunction and lowers ATP generation; it can also lead to apoptosis (Araragi et al. 2003; Shenker et al. 1998). UA at its highest concentration (0.6 mM), significantly maintained lysosomal and mitochondrial membrane integrity showing that it exerts protective effect on organelles too.

Comet assay was used to analyze DNA degradation in lymphocytes (nucleated cell). DNA strand scission produces a 'comet' upon electrophoresis whose tail length is directly proportional to the extent of alkali-labile sites and single strand breaks in DNA. Previous studies have shown that Hg damages the DNA even at low concentrations (Betti et al. 1993; Grover et al. 2001). Direct chemical modification by generating ROS and free radicals is one of several mechanisms by which HgCl_2 damages DNA (Halliwell and Aruoma 1991). It can also work indirectly through free radicals generated by end products of LPO such as unsaturated aldehydes and malondialdehyde that can produce mutagenic lesions in DNA (Marnett 2002). Incubation of lymphocytes with HgCl_2 led to a concentration-dependent treatment increase in comet tail length but presence of UA led to shortening of comet tail. Thus comet assay showed that UA effectively protects human lymphocytes from HgCl_2 -induced DNA damage.

The above results strongly suggest that HgCl_2 generates ROS and induces oxidative stress condition inside the cell. This was confirmed by spectrophotometric and fluorometric analysis. Generation of intracellular ROS and specific ROS like superoxide radicals was greatly enhanced in HgCl_2 treated RBC, as shown by DCFH-DA and DHE assays. Similarly, RNS like NO and peroxynitrite were also elevated in Hg(II) exposed RBC. The long chain sequence of intracellular production of ROS and RNS in Hg(II) incubated RBC was likely hampered by UA which can act on various stages to lower their levels.

There are numerous ways through which UA can exert its protective effect on RBC and lymphocytes. First, UA can directly quench ROS (Sueishi et al., 2014). Second, it can function indirectly by increasing AO enzyme activity or GSH levels. Third, UA can chelate ferrous form of free iron and inhibit free radical generation by Fenton reaction. Fourth, it can directly quench peroxy radical (Kuzkaya et al. 2005) and also exhibits superoxide scavenging activity. Thus, UA attenuates the toxicological effect of Hg(II) in human RBC and lymphocytes, showing its potential effectiveness against Hg-mediated toxicity. To the best of our knowledge, this represents the first study in which the strong AO activity of UA has been shown to protect blood cells from metal-induced oxidative damage.

The protective effect of UA and consequent lowering of HgCl_2 -induced toxicity in human RBC and lymphocytes will have many beneficial results. 1. By lowering MetHb formation, UA will increase oxygen transport by blood. 2. Blood serves as a circulating AO and RBC, due to their abundance, have major role in it. The improved AO capacity of RBC will increase

capacity of circulating blood to scavenge oxidants in cells that come in contact with it. 3. The restoration of PMRS by UA will allow regeneration of plasma ascorbate and increase its AO capacity. 4. ROS leach out of damaged RBC and damage other blood cells and also those cells/tissues that contact it. By quenching ROS and RNS generation in RBC, UA will lower this oxidative damage. 5. Damaged RBC are rapidly cleared, mainly by the spleen (red cell senescence) resulting in anemia. By lowering damage, UA will increase RBC life span in blood. 6. Morphological changes impair the rheological properties of RBC, especially their ability to deform and pass through extremely thin capillaries (some having diameter narrower than RBC) resulting in lowered oxygen delivery to other cells/tissues. By greatly restoring the RBC morphology, UA will allow flow of blood and prevent occlusion of capillaries. 7. Finally, by protecting lymphocytes from HgCl₂-mediated damage, UA will improve the immune function of blood.

A schematic representation of the results is shown in Figure 10.

In conclusion, HgCl₂ generates oxidative stress condition in human RBC and lymphocytes. At physiologically relevant concentrations, UA reduces the HgCl₂-induced ROS and RNS and lowers its cytotoxicity and genotoxicity. Since UA is a biological AO, the protective effect must be due to its AO property. Thus UA likely plays a key role in mitigating Hg toxicity and protecting blood cells from the harmful effects of this metal, and probably other heavy metals too.

Declarations

Ethics approval and consent to participate The study involved human volunteers and was approved by the Ethics Committee of Aligarh Muslim University (Registration number 714/GO/Re/S/02/CPCSEA) which monitors research on human subjects. Informed consent was taken from all the volunteers involved in this study. (Not applicable)

Consent for publication (Not applicable).

Availability of data and materials All data generated or analysed during this study are included in this published article.

Code availability: I have used ANOVA software.

Consent to participate: Not applicable

Authors' contribution RM planned the study, analyzed and interpreted the data and checked the manuscript, SA did the bulk of the experiments and also wrote the manuscript, NT and NP did early experiments. All authors read and approved the final manuscript.

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Tables

Table 1 Protective effect of uric acid on HgCl₂-induced changes in some markers of oxidative stress in RBC.

	PC	AOPP	LPO	Total SH	GSH
Control	14.65±1.63	82.50±3.65	0.36±0.02	0.93±0.10	2.47±0.21
UA (0.6 mM)	13.50±1.04	73.29±6.43	0.28±0.02	0.84±0.08	2.56±0.22
HgCl ₂	34.77±2.39*	237.56±17.68*	1.68±0.11*	0.32±0.04*	1.05±0.09*
UA (0.3 mM) +HgCl ₂	29.56±2.27*	212.30±14.67*	1.31±0.09*	0.43±0.04*	1.31±0.14*
UA (0.4 mM) + HgCl ₂	23.88±2.80*	185.80±10.54*	1.01±0.09*	0.54±0.07*	1.56±0.17*
UA (0.6 mM) + HgCl ₂	17.00±1.57	115.33±8.17*	0.56±0.05*	0.71±0.08*	2.06±0.22

All parameters were assayed in hemolysates and results are mean ± standard error of six different samples. HgCl₂ concentration in all cases is 0.05 mM.

PC, AOPP, LPO and GSH and are in nmoles/mg Hb and total SH in μmoles/mg Hb.

*Significantly different from control at p<0.05.

PC, protein carbonyl; AOPP, advanced oxidation protein products; LPO, lipid peroxidation; GSH, glutathione; SH, sulfhydryl; UA, uric acid.

Table 2 Protective effect of uric acid on HgCl₂-induced changes in antioxidant capacity of RBC.

	FRAP	CUPRAC	PMG	DPPH	ABTS
Control	58.34±3.52	0.48±0.036	0.727±0.107	66.66±4.25	78.44±6.63
UA (0.6 mM)	57.33±3.40	0.42±0.037	0.664±0.085	68.00±4.82	74.84±6.21
HgCl ₂	25.28±1.82*	0.23±0.025*	0.306±0.022*	30.62±2.02*	36.78±2.70*
UA (0.3 mM) + HgCl ₂	32.59±2.10*	0.27±0.038*	0.334±0.029*	37.33±2.84*	44.55±2.98*
UA (0.4 mM) + HgCl ₂	43.14±3.53*	0.33±0.045*	0.402±0.044*	48.00±3.23*	52.70±3.45*
UA (0.6 mM) + HgCl ₂	51.76±4.90	0.36±0.041*	0.500±0.061*	56.45±3.87	65.55±4.86

All assays were done in hemolysates and results are mean ± standard error of six different samples. HgCl₂ concentration in all cases is 0.05 mM.

FRAP, CUPRAC and PMG are in nmoles/mg Hb. DPPH is in percent quenching of DPPH radical while ABTS results are expressed as µM TE/mg Hb.

Results are mean ± standard error of six different samples.

*Significantly different from control at p<0.05.

FRAP, ferric reducing ability of plasma; CUPRAC, cupric reducing antioxidant capacity; PMG, phosphomolybdenum green; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid; TE, Trolox equivalent; UA, uric acid..

Table 3 Protective effect of uric acid on HgCl₂-induced changes in activities of some antioxidant enzymes in RBC.

	SOD	CAT	GPx	GR	TR
Control	18.78±1.59	48.18±4.02	59.93±4.47	23.05±2.23	44.34±3.73
UA (0.6 mM)	17.64±1.41	47.66±3.85	58.33±4.12	22.72±2.10	41.77±3.54
HgCl ₂	8.93±0.56*	20.10±1.09*	24.66±1.45*	11.35±0.95*	15.56±0.98*
UA (0.3 mM) + HgCl ₂	10.61±0.89*	25.77±1.46*	35.60±2.82*	14.41±1.22*	19.97±1.04*
UA (0.4 mM) + HgCl ₂	12.30±1.14*	31.21±2.24*	43.13±3.85*	17.12±1.54*	27.46±2.24*
UA (0.6 mM) + HgCl ₂	14.40±1.21*	38.66±2.86*	52.66±4.83	20.47±1.88	34.00±2.35*

All enzymes were assayed in hemolysates and results are mean ± standard error of six different samples. HgCl₂ concentration in all cases is 0.05 mM.

SOD activity is in U/mg Hb and catalase in µmol/mg Hb/min. GPx, GR and TR activities are in nmol/mg Hb/min.

Significantly different from control at P <0.05.

SOD, Cu,Zn-superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; TR, thioredoxin reductase; UA, uric acid.

Table 4 Protective effect of uric acid on HgCl₂-induced changes in the activity of RBC plasma membrane bound enzymes.

	AChE	Total ATPase	Na,K- ATPase
Control	43.41±2.36	1.63±0.08	0.71±0.05
UA (0.6 mM)	42.21±2.12	1.58±0.08	0.65±0.05
HgCl ₂	12.85±1.50*	0.60±0.02*	0.33±0.02*
UA (0.3 mM) + HgCl ₂	18.41±1.67*	0.78±0.04*	0.40±0.02*
UA (0.4 mM) + HgCl ₂	25.42±1.75*	1.00±0.05*	0.54±0.03*
UA (0.6 mM) + HgCl ₂	32.00±1.88	1.33±0.06	0.60±0.04

All enzymes were assayed in hemolysates and results are mean ± standard error of six different samples. HgCl₂ concentration in all groups is 0.05 mM. AChE activity is in nmol/mg Hb/min while total ATPase and Na⁺ K⁺ ATPase are in μmol/mg Hb/h.

Significantly different from control at P <0.05.

AChE: acetylcholinesterase; Na⁺K⁺ATPase : sodium potassium adenosine triphosphatase; UA, uric acid.

Table 5 Protective effect of uric acid on HgCl₂-induced changes in the activity of some metabolic enzymes in RBC.

	HK	PK	LDH	G6PD	5-NT	GLO-1	ADA
Control	0.820±0.06	51.51±3.76	40.96±4.75	3.16±0.90	1.139±0.176	17.88±2.31	1.47±0.08
UA (0.6 mM)	0.802±0.05	53.66±3.64	37.21±4.16	2.96±0.80	1.10±0.160	15.60±2.18	1.62±0.10
HgCl ₂	0.466±0.01*	17.44±1.04*	16.22±1.68*	1.63±0.25*	0.619±0.07*	95.00±10.78*	4.58±0.25*
UA (0.3 mM) + HgCl ₂	0.505±0.03*	24.66±1.45*	19.70±2.00*	1.96±0.30*	0.712±0.113*	78.08±7.16*	3.99±0.241*
UA (0.4 mM) + HgCl ₂	0.544±0.04*	31.33±2.00*	24.18±2.76*	2.30±0.43*	0.824±0.132	69.98±5.26*	3.61±0.228*
UA (0.6 mM) + HgCl ₂	0.664±0.05*	39.07±2.63*	30.78±3.65*	2.66±0.69	0.930±0.158	45.84±3.10*	2.54±0.196*

All enzymes were assayed in hemolysates and results are mean $\pm$ standard error of six different samples. HgCl_2 concentration in all cases is 0.05 mM

PK, LDH, G6PD, and GLO-I activities are in nmol/mg Hb/min. HK and 5'-NT is in $\mu\text{mol/mg Hb/min}$ and ADA is in units/gm Hb.

Significantly different from control at $P < 0.05$.

HK, hexokinase; PK, pyruvate kinase; LDH, lactate dehydrogenase; G6PD, glucose 6-phosphate dehydrogenase; 5'-NT, 5'-nucleotidase; GLO-I, glyosylase I; ADA, AMP deaminase; UA, uric acid.

Figures

Figure 1

(A) Methemoglobin level and (B) methemoglobin reductase activity in RBC. Results are mean $\pm$ standard error of six different samples. *Significantly different from control at $p < 0.05$.

Figure 2

(A) Heme degradation and (B) release of free iron in RBC. Heme degradation was determined from fluorescence of hemolysates and is given as percent of control. Free iron was quantified in hemolysates using ferrozine and is given in nmoles/mg Hb. Results are mean $\pm$ standard error of six different samples. *Significantly different from control at $p < 0.05$.

Figure 3

ROS generation in human RBC studied by (A) dihydroethidium and (B) DCFH-DA methods. Fluorescence of samples is shown as percent of control. Results are mean $\pm$ standard error of six different samples. *Significantly different from control at $p < 0.05$.

Figure 4

RNS generation in RBC. (A) Nitric oxide and (B) peroxynitrite were determined in hemolysates using Greiss reagent and folic acid, respectively. Peroxynitrite fluorescence in samples is given as percent of control. Results are mean $\pm$ standard error of six different samples. *Significantly different from control at $p < 0.05$.

Figure 5

(A) PMRS and (B) AFR reductase activity in RBC. PMRS and AFR reductase were determined in packed RBC and hemolysates, respectively. PMRS is in μmoles ferrocyanoide/30 min /ml PRBC and AFR reductase in μmoles NADH oxidized/min/ml PRBC. Results are mean± standard error of six different samples. *Significantly different from control at p<0.05. PRBC- packed red blood cells, AFR- ascorbate free radical, PMRS- plasma membrane redox system.

Figure 6

Scanning electron microscopic images of human RBC. (A) Control (untreated) RBC. Cells treated with (B) 0.6 mM UA alone (C) 0.05 mM HgCl₂ alone (D) 0.3 mM UA+0.05 mM HgCl₂ (E) 0.6 mM UA+0.05 mM HgCl₂. Magnification is 1500 fold.

Figure 7

DNA damage in lymphocytes by comet assay. (A) Comet images of (a) Control (b) 0.6 mM UA (c) 0.05 mM HgCl₂ (d) 0.3 mM UA+ 0.05mM HgCl₂ (e) 0.4 mM UA + 0.05 mM HgCl₂ (f) 0.6 mM UA + 0.05 mM HgCl₂ (B) Comet tail length. The tail lengths of comets were determined by Komet 5.5 (Kinetic Imaging, UK) the image analysis system. Results are mean $\pm$ standard error of six different samples. *Significantly different from control at $p < 0.05$.

Figure 8

Lysosomal membrane damage in lymphocytes. Cells were loaded with acridine orange, washed and then fluorescence was recorded. Fluorescence of samples is shown as percent of control. Results are mean $\pm$ standard error of six different samples. *Significantly different from control at $p < 0.05$.

Figure 9

Mitochondrial membrane potential (MMP) in lymphocytes. The MMP was determined from fluorescence of Rhodamine 123 and is shown as percent of control. Results are mean $\pm$ standard error of six different samples. *Significantly different from control at $p < 0.05$.

Figure 10

Schematic representation of UA protection against Hg(II)-induced oxidative damage in human blood cells. Hg(II) enters the cell where it enhances the formation of ROS and RNS. UA can protect cells from the damaging effects of Hg(II), either by preventing the generation of ROS/RNS and/or directly quenching the ROS/RNS generated. This will prevent oxidation of proteins and lipids, damage to DNA and cell organelles (mitochondria and lysosomes). The overall result will be attenuation of Hg(II)-induced cytotoxicity and genotoxicity. (ROS, reactive oxygen species RNS, reactive nitrogen species Hb, hemoglobin UA, uric acid).