Hepatoprotective Activity of Liposomal Flavonoid against Arsenite-Induced Liver Fibrosis

Ardhendu K. Mandal, Subhankar Das, Mukul K. Basu, Rohini N. Chakrabarti, and Nirmalendu Das
Biomembrane Division, Indian Institute of Chemical Biology, Kolkata, India (A.K.M., S.D., M.K.B., N.D.); and Ashok Laboratory, Kolkata, India (R.N.C.)
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ABSTRACT
Arsenic, the environmental metalloid toxicant, is known to induce oxidative damage to liver and produce hepatic fibrosis. The theme of our study was to optimize and evaluate the therapeutic efficacy of galactosylated liposomal flavonoidal antioxidant, quercetin (QC), in combating arsenic-induced hepatic fibrogenesis. The rats of the hepatic damage group were injected s.c. a single dose of sodium arsenite (NaAsO₂) (100.06 μM/kg b. wt. in 0.5 ml of physiological saline). Hepatocytes and stellate cells were separated. Mitochondrial membranes were isolated from all those separated cells. Oxidative damage was monitored at different isolated subcellular parts of different hepatic cells. Liver fibrosis was also induced by the injection of NaAsO₂. Galactosylated liposomal QC injection before NaAsO₂ treatment checked fibrogenesis completely by protecting the liver from oxidative attack in cellular and subcellular levels. The maximal protections from hepatocellular and fatty metamorphosis, necrosis, Kupffer cell hyperplasia, fibrosis, and in the deposition of collagen contents were observed and confirmed by our histopathological and histochemical analysis when rats were treated with galactosylated liposomal QC before NaAsO₂ injection. Application of galactosylated liposomal QC may be a potent therapeutic approach for NaAsO₂-induced fibrogenesis through a complete protection against oxidative attack in cellular and subcellular parts of rat liver.

Arsenic, the naturally occurring metalloid, has been recognized as an environmental toxicant, and its contamination of drinking water is a serious environmental calamity worldwide because of the million of people at risk, particularly in developing countries such as West Bengal in India and Bangladesh (Chatterjee et al., 1995). An important feature in arsenic toxicity is in a form of hepatic fibrosis that causes portal hypertension (Guha Mazumder et al., 1998). However, although mechanism(s) by which arsenic induces hepatic fibrosis remains poorly understood, the common theme that was emerged is the role of reactive oxygen species (ROS) in the pathogenesis of arsenic-induced hepatic fibrosis. Hepatocyte necrosis itself may activate lipocytes. Such activation could be mediated by the lipid peroxides formed after the membrane of the hepatocyte is injured (Greenwel et al., 2000). The critical event in hepatic fibrosis is the activation of lipocytes (stellate, fat-storing, or Ito cell), the main source of extracellular matrix in fibrosis formation (Du et al., 1999). A complementary approach that delineates the NaAsO₂-induced contribution of ROS includes the use of antioxidants, superoxide dismutase (SOD), and catalase. However, introduction of either SOD or catalase can only suppress a little part of toxic oxygen species that is generated by the induction of NaAsO₂ (Kessel et al., 2002).

Nonenzymatic supplementation as a protective strategy has also resulted in conflicting outcomes in in vivo experiments. Administration of D-α-tocopherol to rats provided no protection against ROS in liver diseases. Therefore, the present challenge is to counter NaAsO₂-induced oxidative attack and to isolate a nontoxic antioxidant that can be selectively targeted to liver, which provides an even distribution in intrahepatic membrane.

Liposomes are accepted as potent drug carriers not only for their biocompatible nature but also for their negative biological responses in the biological system. Furthermore, because of the presence of galactosyl receptor on the surface of hepatocytes, galactosylated liposomes are effective in the site-specific drug delivery to hepatic tissue with a homogenous distribution.
Liposomes Intercalated with QC and others in forming monomethyl arsenous acid (Csanaky and report on rats because this species is known to be superior to isolated and purified from E. Merck (India) Ltd. (Mumbai, India). Quercetin was (Buchs, Switzerland), respectively, whereas chloroform and metha-
diphenyl hexatriene (DPH), Triton X-100, and glutathione reductase were purchased from Sigma Chemicals (St. Louis, MO). Sodium arsenite (Merck, Darmstadt, Germany) was used for experimental oxidative damage in cellular or subcellular parts of hepatic tissue and provide a protective mechanism against liver fibrosis.

Despite the fact that rats, unlike other mammals, retain the dimethyl arsenic acid, one of the intermediate metabo-
ites of NaAsO₃ in the blood, studies were performed in this report on rats because this species is known to be superior to others in forming monomethyl arsenous acid (Csanaky and Gregus, 2002). Monomethyl arsenous acid is most toxic among other NaAsO₃ metabolic intermediates to rats as well as human hepatocytes (Styblo et al., 2000). The purpose of our in vivo studies was to ascertain whether treatment with QC intercalated in galactosylated liposomes exerts any hepatoprotective effect against NaAsO₂-induced fibrosis in liver.

Materials and Methods

Materials

Phosphatidylethanolamine (PE), cholesterol, dicetyl phosphate (DCP), p-amino phenyl α-D-galactoside, glutaraldehyde, collagenase, diphenyl hexatriene (DPH), Triton X-100, and glutathione reductase were purchased from Sigma Chemicals (St. Louis, MO). Sodium arsenite (Merck, Darmstadt, Germany) was used for experimental purposes. Chloramine-T, Fast Green FCF, and Sirius Rose BB were obtained from Loba Chemie Pvt. Ltd. (Mumbai, India) and Fluka (Buchs, Switzerland), respectively, whereas chloroform and methanol were from E. Merck (India) Ltd. (Mumbai, India). Quercetin was isolated and purified from *Fagopyrum esculentum* (buckwheat). All other reagents were of analytical grade.

Preparation of Liposomal QC and Coupling of p-Aminophenyl α-D-Galacto-Pyranoside with Liposomes Intercalated with QC

Multilamellar liposomes were prepared with PE, cholesterol, DCP, and QC in molar ratio 7:1:1:1 (Budai and Szoqvi, 2001). In short, PE, cholesterol, DCP, and QC were dissolved in chloroform and methanol mixture (2:1, v/v) in a round-bottom flask. The lipid film was made by drying the organic solvents and was desiccated overnight. The thin film was swollen in phosphate-buffered saline, pH 7.2, for 1 h and sonicated in a probe-type sonicator for 30 s. Liposomal QC was coupled with p-aminophenyl α-D-galacto-pyranoside by using glutaraldehyde as the coupling agent (Mitra et al., 2005).

Animal and Experimental Design

Adult male Swiss albino rats, each weighing 120 to 150 g, were acclimatized to conditions in the laboratory (26–28°C, 60–80% relative humidity, 12-h light/dark cycle) for 10 days before the commencement of the treatment, during which they received food (purchased from Hindustan Lever Limited, Maharahstra, India) and drinking water. Rats were divided into six groups with five animals in each group. Rats in the normal group were injected s.c. with a single dose of physiological saline (0.5 ml) at multiple sites. Rats in the NaAsO₃-treated group were injected s.c. with NaAsO₃ (100.06 μg/kg body weight in 0.5 ml of physiological saline) at five different sites of its abdominal skin by making a 10-s time gap between one injection to its next. Free drug (0.5-ml suspension of 0.2% Tween 80 aqueous solution containing 0.898 μM QC) was injected into the tail vein of a third group of rats 2 h before NaAsO₂ treatment. Liposome with QC (0.5-ml suspension containing 0.898 μM QC), empty galactosylated liposome, or galactosylated liposome intercalated with QC (0.5-ml suspension containing 0.222, 0.445, 0.898, or 1.335 μM QC) was injected into the tail vein of rats in those different experimental groups 2 h before NaAsO₂ treatment. QC uptake by liver was esti-

Biomedical Analysis and Enzyme Assays

Lipid Peroxidation Assay. Lipid peroxidation in the mitochondrial membrane was determined by measuring the amount of conjugated diene. The mitochondrial membrane was extracted twice in a chloroform-methanol mixture (2:1, v/v). The pooled extract was evaporated to dryness under nitrogen atmosphere at 25°C and redisolved in cyclohexane. Lipids in cyclohexane solvent was assayed at 234 nm, and the results were expressed as micromoles of lipohydroperoxide per milligram of protein by using an ε of 2.52 × 10⁴ l/mole/cm (Mandal et al., 2002).

Quantitation of Quercetin Level in Liver. Liver homogenates of normal and experimental rats were diluted with an equal volume of absolute ethanol containing 1 μg/ml butylated hydroxyanisole. One milliliter of n-heptane was added, and the whole suspension was vortexed. This sample was centrifuged at 1000 rpm for 5 min at 4°C. The heptane layer was removed, and another 1 ml of fresh n-heptane was added. The sample was centrifuged as earlier until 3 volumes of heptane were added. These volumes were combined and dried under nitrogen atmosphere. The residue was dissolved in 0.2 ml of methanol, 20 μl was injected onto a high-performance liquid chromatography column, and quantitation of quercetin in liver homogenate was performed spectrophotometrically (Mandal and Das, 2005).
Different Enzyme Activities. Rat liver was perfused with the perfusion buffer (125 mM NaCl, 2.47 mM KH₂PO₄, 15 mM Na₂HPO₄, 5 mM NH₄Cl, 34.01 mM CaCl₂, 2H₂O, 5 mM glucose, and 0.2% bovine serum albumin, pH 7.2) containing collagenase and CaCl₂ at 37°C through the portal vein of the animal for 10 min at a rate of 10 to 15 ml/min. The tissue was chopped, homogenized in ice-cold perfusion buffer containing bovine serum albumin (2 g/l), and filtered through a nylon gauge (110 mesh). The cell suspension was centrifuged at 50g for 4 min. The pellet was resuspended, washed, and collected as hepatocytes. The supernatant was then centrifuged at 500g for 9 min to precipitate stellate cells. The isolated stellate cells were further purified with Percoll gradient. The cells were resuspended in 9.3 ml of perfusion buffer and mixed with 12.7 ml of Percoll containing 1/10 volume of 10-fold concentrated perfusion buffer. After centrifugation at 20,000g for 10 min at 4°C, the cell layer above the 1.07 gradient was carefully recovered. Thus, stellate cells were collected and re-suspended in buffer. The hepatocytes and stellate cell suspensions were homogenized at 4°C with 10 up-and-down strokes in a glass homogenizer fitted with a Teflon pestle. The homogenates were centrifuged at 105,000g for 60 min to get cytosolic fractions.

Catalase Activity. The cytosolic fractions, thus obtained, were used for enzyme activities. Catalase activity was assayed (Moragon et al., 2005). The reaction mixture contained sodium phosphate buffer (pH 7.0), 50 mM H₂O₂, and 50 μl of enzyme extract in a 3-ml volume. The activity was assayed by monitoring the decrease in absorbance at 240 nm as a consequence of H₂O₂ consumption and enzyme activity expressed as amount of H₂O₂ decomposed per minute per milligram of protein.

Glutathione Peroxidase Activity. The glutathione peroxidase (GPx) activity was determined (Sarkar and Das, 2006). The cytosol containing the enzyme source was mixed with 0.25 M potassium phosphate buffer, 25 mM EDTA, glutathione reductase, 40 mM glutathione (GSH), and 20 mM NADPH. The mixture was mixed and then incubated for 2 min at 37°C. The reaction was initiated by adding t-butyl hydroperoxide at the final concentration of 0.3 mM. The mixture was stirred, and the absorbance was read immediately at 340 nm at 1-min intervals for 4 min. The absorbance change during the 2- to 4-min interval was used to calculate enzyme activity. The activity was determined and expressed as micromoles of NADPH oxidized per minute per milligram of protein.

Glutathione Reductase Activity. Glutathione reductase was assayed (Castro et al., 1990). A 3 mL reaction mixture contained 100 mM phosphate buffer, pH 7.0, 1 mM oxidized glutathione, 1 mM EDTA, 0.1 mM NADPH, and 25 to 50 μl of enzyme extract. The reaction was started by adding the enzyme extract. The rate of NADPH oxidation was followed by monitoring the decrease in absorbance at 340 nm with a recording spectrophotometer. The activity was expressed as micromoles of NADPH oxidation per minute per milligram of protein.

Glutathione S-Transferase Activity. Glutathione S-transferase activity was determined in a total volume of 1.0 ml, containing 100 mM potassium phosphate buffer, pH 6.5, and 2 mM each of GSH and 1-chloro-2-4-dinitrobenzene (final concentration). The rate of formation of S-2,4-dinitrophenylglutathione (a GSH-1-chloro-2,4-dinitrobenzene conjugate) by enzyme extract was quantified at 340 nm using the extinction coefficient of 9.6 l/mmol/cm (Maiti and Chatterjee, 2000), and the activity was expressed as nanomoles per minute per milligram of protein.

Glucose-6-Phosphate Dehydrogenase Activity. The glucose-6-phosphate dehydrogenase (G6PDH) activity was determined using a Sigma Diagnostics Kit that is based on a modification of a spectrophotometric method (Maiti and Chatterjee, 2000). G6PDH catalyzes the first step in the pentose phosphate pathway, oxidizing glucose-6-phosphate to 6-phosphogluconate and reducing NADP to NADPH. The rate of formation of NADPH is proportional to G6PDH activity and was measured spectrophotometrically as an increase in absorbance at 340 nm. One unit of G6PDH activity was defined as 1 μM NADPH produced/min.

Fluorescence Depolarization Measurements of the Fluidity of Mitochondrial Membrane

The fluorescence depolarization, associated with the hydrophobic fluorescence probe DPH, was used to monitor the changes in the fluidity of the lipid matrix accompanying the gel to liquid crystalline phase transition. Mitochondrial membrane fractions of hepatic cells were incubated at 37°C by the addition of DPH dissolved in tetrahydrofuran (DPH/lipid molar ratio, 1:500). The excitation and the emission maxima were kept at 355 and 430 nm, respectively. The fluorescence anisotropy was calculated by using the equation:

\[ r = \frac{I_{II} - I_{I}}{I_{II} + 2I_{I}} \]

where \( I_{II} \) and \( I_{I} \) are the fluorescence intensities in parallel and perpendicular to the direction of polarization of the excited light. The microviscosity parameters \( (\rho_{I}/\rho_{II})^{-1} \) were calculated in each case, knowing the maximal limiting fluorescence anisotropy, \( r_{\text{m}} \), which for DPH is 0.362 (Sarkar et al., 2002).

Estimation of Hepatic 4-Hydroxyproline

The liver was cut into small pieces and homogenized in sufficient deionized water to yield 10% homogenate (w/v). Aliquots (2 ml) of the homogenate were added to an equal volume of 12 N HCl and hydrolyzed in Teflon-capped vials at 105°C for 18 h. 4-Hydroxyproline (4-HP) levels from those hydrolysates were measured (Sarin et al., 1999). The absorbance at 558 nm was read, and values were plotted against a standard graph using known amounts of 4-HP.

Estimation of Total Hepatic Collagen

The technique (Sarin et al., 1999) was used that is based on selective binding of Sirius Rose BB and Fast Green FCF to collagen and noncollagen components, respectively. When the sections are stained with both dyes dissolved in aqueous saturated picric acid, they are eluted readily with NaOH; simultaneously, the absorbance obtained at 550 and 625 nm can be used to determine the amount of collagen and total protein, respectively.

Arsenic Analysis

Subcellular fractions were digested with acid mixture containing nitric acid, sulfuric acid, and perchloric acid in the ratio of 6:1:1 over a regulated heater. After the digestion, the acid mixture was evaporated with occasional addition of triple distilled water, and the solution thus obtained was employed for estimation of arsenic content. Estimation was carried out using the atomic absorption spectrophotometer (Spectra AA 30/40; Varian, Inc., Palo Alto, CA) fitted with a graphite furnace (Flora et al., 1995).

Statistical Analysis

Statistical analysis was performed with Student’s t test. Linear correlations between two data were calculated by means of Pearson’s correlation coefficient r. In all instances, \( P < 0.05 \) was considered as the minimum level of significance.

Results

Acute NaAsO₂-Induced Mortality in Rats. A single injection of NaAsO₂ produced a dose-dependent mortality in rats (Fig. 1). Arsenite induced mortality at a dose of arsenic higher than 84.67 μM/kg b. wt., and no rats survived the dose of 130.8 μM/kg. The LD₅₀ value for arsenic-induced lethality in the rats was 107.75 μM/kg.

Effect of QC on Galactosylated Liposome on Arsenic Deposition in Mitochondria of Different Hepatic Cells.

A single dose of NaAsO₂ injection (s.c.) (100.06 μM/kg b. wt.) resulted in a deposition of arsenic in mitochondria of hepatocytes and stellate cells at the level of 264 ± 24 and 252 ±
18 μg/g mitochondrial protein. The elevated arsenic level in experimental animals was found to be reduced maximally by the treatment with QC in galactosylated liposomes (Fig. 2, a and b), where arsenic content was not detected by atomic absorption spectrophotometer in normal rats.

**Correlation of Mitochondrial Arsenic Content of Hepatocytes and Stellate Cells with Lipid Peroxidation.** Figure 3a shows positive correlation ($r = 0.97, P < 0.001$) between hepatocytic mitochondrial arsenic level and its conjugated diene level. The increased stellate mitochondrial arsenic level was also found to be associated with its increased lipid peroxidation as was evident from a significant positive correlation ($r = 0.93, P < 0.001$) (Fig. 3b) between its arsenic and conjugated diene content.

**Effect of QC in Galactosylated Liposomes on NaAsO₂-Induced Antioxidant Enzymes, Protection in Hepatocytes, and Stellate Cells.** A single injection of NaAsO₂ (100.06 μM/kg b. wt.) resulted in significant depletion of antioxidant level both in hepatocytes and stellate cells (Table 1). No significant protection was observed in the case of rats treated with free QC before arsenic treatment. Liposomal QC treatment prevented thiol depletion in stellate cells completely, but QC in galactosylated liposome treatment prevented the depletion completely both in hepatocytes and stellate cells.

**Effect of QC in Galactosylated Liposome Treatment on NaAsO₂-Induced Hepatic 4-HP and Collagen Contents in Rat Liver.** A single injection of NaAsO₂ (100.06 μM/kg b. wt.) induced an appreciable increase in hepatic collagen protein and 4-HP (Table 2). These increments of collagen and 4-HP were not reduced more by the treatment of free QC, whereas these levels were decreased appreciably by the treatment of liposomal QC. QC in galactosylated liposome injection inhibited completely the collagen protein deposition and 4-HP increase in rat liver.

**Effect of QC-Entrapped Galactosylated Liposomes on NaAsO₂-Mediated Hepatocellular Toxicity.** Rats treated with a single dose of NaAsO₂ developed significant hepatic damage as observed from elevated hepatospecific enzymes in serum. The activity of AST and AP in serum was increased in NaAsO₂-intoxicated animals. The serum urea and creatinine levels were also increased upon NaAsO₂ administration. A single injection (i.v.) of liposome-entrapped QC exerted a significant protection compared with free QC against NaAsO₂-induced liver toxicity. The degree of protection was observed maximally when galactose-coated liposomal QC was injected (Table 3).

**Effect of Galactosylated Liposomal QC on NaAsO₂-Induced Mitochondrial Membrane Microviscosity of Hepatocytes and Stellate Cells.** Arsenite induced a decrease of mitochondrial membrane microviscosity both in hepatocytes and stellate cells of rat liver. Liposomal QC treatment prevented the alteration in mitochondrial membrane microviscosity of stellate cells, whereas QC in galactosylated liposome treatment protected mitochondrial membrane of both hepatocytes and stellate cells completely from any decrease mediated by NaAsO₂ treatment (Table 4).

**Quantitation of Quercetin in Liver Homogenate.** In Table 5, QC levels were expressed per milligram of protein.
**Discussion**

Exposure to the metalloid arsenic has become an increasingly recognized source of illness worldwide. Arsenic has a direct toxic effect on cellular respiration in liver mitochondria with an evidence of oxidative stress and hepatic collagenesis in human (Pi et al., 2002; Das et al., 2005). Hepatic fibrosis is a disease state characterized by exuberant synthesis and deposition of collagen in the extracellular matrix. Fibrogenesis is expressed by an increase in the hepatic hydroxyproline levels (Testa et al., 1993). The present observation also indicates that fibrogenesis could be induced with an increment of 4-HP by a single injection (s.c.) of NaAsO₂ (100.06 μM/kg b. wt.) (Table 2). The exact mechanism of

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**TABLE 2**

Effect of QC and liposomal QC treatment on collagen and 4-HP levels in liver following NaAsO₂ treatment

Results are expressed as mean ± S.D. NaAsO₂-treated group was compared with normal, and the values were significantly different where *P < 0.001 for hepatic collagen and 4-HP. Experimental groups QC entrapped in liposome and QC in galactosylated liposome were also compared with the NaAsO₂-treated group, and in all those cases, *P < 0.001 for hepatic collagen and 4-HP.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hepatic Collagen</th>
<th>Hepatic 4-HP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/mg protein</td>
<td>µg protein</td>
</tr>
<tr>
<td>Normal</td>
<td>10.93 ± 1.86</td>
<td>22.18 ± 3.14</td>
</tr>
<tr>
<td>NaAsO₂-treated (A)</td>
<td>19.27 ± 2.13*</td>
<td>83.21 ± 7.19*</td>
</tr>
<tr>
<td>A + empty galactosylated liposome-treated</td>
<td>19.02 ± 1.14</td>
<td>81.78 ± 5.45</td>
</tr>
<tr>
<td>A + free QC-treated</td>
<td>18.52 ± 1.96</td>
<td>76.52 ± 5.77</td>
</tr>
<tr>
<td>A + liposomal QC-treated</td>
<td>15.19 ± 1.67*</td>
<td>61.73 ± 4.88*</td>
</tr>
<tr>
<td>A + QC in galactosylated liposome-treated</td>
<td>11.21 ± 1.73*</td>
<td>26.36 ± 4.11*</td>
</tr>
</tbody>
</table>

*P < 0.001.
TABLE 3
Effect of liposomal-encapsulated QC injection (i.v.) on blood serum biochemical parameters in NaAsO₂-induced hepatocellular injury
Results are expressed as mean ± S.D. of five animals. NaAsO₂-treated group was compared with normal, and the value was significantly different where P < 0.001. Experimental groups (galactosylated liposomal QC and liposomal QC group) were also compared with NaAsO₂-treated group, and in all those cases, P < 0.001.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>NaAsO₂-Treated Group (A)</th>
<th>A + Empty Galactosylated Liposome-Treated</th>
<th>A + Free QC-Treated (8.9 μM/kg b. wt.)</th>
<th>A + Liposomal QC-Treated (8.9 μM/kg b. wt.)</th>
<th>A + Galactosylated Liposomal QC-Treated (8.9 μM/kg b. wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>280 ± 11.70</td>
<td>818 ± 36.60*</td>
<td>808 ± 16.50</td>
<td>824 ± 33.81</td>
<td>513.55 ± 11*</td>
<td>307.78 ± 14.43*</td>
</tr>
<tr>
<td>Serum aspartate (IU/l)</td>
<td>36.86 ± 2.18</td>
<td>89.71 ± 6.42*</td>
<td>85.52 ± 4.51</td>
<td>94.11 ± 4.61</td>
<td>67.43 ± 3.97*</td>
<td>49.14 ± 2.21*</td>
</tr>
<tr>
<td>Urea (g/l)</td>
<td>0.45 ± 0.04</td>
<td>1.48 ± 0.08*</td>
<td>1.40 ± 0.02</td>
<td>1.61 ± 0.08</td>
<td>0.71 ± 0.09*</td>
<td>0.46 ± 0.03*</td>
</tr>
<tr>
<td>Creatinine (mg/l)</td>
<td>13.4 ± 1.84</td>
<td>74.17 ± 5.49*</td>
<td>72.43 ± 2.67</td>
<td>76.8 ± 4.86</td>
<td>44.7 ± 3.18*</td>
<td>14.7 ± 3.18*</td>
</tr>
</tbody>
</table>

* P < 0.001.

TABLE 4
Effect of QC in galactosylated liposomal treatment on the NaAsO₂-induced mitochondrial membrane microviscosity, \((r_0/r - 1)^{-1}\) of rat hepatocyte (HP) and stellate (ST) cells
Values are mean ± S.D. for five rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>NaAsO₂-treated (A)</th>
<th>A + empty galactosylated liposome-treated</th>
<th>A + free QC-treated</th>
<th>A + liposomal QC-treated</th>
<th>A + QC in galactosylated liposome-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane Microviscosity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(r_0/r - 1)^{-1} HP</td>
<td>0.677 ± 0.073</td>
<td>0.867 ± 0.024</td>
<td>0.216 ± 0.021*</td>
<td>0.274 ± 0.019*</td>
<td>0.231 ± 0.018*</td>
<td>0.286 ± 0.027*</td>
</tr>
<tr>
<td>ST</td>
<td>0.340 ± 0.089*</td>
<td>0.382 ± 0.048*</td>
<td>0.312 ± 0.099*</td>
<td>0.606 ± 0.052**</td>
<td>0.661 ± 0.045**</td>
<td>0.666 ± 0.061**</td>
</tr>
</tbody>
</table>

* P < 0.001 significantly different from normal.
** P < 0.001 significantly different from NaAsO₂-treated.

Arsenic toxicity was reported as an inhibitory effect on cellular respiration at the level of mitochondria (Stanton et al., 2006). In our observations, the increased arsenic deposition in hepatocytes and stellate cell mitochondria from NaAsO₂-treated rats are generating more ROS than normal animals (Fig. 2; Table 1). When arsenic deposition in mitochondria is prevented by galactosylated liposomal QC treatment, NaAsO₂-induced fibrogenesis is markedly reduced. Maintenance of mitochondrial membrane microviscosity, i.e. reciprocal of membrane fluidity of hepatocytes and stellate cells, could be achieved by the protective action of QC in galactosylated liposomes in cases where significant fall of mitochondrial membrane microviscosity takes place by the induction of NaAsO₂ (Table 4).

Previous investigators have shown a deviation of the pro-oxidant/antioxidant balance in NaAsO₂-treated rats with development of oxidative damage and a reduction in antioxidant status (Das et al., 2005). We also observed that liver injury was accompanied by the accumulation of arsenic with impaired activity and depletion of antioxidant status in NaAsO₂-induced rats. Reduction of liver injury was noticed by the improvement of antioxidant status with an increased concentration of QC and a marked reduction of arsenic content in liver by QC in galactosylated liposomes.

Our results indicate that galactosylated liposomal QC prevents arsenic deposition and protects liver from NaAsO₂-induced collagen deposition and fibrogenesis (Fig. 2; Table 2). Administration of QC in galactosylated liposomes to rats protects those animals from arsenic-induced liver fibrosis, whereas free QC does not. It is expected that QC in galactosylated liposomes may be more protective than free QC or liposomal QC because of the enhanced intracellular accumulation of QC by selective tissue targeted delivery of galactosylated liposomes (Table 5). We demonstrated a positive correlation between arsenic accumulation and lipid peroxidation level in liver (Fig. 3) as reported earlier (Ramos et al., 1995). A single injection (s.c.) of NaAsO₂ (100.06 μM/kg) to rats resulted in impairment of antioxidant status with a marked increase in arsenic content in liver. Our observation suggests that the mechanism of the protective effect of galactosylated liposomal QC against arsenic-induced liver injury could be related primarily to the reduction of arsenic accumulation in liver.

As the levels of AST and AP increase in NaAsO₂-induced hepatocellular injury, it seems that targeting of QC prevents hepatic cells against NaAsO₂-induced membrane damage by decreasing the leakage of AST and AP in the circulation (Table 3). The protective role of QC in galactosylated lipo-
somes against NaAsO₂-induced hepatotoxicity has also been confirmed by our histopathological and histochemical analysis (Fig. 4). This approach of delivering a nontoxic herb origin polyphenolic compound QC selectively to the liver might be useful in therapeutic application to prevent NaAsO₂-induced liver fibrogenesis. Further studies are planned to perform subchronic and chronic studies to substantiate our claims that QC might be useful in therapeutic application in combating NaAsO₂-induced fibrogenesis resultant from chronic arsenic exposure.

Acknowledgments

We acknowledge Suman Bhattacharya (University of Pittsburgh) for cooperation.

TABLE 5
Quercetin level in liver homogenate from rats treated with QC (in Tween 80), liposomal QC, and galactosylated liposomal QC

<table>
<thead>
<tr>
<th>Free QC</th>
<th>Liposomal QC</th>
<th>Galactosylated Liposomal QC</th>
</tr>
</thead>
<tbody>
<tr>
<td>QC concentration in liver homogenate (nM/mg protein)</td>
<td>0.83 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.63 ± 0.32&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Injected QC in whole liver (%)</td>
<td>25.61</td>
<td>50.29</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values expressed as mean ± S.D. (n = 5).
<sup>b</sup> Values are significantly different (P < 0.001) from free QC.

References


Address correspondence to: Dr. Nirmalendu Das, Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Jadavpur, Kolkata 700032, India.