Novel Use of Silymarin as Delayed Therapy for Acetaminophen-Induced Acute Hepatic Injury

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Keywords
Acetaminophen · Hepatic injury · N-Acetyl cysteine · Silymarin

Summary
Aim: Recently, we have demonstrated that silymarin has a comparable pharmaceutical activity as Phyllanthus uri-naria extract when used to rescue mice from acetami-nophen-induced acute liver injury. In the present study, we further compared the therapeutic action of silymarin with N-acetyl cysteine (commonly used in clinical prac-tice for emergency treatments) as a rescuer in mice after administering a lethal dose of acetaminophen for 24 h.

Methods: Acute liver injury was induced in the treatment groups by intraperitoneally administered acetaminophen at a dose of 550 mg/kg body weight on day 1. The control group received an equal volume of physiological saline intraperitoneally. From day 2 to 4, the treatment groups received various doses of silymarin or N-acetyl cysteine orally once daily, while the control group and the acetaminophen group received an equal volume of water orally. The mortality rate was recorded in all groups. On day 5, all mice were sacrificed for examination.

Results: Silymarin greatly improved the counteracting effects on mortality rate as compared to N-acetyl cysteine. Conclusion: Silymarin should be further considered as an antidote for patients with acetaminophen-induced acute hepatic injury and delayed treatment.

Schlüsselwörter
Acetaminophen · Leberversagen · N-Acetylcystein · Silymarin

Zusammenfassung
Introduction

An overdose of acetaminophen (APAP) is one of the common types of drug poisoning. It can lead to fatal damage of vital organs including liver, heart, and kidneys. An overdose of about 10 g/kg body weight (BW) can be fatal, as liver damage may develop after several hours of intake. The culprits are found to be the oxidative products of APAP including N-acetyl-benzoquinone imine. Such oxidative metabolites may completely reduce the intracellular glutathione and thus damage hepatocytes [1].

Recently, we have conducted experiments aimed to explore the hepatoprotective activity of Phyllanthus urinaria (P. urinaria) in a mice model 24 h after administering a lethal dose of APAP (550 mg/kg BW). This APAP dosage causes in C57Bl6 mice a very poor prognosis: the majority of the mice did not survive for 3 days. The experimental model employed mimics a clinical condition similar to that found in patients admitted to the accident and emergency divisions of hospitals with acute liver toxicity. We have shown that P. urinaria extract may protect hepatocytes from APAP-induced necrosis after delayed therapy [2]. In the same article we have also shown that silymarin could yield similar survival rates as P. urinaria extract. In the present study, we compare the therapeutic action of silymarin with that of N-acetyl cysteine (NAC) (which is commonly used in clinical practice for emergency treatment) as a rescuer in mice after administration of a lethal dose of APAP for 24 h. In addition to comparing the mortality rate, we analyzed the possible occurrence of necrotic features in a biopsy of liver section as well as liver function enzymes in the peripheral circulation.

Materials and Methods

Chemicals and Reagents

Unless otherwise stated, all reagents, including APAP, were purchased from Sigma chemicals. The physiological saline for APAP injection was obtained from Baxter. NAC was obtained from Zambon (Switzerland).

Animal Care

8-week-old C57Bl6 mice, weighing approximately 20–25 g, were purchased from the animal unit of The Chinese University of Hong Kong and maintained in a conventional sanitary facility, in accordance with the institutional guidelines on animal care, with the required consistent temperature and relative humidity. All procedures were approved by the Animal Research Ethics Committee.

APAP Treatment on Mice

APAP was dissolved in physiological saline. A total of 25 mice were included in the study. On day 1, acute liver injury was induced by intraperitoneally administered APAP at a dose of 550 mg/kg BW in 4 groups of mice. A control group of 3 mice was given intraperitoneally equal volumes of physiological saline. From day 2 to 4, treatment groups received various doses of silymarin (100 or 200 mg/kg BW) or NAC orally once daily, while the control group and the APAP group received equal volumes of water orally (table 1). The treatment dosages of silymarin and NAC were based on the references [2] and [3], respectively. The mortality rate in each group was monitored and recorded. On day 5, all surviving mice were sacrificed for examination.

Proposal for Histopathologic Examination and Biochemical Evaluation of Liver Injury

Sections of mouse liver from autopsy samples were dewaxed, washed with phosphate buffered saline and then stained with hematoxylin and eosin (H and E) for nucleus and cytoplasm staining using the conventional protocol reported elsewhere. Slides were then premounted and inspected under a light microscope.

Whole blood was collected after the mice were sacrificed and plasma was isolated after centrifugation. Afterwards, plasma liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by the Vet biochemistry assay kits for the IDEXX laboratories machine in order to determine whether there were symptoms of liver failure in the silymarin-treated groups and in the control group.

Results

Silymarin Inhibits APAP Induced Hepatotoxicity in vivo

NAC administered at 200 mg/kg BW per day was found to be helpless in APAP overdose-treated mice if treatment was delayed by 24 h. Only 1 out of 6 mice survived until day 3, dying anyhow on the morning of day 4 (table 2). The mortality rate was more or less similar to that in the APAP group. If mice treated with APAP received orally administered silymarin, a significant improvement of the survival rate was observed (table 2) as compared to NAC. If the daily dose of sily-
Silymarin as Delayed Therapy for Overdose Acetaminophen

Results
Liver function enzymes including ALT and AST in the plasma were analyzed in all mice of the control group and the silymarin-treated groups. It was found that both markers of liver function in the 100 mg/kg BW silymarin-treated group were noticeably higher than in the control group. However, in the control group and in the 200 mg/kg BW silymarin group they were similar (table 3). For the APAP and the NAC group no biochemical analyses were performed, as the animals had died before the end of the study.

Discussion
In clinical practice, NAC is the antidote of choice in cases of APAP overdose. It has been reported from clinical practice that NAC given within 8 h of APAP ingestion has a protective effect regardless of initial plasma APAP concentrations.
Table 3. Plasma liver functional assays from mice treated with APAP (single dose of 550 mg/kg BW on day 1) and 2 different doses of silymarin (single dose daily from day 2–4). Student’s t test was used and data was considered to be statistically significant when p < 0.05. No statistically significant difference was found between the APAP and the two silymarin treatment groups (p > 0.05).

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Silymarin 100 mg/kg BW</th>
<th>Silymarin 200 mg/kg BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>51.1 ± 9.6</td>
<td>79.8 ± 12.4</td>
<td>63.2 ± 12.8</td>
</tr>
<tr>
<td>AST</td>
<td>66.9 ± 5.4</td>
<td>96.5 ± 30.7</td>
<td>57.8 ± 14.3</td>
</tr>
</tbody>
</table>

However, efficacy of NAC treatment was found to be significantly decreased if it was delayed further. It was concluded that NAC therapy should be started within 8 h after an APAP overdose; despite this limitation, the treatment is currently still indicated at least as late as 24 h after ingestion. It has also been noticed that delayed NAC administration after APAP intoxication significantly increases the risk of mortality [4].

Silymarin is a collection of complex flavonoids found in milk thistle and has been shown to increase liver glutathione levels in rats [5]. Milk thistle is recommended clinically as a supplementation for people who take acetaminophen in large doses for >1 year and/or with other risk factors for liver problems. The ‘antidote’ activity of silymarin has been demonstrated in many in vitro and in vivo models, including its protective effect against the strongest poison phalloidine [6]. Silymarin injected at a dose of 100 mg/kg BW 10 min after phalloidine could provide a total protection of the experimental animals. However, as the time span between administration of phalloidine and start of treatment increased, the therapeutic activity of silymarin decreased as well, and after 30 min its ‘antidote’ effect was negligible. Silymarin has also been shown to be active in the treatment of hepatotoxicity [7, 8]. In the study presented here, we compared the therapeutic action of silymarin and NAC as an antidote in mice after administering a lethal dose of APAP for 24 h. In the mouse, maximal toxicity following APAP occurred after approximately 6–8 h. As maximal toxicity occurred so early, the antidote administered after 24 h had no effect on the development of toxicity. Our results have clearly demonstrated that treatment using NAC alone was not effective: the mortality rate was 100%, i.e. the same as in the APAP group. NAC thus had no effect in terms of preventing lethality under our experimental design. Silymarin, in contrast, has been demonstrated to be highly effective. In fact, both the 100 mg/kg BW and the 200 mg/kg BW treatment of silymarin produced a 100% survival rate.

What we still have to discuss are the histochemical findings from the liver section autopsy and serum liver enzyme levels. In the present study, we presented the histopathological data with special focus on the centrolobular area because APAP-induced hepatotoxicity is pathologically characterized by its centrolobular hepatic necrotic features. In both silymarin-treated groups, most of the APAP-treated mice showed observable necrotic features of their liver in H and E histochemical staining samples (100% of the 100 mg/kg BW and 66% of the 200 mg/kg BW silymarin treatment groups). The mean plasma ALT and AST in the 100 mg/kg BW silymarin group were also considerably higher than in the control group. However, these plasma liver enzyme markers were comparable to those of the control group if the 200 mg/kg BW silymarin group was considered.

Other experiments on animals have shown that the combination of NAC and cimetidine (an H2-receptor-antagonist drug and an inhibitor of hepatic microsomal oxidative enzymes) might also have an additive effect in the treatment of APAP overdose: in mice, the concomitant administration of NAC and cimetidine resulted in a zero mortality rate and significantly raised hepatic glutathione concentrations to values comparable to those of a saline-treated control group [9]. Similarly, co-administration of silybinin (a component of silymarin), cimetidine, and NAC, 30 min after APAP administration, was reported to be an effective antidote combination in a rabbit demonstration model [10]. Accordingly, we assume that a concomitant administration of NAC and silymarin may be an effective antidote for the treatment of subjects with delayed therapy after APAP overdose.

We assume that, in the future, silymarin extract can be used as a complementary medicine in the emergency treatment of APAP overdose with delayed therapy provided further preclinical and clinical data will support our hypothesis.

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Conflict of Interest

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References