Biochemical Studies on the Role of Curcumin in the Protection of Liver and Kidney Damage by Anti-Malaria Drug, Chloroquine

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Abstract: Some parts of Malaysia are endemic areas, whereas malaria exists and its control has become a formidable task. Chloroquine phosphate (CQ) on account of its rapid action on blood schizontoicide of all the malarial parasite strains has become the most widely prescribed drug for prophylaxis and treatment of malaria. Toxicity of CQ is most commonly encountered at therapeutic and higher doses of treatment. Thus, the present study was undertaken to evaluate the protective effect of curcumin, a herbal antioxidant obtained from Curcuma longa, on hepatic and renal biochemical status of CQ-induced Balb/c mice. It has been shown that administration of CQ brought about a significant decrease in antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSHpx) activities, whereas alanine aminotransferase (ALT) activity was found to be significantly increased following CQ treatment. Lipid peroxidation (LPO) was found to be elevated in a significant manner in the CQ treated group as compared to control. Body, liver and kidney weights declined significantly following CQ treatment. Administration of curcumin exhibited significant reversal of CQ induced toxicity in hepatic and renal tissues. Thus obtained results led us to conclude that curcumin has a highly protective power against CQ toxicity and the protective action relates, at least in part to its direct free radical scavenging ability.

Key words: Chloroquine · Curcumin · Antioxidant · Liver damage · Kidney damage

INTRODUCTION

Chloroquine (4-aminquinoline) (CQ) is used to treat malaria and a variety of inflammatory diseases including systemic lupus erythematosus and rheumatoid arthritis [1, 2]. However, CQ is known to cause cytotoxicity of which mechanism is still uncertain.

The liver regulates many important metabolic functions [2]. Hepatic injury is associated with distortion of these metabolic functions especially xenobiotics [3, 4]. The toxins absorbed from the intestinal tract gain access first to the liver resulting in a variety of liver ailment. To study the extent of liver damage by xenobiotics, clinical pathologists are trained to carefully evaluate serum chemistry alterations and interpret the observed changes in the context of the predicted relationships between the changes and pathogenesis that may potentially produce the changes.

Drug/chemical – mediated hepatic injury is the most common manifestation of drug toxicity [5] and account for greater than 50% of acute liver failure cases [2]. Most of the toxicity of drugs come from the induction of the generation of reactive oxygen species (ROS) [6, 7].

Oxidative stress, defined as the state of imbalance between the concentrations of ROS and the antioxidant system [8]. It was reported that chloroquine could induce oxidative stress and causes oxidative disorders in the cells [9, 10].

In the last years an extensive research has been done to evaluate several natural antioxidants regarding their chemoprotective effects [6, 11]. One of the recently most studied chemopreventive agent is curcumin (diferucyl methane), which is used widely as a food spece and colouring agent, as well as using in traditional Asian medicine [12]. Curcumine was proved by others that exhibits a protective effects against oxidative damage [13]. The aim of this study was to investigate the extent of the hepato and renaltotoxicity of chloroquine and the role of curcumin in attenuation of this state.

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MATERIALS AND METHODS

Chemicals: All chemicals used in this study were of analytical grade and purchased from Sigma and Aldrich Co. Malaysia. Chloroquine was obtained from University Hospital. Curcumin (Corma longa, tumeris) was purchased from local food market.

Measurement of Reducing Power of Curcumin: The reducing power of curcumin was quantified by the method described before [14].

Measurement of DPPH Radical Scavenging Capacity of Curcumin: The DPPH radical scavenging capacity of curcumin was determined according to the method reported before [15].

Toxicological Studies: Forty rats were divided into four groups (ten rats each, 8-9 weeks old, weight 60-80 g each), each group was housed in stainless cage and put them in tap water ad libitum in 12-12h light-dark periods. These groups are: control group (CON) without any supplementation either chloroquine or curcumin. The second group was Chloroquine group (CQ), supplemented with standard dose of Chloroquine, 200 mg/kg b.wt/day orally for three days then sacrificed. The third group was curcumin control (CUR) group, which fed with 300 mg/kg b.wt/day orally for 14 days then sacrificed. The fourth group (CURCQ) was supplemented with curcumin (300 mg/kg b.wt/ day orally) for 14 days then supplied with 200 mg/kg b.wt/day orally for further 14 days then sacrificed.

Rats were sacrificed by cervical dislocation, liver and kidney were excised and washed in ice-cold saline. Blood was removed immediately and serum was separated. Livers and kidneys were homogenized separately in ice-cold 0.1 M Tris-HCl buffer (pH 7.4) using Ultra homogenizer. Each homogenate was centrifuged at 10,000xg for 15 min and the supernatants were then centrifuged at 100,000 xg for 1 h. Supernatant (cytosolic fraction) was recovered and the protein concentration was determined by Biuret using bovine serum albumin as standard and aliquoted were used for the determination of enzymatic activities and lipid peroxidation as malondialdehyde (MDA) from the thiobarbituric acid reaction in both homogenates [16].

Blood was obtained by heart puncture technique into centrifuge tubes. Serum was prepared by centrifugation for ten min at 3000xg. Heparinized blood samples were centrifuged at 1500xg for 10 min and plasma was removed.

Blood urea nitrogen (BUN) [17] and creatine [18] were determined. Gamma-glutamyl transferase [19], Catalase (CAT) [20] and Superoxide dismutase [21] were determined. GSH peroxidase (GSH-px) was determined in both homogenates [22]. GSH was determined in the 10,000xg supernatant fraction of both homogenates [23]. Lipid peroxidation was determined as malondialdehyde (MDA) in plasma as well as liver and kidney cytosol [24]. Protein carbonyl contents was determined as previously described [25]. The extent of DNA-protein cross-links were assayed by the method of Carmichael [26].

Statistical Analysis: All results were expressed as the means±S.E.M. from ten rats per group. One way analysis of variance (ANOVA) followed by Tukey test was used to determine the significance of the differences between the groups. Statistical significance was declared when P value was equal to or less than 0.05. The statistical analysis was performed using the Sigma State Statistical Software version 3.5.

RESULTS

Results revealed non significant (p<0.005) alterations in the body weights of rats of the various treated groups. CQ treated mice liver showed a little increase in the liver body mass index ratio due to massive intra-hepatic hemorrhage and pooling of blood in the liver, making the liver appear darker in colour when compared with the other groups, which were all within the normal values. Serum values of ALT was utilized to evaluate liver injury. CQ administration increased serum values of ALT about 4 fold compared to those in CON rats, while pretreatment with curcumin significantly inhibit the rise of this enzyme induced by CQ as shown in table 1.

Antioxidant enzymes (CAT, SOD and GSHpx) activities significantly decreased in liver and kidney homogenates in rats treated with CQ only, while these activities were near normal after pretreatment with curcumin (Table 2).

MDA is a product of oxidative damage to lipids and in this study, the concentration of MDA in liver and kidney homogenates is considered as a biomarker of CQ toxicity. Results in table 2 show that liver and kidney homogenates of rats exposed to CQ contained higher levels of MDA (about 18-fold in liver and 12 fold in kidney), as well as protein carbonyl contents ( 20 fold in liver and 9 fold in kidney) when compared with CON values. These levels decreased significantly (P ≤0.05) in group that received CQ with curcumin.
Table 1: Effect of curcumin on the alterations in the serum induced by administration of CQ (200 mg/kg body weight / day) orally for 14 days

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>CUR</th>
<th>CQ</th>
<th>CQ+CUR</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>98.3±4.18</td>
<td>91.72±3.22</td>
<td>197.61±7.21</td>
<td>128.61±5.60</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>71.27±3.86</td>
<td>70.42±3.41</td>
<td>138.66±5.62</td>
<td>96.51±3.55</td>
</tr>
<tr>
<td>Creatinine mg/dl</td>
<td>0.92±0.004</td>
<td>0.88±0.003</td>
<td>2.88±0.072</td>
<td>1.22±0.022</td>
</tr>
<tr>
<td>BUN mg/dl</td>
<td>33.7±2.26</td>
<td>33.7±2.11</td>
<td>97.22±6.31</td>
<td>76.83±4.44</td>
</tr>
</tbody>
</table>

Table 2: Effect of curcumin on the levels of some biomarkers in liver and kidney homogenates

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>Kidney</th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>CUR</td>
<td>CQ</td>
<td>CUR+CQ</td>
<td>CON</td>
<td>CUR</td>
<td>CQ</td>
<td>CUR+CQ</td>
</tr>
<tr>
<td>GSH(μmol/kg)</td>
<td>6.82±0.42</td>
<td>6.88±0.51</td>
<td>2.10±0.18</td>
<td>5.82±0.44</td>
<td>3.31±0.21</td>
<td>3.22±0.20</td>
<td>1.16±0.12</td>
<td>2.71±0.17</td>
</tr>
<tr>
<td>GSHpx(μmol/min/mg)</td>
<td>0.87±0.004</td>
<td>0.89±0.008</td>
<td>0.22±0.002</td>
<td>0.67±0.003</td>
<td>0.81±0.003</td>
<td>0.89±0.008</td>
<td>0.23±0.002</td>
<td>0.71±0.003</td>
</tr>
<tr>
<td>LPO (μM)</td>
<td>0.10±0.12</td>
<td>0.09±0.08</td>
<td>0.62±1.54</td>
<td>0.74±0.14</td>
<td>0.08±0.007</td>
<td>0.07±0.005</td>
<td>1.98±0.52</td>
<td>3.88±0.007</td>
</tr>
<tr>
<td>PCC (μM)</td>
<td>2.81±0.11</td>
<td>2.10±0.10</td>
<td>4.43±3.56</td>
<td>7.44±8.22</td>
<td>1.66±0.33</td>
<td>1.60±0.33</td>
<td>18.22±2.42</td>
<td>6.24±2.16</td>
</tr>
<tr>
<td>DNA protein cross link%</td>
<td>0.92</td>
<td>0.88</td>
<td>11.3</td>
<td>2.64</td>
<td>0.91</td>
<td>0.87</td>
<td>7.4</td>
<td>2.71</td>
</tr>
<tr>
<td>CAT U/mg protein</td>
<td>365.21±31.22</td>
<td>381.33±38.41</td>
<td>77.31±4.66</td>
<td>271.12±21.45</td>
<td>217.12±11.23</td>
<td>220.41±3.79</td>
<td>124.32±5.07</td>
<td>166.71±5.94</td>
</tr>
<tr>
<td>SOD U/mg protein</td>
<td>18.63±3.18</td>
<td>20.55±3.46</td>
<td>9.42±1.78</td>
<td>15.44±2.73</td>
<td>37.81±3.88</td>
<td>37.72±3.88</td>
<td>16.12±2.14</td>
<td>30.47±3.22</td>
</tr>
</tbody>
</table>

The percentage of DNA-protein cross link increased significantly in group supplemented with CQ by 12 folds in liver homogenate and about 8 fold in kidney homogenate and these values were altered to near the normal when curcumin was used.

**DISCUSSION**

Chloroquine is a 4-aminoquinoline approved for the treatment and prophylaxis of malaria caused by susceptible strains of *Plasmodium falciparum*, *P. ovale*, *P. vivax* and *P. malariae* [27]. It is cheap, readily available, which explains its widespread use in areas where malaria is endemic [2, 3]. Recently, it has received much attention concerning its variety of toxicities [28].

In this study, QC - induced hepatotoxicity and nephrotoxicity were evidenced by biochemical measurements changes that coincide with other observations of other investigators [29-32]. QC toxicity is thought to be due to the formation of some oxidative metabolites, which raised the production of ROS [32,33]. However, there is a tendency now to limit the clinical use of this drug because of its several adverse effects, mainly idiosyncratic hepatotoxicity [34, 35]. The increased level of serum ALT activity reflects damage to hepatocytes and indicated the increased cellular permeability [36, 37] and considered to be highly sensitive and fairly specific preclinical and clinical biomarkers of hepatotoxicity [38]. In the present study, administration of hepatotoxic and nephrotoxic doses of QC to rats resulted in development of oxidative stress damage in hepatic and renal tissues.

This effect was indicated by increasing the degree of lipid peroxidation, inhibiting of enzymatic antioxidants, depleting non-enzymatic antioxidants (e.g. intracellular GSH) and increasing the level of methylglyoxal in liver and kidney.

Curcumin is unique in its ability to act as an antioxidant because of its ability to chelate transition metals thus inhibiting the formation of hydroxyl radicals [39], as well as its capacity to scavenge reactive oxygen species [40], also, its capacity to assist generating endogenous antioxidants such as vitamin C, vitamin E and GSH [41, 42]. In view of these considerations, the effect of curcumin on QC - induced hepatotoxicity and nephrotoxicity was evaluated in this study. Our results demonstrated that pretreatment of rats with curcumin markedly protected against hepatic and renal damage induced by a toxic dose of QC as assessed by biochemical measurements. Thus, pretreatment of rats with curcumin prevented QC-induced mortality. At the same time, reduction of GSHpx activity and depletion of intracellular GSH level induced by QC in liver and kidney were inhibited by treatment of rats with curcumin. Lipid peroxidation was assayed as marker of oxidative damage in the liver and kidney of rats treated with QC. Results of our investigation revealed a significant increase in the TBARS level in the liver and kidney of treated rats. This suggested an increased peroxidation of lipids, with contaminant loss of cellular functions in these organs by CQ feeding. Curcumin a known scavenger of free radicals [43-45], when administered in the current study, efficiently lowered the peroxidation levels thus protecting tissues from oxidative stress.
The antioxidant mechanism of curcumin is attributed to its unique conjugated structure, which includes wqo methyloxylated phenols and an enol form of B-diketone; the structure shows typical radical-trapping ability as a chain-breaking antioxidant [46].

It is evident therefore that curcumin exerts significant protection against CQ induced toxicity due to its antioxidant activity. Thus CQ can bring about irreversible toxic effect in tissue, hence use of strong antioxidants like curcumin should be recommended with CQ for treating malaria, so as to avoid the toxic influences of the above mentioned drug.

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