Effect of a Toxic Dose of Acetaminophen on Electrolytes and Histopathological Changes in the Kidney

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Abstract: Introduction: Acetaminophen in toxic doses can cause renal failure. The actual mechanism of acetaminophen-induced renal failure is still unknown. The aim of the current study is to investigate the effect of single toxic dose of acetaminophen on renal function and renal histopathological structure. Method: A single toxic dose of acetaminophen (500 mg/kg) was injected intraperitoneally into male Wistar rats. Serum acetaminophen, biochemical markers of renal and liver functions, serum and urine electrolytes were measured at 4, 12 and 24 h after drug injection. The histopathology of renal tissue was investigated. Results: Serum ALT, AST, BUN and creatinine were significantly deteriorated in the experiment group. Acetaminophen injection significantly caused serum sodium retention, hypokalemia and hyperosmolality. In the urine, there was significantly higher excretion of sodium, potassium and phosphate. Renal biopsy showed structural changes resembling acute tubular necrosis. The control group did not show any of the changes observed in the case group. Discussion: Acetaminophen in toxic doses can cause renal injury starting 12 to 24 h post-injection. The histopathological changes in kidney structure resemble acute tubular necrosis. Acetaminophen also alters the renal handling of electrolytes observed by sodium retention, an increase in serum osmolality, hypokalemia and an increase in the urinary excretion of sodium, potassium and phosphate.

Keyword: Acetaminophen, nephrotoxicity, cyclooxygenase, prostaglandin, renal failure, acute tubular necrosis.

INTRODUCTION

Acetaminophen (APAP) is one of the most commonly taken drugs that result in overdoses in the UK and US [1]. It is an over-the-counter analgesic and antipyretic frequently used for minor aches and pains. It is believed that the analgesic and antipyretic effects of APAP are due to the selective inhibition of COX-III (cyclooxygenase III), a variant of COX-I, leading to the central inhibition of prostaglandin synthesis [2]. Prostaglandins in the kidney, mainly produced by the COX enzyme, play a major protective role in renal hemodynamics. Prostaglandin inhibitors such as NSAIDs, and potentially APAP in toxic doses, may have various effects on the kidney. Renal injury may partly be due to vasoconstriction as a consequence of inhibition of kidney prostaglandin-mediated vasodilatation, resulting in renal hypoperfusion and consequent reduction in the glomerular filtration rate [3]. NSAID-induced renal injury is affected by the dose of drug, the duration of the pharmacologic effect, and the health status of the patient. Individuals who are poorly hydrated and have compromised renal function are more likely to develop acute renal injury [4].

APAP in a toxic dose induced renal failure. The incidence of APAP-induced renal failure has been reported to be approximately 2%, and it reaches 10% in severe cases [5]. Although most cases of renal failure are associated with hepatic injury, renal damage in the absence of hepatic injury has been reported [6]. It is still unknown whether this effect is due to a direct APAP nephrotoxic effect or as a consequence of hepatic failure. A single nonlethal dose of APAP on animals with high levels of renal microsomal P-450 activity caused proximal convoluted tubular necrosis, possibly as a result of local production of the toxic intermediate metabolite known as para-benzo-quinone imine [7, 8]. An increase in blood urea nitrogen (BUN) and serum creatinine (Cr) after a toxic dose of APAP has been shown in other studies [9]. Hemodynamic changes in the kidney through COX inhibition, similar to the action of classical NSAIDs, are another suggested mechanism for APAP-induced nephrotoxicity.

A previous experimental study on Wistar rats showed that single doses of APAP significantly
Reduced the glomerular filtration rate and renal blood flow [10]. Another study reported phosphaturia following APAP toxicity, possibly due to its tubular effect [11,12]. In a study of patients presenting with APAP overdose at the hospital, we explored the effect of APAP toxicity on serum and urine electrolytes and showed that a toxic dose of APAP is associated with dose-related phosphaturia, hypokalemia, and short-duration kaliuresis (less than 24 h). We concluded that this effect may suggest a specific renal effect of APAP in overdose conditions, perhaps via cyclooxygenase inhibition. This effect seems to be distinct from any other nephrotoxic effect of APAP [13]. To better understand the effect of APAP on renal function and serum electrolytes and its pathological effect on kidney structure, we performed an experimental study on male Wistar rats exposed to a toxic dose of APAP.

METHODS

Animals and Treatment

Male Wistar rats (150-200 g body weight) were used. They were housed in rooms with controlled temperature (21-24 °C) and humidity and a 12 h light cycle (6.00-18.00), and maintained on standard diet and water. They were fasted 12 h before the experiment but had free access to water. The study protocol was approved by the local ethical committee.

Several experimental groups were studied. Rats were grouped into the following two categories: case and control. Each rat in the case groups received a single dose of 500 mg/kg APAP (acetaminophen) via IP (intraperitoneal) injection. To solvate APAP, 1 ml of 0.9% normal saline and 1 ml of polyethylene glycol 400 were used. The case and control groups each had three subgroups, 4 h, 12 h and 24 h. fifteen rats were used in each case subgroup. In the control subgroups, 8 rats were used for 4 h and 24 h, and 15 rats were used in the 12 h group. The control group only received 0.9% normal saline (1 ml) and polyethylene glycol 400 (1 ml). Samples of blood and urine were taken at 4, 12 and 24 h post-injection in both the case and control groups. Serum ALT, AST, BUN and Cr were measured at 4, 12 and 24 h post-injection in both the case and control groups.

Statistical Analysis

Data are presented as the means±Sem (standard error of the mean). Student's T test was used for two group comparisons. One way ANOVA was used for three group comparisons. P values less than 0.05 were considered significant. SPSS version 13 was used for statistical analysis.

RESULTS

Biochemical Analysis of the Serum

In the case group, the serum APAP (acetaminophen) concentration was the highest at 4 h post-injection (70.32 ± 21.2 mg/l). There was no significant difference between the case and control groups in serum Na, Cl, PO4, AST, ALT, and osmolality at 4 h post-injection (Table 1). There was a significant difference between serum K and BUN in the case and control groups at 4 h. Serum K and BUN were higher in the case group. Serum BUN and Cr were significantly increased at 12 and 24 h (Cr: 3.31 ± 0.43 vs. 0.77 ± 0.21 in case and control group at 24 h, respectively). There was no significant change in serum BUN and Cr in the control group. Serum BUN and Cr significantly increased from 4 h to 24 h post-injection.
administration, whereas there was no significant difference in BUN and Cr in the control group at the different time points after injection. The serum BUN and Cr were even higher at 24 h compared to 12 h in the case group. The AST and ALT values were significantly higher in the case group at 12 h and 24 h compared to 4 h; however, there was no significant difference between AST and ALT at 12 h and 24 h. Serum Na significantly increased at 24 h compared to 4 h and 12 h in the case group. There were no significant changes in serum Na over time in the control group. Serum K decreased significantly at 12 h compared to 4 h and was restored at 24 h in the case group. Serum K in the control group was slightly increased at 12 and 24 h; however, these changes were insignificant. Serum PO₄ and Cl significantly increased at 12 h and 24 h compared to 4 h in the case group. There was no significant change in serum PO₄ and Cl at 12 and 24 h in the control group. The calculated serum osmolality significantly increased at 12 h and 24 h in the case group. No change was observed in the calculated serum osmolality in the control group (Table 1).

### Biochemical Analysis in the Urine

The urine pH was significantly decreased at 12 and 24 h compared to 4 h in the case group. The urine pH was significantly lower at 4, 12 and 24 h in the case group compared to the control group. Urinary excretion of Na, K and PO₄ was significantly higher in the case group at 4, 12, and 24 h compared to the control group. These figures were significantly higher at 12 and 24 h compared to 4 h in the case group. Although urinary excretion of Cl was higher in the case group than the control group, there was no significant difference between urinary Cl at 4 h, 12 h and 24 h in the case group (Table 2).

### Table 1: Time Course of the Changes in Different Biochemical Variables in the Serum of Male Wistar Rats from the Case and Control Groups. The case group received a single dose of 500 mg/kg acetaminophen (APAP) by intraperitoneal injection. n = number

<table>
<thead>
<tr>
<th>Variables/ time/groups</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 h, n=15</td>
<td>12 h, n=15</td>
</tr>
<tr>
<td>APAP ppm mean±sem</td>
<td>70.32±21.2</td>
<td>0.49±0.25</td>
</tr>
<tr>
<td>Serum BUN Mg/dl</td>
<td>18.10±1.20</td>
<td>75.53±7.09</td>
</tr>
<tr>
<td>Serum Cr mg/dl</td>
<td>0.81±0.24</td>
<td>2.48±0.37</td>
</tr>
<tr>
<td>Serum ALT IU/l</td>
<td>188.50±9.90</td>
<td>703.30±19.06</td>
</tr>
<tr>
<td>Serum AST IU/l</td>
<td>82.46±9.32</td>
<td>428.00±113.40</td>
</tr>
<tr>
<td>Serum Na mEq/l</td>
<td>139.60±1.78</td>
<td>140.40±1.00</td>
</tr>
<tr>
<td>Serum K mEq/l</td>
<td>3.76±0.30</td>
<td>3.30±0.16</td>
</tr>
<tr>
<td>Serum PO₄ mEq/l</td>
<td>3.41±0.38</td>
<td>4.00±0.36</td>
</tr>
<tr>
<td>Serum Cl mEq/l</td>
<td>97.70±1.97</td>
<td>101.90±3.50</td>
</tr>
<tr>
<td>Calculated Osmolality</td>
<td>285.67±3.69</td>
<td>307.77±3.33</td>
</tr>
</tbody>
</table>

### Table 2: Time Course of Changes in Different Biochemical Variables in the Urine of Male Wistar Rats, in the Case and Control Groups. The case group received a single dose of 500 mg/kg acetaminophen by intraperitoneal injection. n = number

<table>
<thead>
<tr>
<th>Variables/time/groups</th>
<th>Case n=15</th>
<th>Control n=8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 h</td>
<td>12 h</td>
</tr>
<tr>
<td>Urine Na mEq/l, mean±sem</td>
<td>165.2±24.83</td>
<td>181.6±17.99</td>
</tr>
<tr>
<td>Urine K mEq/l, mean±sem</td>
<td>40.4±2.65</td>
<td>47.4±4.8</td>
</tr>
<tr>
<td>Urine PO₄ mEq/l, mean±sem</td>
<td>58.2±4.53</td>
<td>72.8±8.93</td>
</tr>
<tr>
<td>Urine Cl mEq/l, mean±sem</td>
<td>187.2±16.77</td>
<td>186.6±15.7</td>
</tr>
<tr>
<td>pH</td>
<td>7.90±0.96</td>
<td>6.20±0.24</td>
</tr>
</tbody>
</table>
Histopathological Investigation

Light microscopy of the renal biopsy (glomerulus and tubule) of rats in the control group showed a normal pattern. Moreover, no significant change was observed at 4 h in the case group. However, there were significant histopathological changes in the nephron structure at 12 and 24 h post-injection. Changes in the nephron at 12 h were interstitial edema, peri-tubular bleeding, glomerular bleeding, glomerular proliferation, peri-glomerular vasodilatation and congestion, lymphocyte proliferation and interstitial nephritis (Figure 1). At 24 h, tubular epithelial necrosis, debris and damage to the basement membrane were observed (Figure 2).

DISCUSSION

High doses of APAP (acetaminophen) are capable of causing hepatic necrosis and renal tubular necrosis [15-17]. The hepatotoxic effect of APAP has been widely studied; however, APAP-induced nephrotoxicity and the actual mechanism of nephrotoxicity are not fully understood. In the current experiment, the effect of a single, toxic and non-lethal dose of APAP (500 mg/kg) on kidney function and nephron structure on male Wistar rats was studied. The main finding of this study is that a single toxic dose of APAP caused renal dysfunction, changed electrolyte handling, and had a pathological effect on kidney structure. According to the results and as expected, liver injury occurred in all
cases, and ALT and AST, two main markers of liver function, significantly increased at 12 and 24 h post-injection. Moreover, renal function was also observed to deteriorate by an increase in BUN at 12 h followed by a later increase in serum Cr at 24 h post-injection.

Renal failure is less common than liver failure after an APAP overdose. The occurrence of renal failure is greater in severely poisoned patients and is often observed in those that develop significant liver injury. The timing of the onset of renal dysfunction is later than that of liver injury [18, 19]. Nonetheless, the occurrence of renal failure cannot be attributed solely to coexistent hepatic damage and has been reported as an isolated manifestation of APAP toxicity [20, 21].

Our previous studies showed that APAP may have two different effects on the kidney: early and late effects [13, 22]. The early effect is thought to mainly be due to hemodynamic compromise, and it usually improves within 24 h. However, in some cases hemodynamic compromise might cause hypoperfusion and renal ischemia resulting in acute tubular necrosis. The later renal effect of APAP, which occurs 4-5 days after ingestion of the toxic dose, might be either a consequence of liver injury or caused by the direct nephrotoxic effect of APAP on renal tissue through the same mechanism that causes liver injury [23]. One possible explanation for the early renal effects is that APAP at high doses may cause local hemodynamic changes, perhaps through COX inhibitory effects in the kidney, similar to classical NSAIDs. A previous experimental study also showed that single doses of APAP caused a significant reduction in glomerular filtration rate (GFR) and renal blood flow in a dose-dependent manner [10]. This altered tubular function, mainly in the distal tubules, was observed as a change in renal concentrating ability. The maximum changes were observed at 16 h post-injection, and after 24 h, renal function was restored. The authors suggest that the early stage of APAP-induced nephrotoxicity might be due to vasoconstriction. In the current study, renal blood flow and glomerular filtration were not measured; however, calculated serum osmolality was significantly increased at 12 h and further increased at 24 h in the case group, which is consistent with the results of a previous study, suggesting the possibility of renal hemodynamic compromise and tubular function impairment.

In another previous study on patients, we reported that serum APAP causes a dose-dependent reduction in serum K and an increase in urinary excretion of Na and K. The results of the current study also confirmed that a single toxic dose of APAP caused serum Na retention at 24 h and a serum K reduction at 12 h. The effect of APAP on urinary excretion of Na, K and PO4 showed an increase in the urinary excretion of Na, K and PO4 at 12 h and a larger increase at 24 h post-injection. Another study on patients presenting with APAP toxicity reported similar results, showing an increase in serum Cr and a decrease in serum K [24]. A study on overdoses of classical NSAIDs, such as ibuprofen, in humans reported an increased fraction of K excretion and Na retention, an effect that might to be due to renal vasoconstriction and consequent activation of the renin-angiotensin-aldosterone system [25]. Other works reported phosphaturia after APAP overdose, possibly due to its tubular effects, and hypophosphatemia as a result of liver injury in severe APAP poisoning [11, 26]. In the current study, the increase in urinary excretion of PO4 is consistent with the results of previous studies; however, an increase in serum PO4 does not support previous findings. One possible explanation might be that hypophosphatemia occurs only in severe cases where significant liver injury occurs. Further studies are required to investigate the actual effect of APAP on the serum and urinary excretion of PO4.

A renal biopsy is rarely performed for the diagnosis of APAP-induced renal injury. However, histopathological studies have shown a pattern of acute tubular necrosis (ATN) with the proximal tubule being the most commonly involved segment. Renal biopsy of patients who developed renal failure after APAP overdose showed tubular epithelial necrosis in both the proximal and distal tubules [27]. Renal biopsy of some patients
Acetaminophen-Induced Nephrotoxicity

Using light microscopy have shown normal glomeruli and vessels, but with debris and evidence of damage to the basement membrane. Electron microscopy has shown a significant loss of the tubular brush border with tubular swelling and distortion of mitochondrial organization [17, 27]. Light microscopy of renal tissue in the current study confirmed the results of previous studies and showed glomerular and tubular pathology consistent with acute tubular necrosis possibly due to renal ischemia induced by renal hemodynamic compromise. Whether other mechanisms are involved is still unknown and requires further studies.

CONCLUSION

Acetaminophen in a toxic dose can cause renal injury starting 12 to 24 h after toxic exposure as observed by an increase in serum blood urea nitrogen and creatinine. Histopathological changes in kidney structure resemble the pattern of acute tubular necrosis. Acetaminophen also alters the renal handling of electrolytes as observed by sodium retention, an increase in serum osmolality, hypokalemia and an increase in the urinary excretion of sodium, potassium and phosphate. The possible mechanism of the early renal effect of acetaminophen might be via cyclooxygenase inhibition, causing renal vasoconstriction, hemodynamic compromise and renal tubular ischemia. Measurement of urinary prostaglandin and serum rennin activity and glutathione storage in the kidney in future studies may provide a better understanding of the potential mechanism of the renal effects of acetaminophen.

REFERENCE


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