Early biomarkers predicting outcome in a porcine model of acetaminophen intoxication: A pilot study

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Abstract

Background. Acetaminophen intoxication has become the leading cause of acute liver failure (ALF) in Europe and the USA.

Objectives. To identify early biomarkers in order to predict the development of ALF in a porcine model of acetaminophen intoxication.

Materials and methods. Six German Landrace pigs received a single acetaminophen bolus of 1 g/kg body weight via a jejunal catheter. Cytokines and laboratory parameters were analyzed at 8-hour intervals for a total of 40 h.

Results. Three of the 6 animals survived the intoxication. The nonsurviving animals had an increase in serum lactate and interleukin (IL)-6, with a simultaneous decrease in prothrombin time (PT) and albumin concentration 8 h after intoxication. In all nonsurviving animals, elevated levels of tumor necrosis factor alpha (TNF-α) at baseline before intoxication and during the course of ALF were observed. The acetaminophen serum concentrations and toxicokinetics did not differ between the nonsurviving and surviving animals. Methemoglobinemia was proportional to the administered doses and acetaminophen blood levels, but methemoglobinemia did not affect survival.

Conclusions. Tumor necrosis factor alpha, IL-6, lactate, prothrombin time, and albumin blood concentration were identified as early predictors of outcome after acetaminophen intoxication. An elevated TNF-α level before acetaminophen exposure was the earliest prognostic marker for a lethal outcome. Therefore, it could serve as a very early indicator of prognosis.

Key words: cytokines, acute liver failure, prognostic markers, porcine model, acetaminophen intoxication

Background

Acetaminophen (Paracetamol®) intoxication has become the number one cause of acute liver failure (ALF) in Europe and the USA.[1,2] It is very important to make the diagnosis at an early stage so that specific treatment can be applied.[3] An overdose of acetaminophen, either deliberate or accidental, does not necessarily result in ALF. On the other hand, therapeutic doses of acetaminophen may cause severe hepatic injury in certain patients. Several risk factors, including concomitant use of other drugs, comorbidities, advanced age, and specific variants of genes encoding metabolic enzymes, are known to increase the probability of developing ALF.[4–8] Alcohol consumption is another crucial factor, but it plays a somewhat contradictory role.[9] While chronic alcoholism has been postulated to increase acetaminophen-related hepatotoxicity, acute administration of ethanol seems to protect against acetaminophen-induced liver injury.[10,11] Prognostic scores, such as the King's College Criteria for patients with ALF following acetaminophen intoxication, are commonly used to distinguish between patients who are likely to recover spontaneously and those who might require emergency liver transplantation as the only therapeutic life-saving option.[12] However, these late prognostic scores are based on clinical and laboratory data obtained after the onset of ALF. To date, there are no clinical tests or biomarkers to predict which individuals are prone to develop ALF from acetaminophen exposure before the onset of clinical disease.

The ideal biomarkers for predicting acetaminophen-induced liver failure should be easy to measure in a routine clinical setting using precisely quantifiable blood or urine markers, with results available within a short time. Certain cytokines, which are known to be promising candidates for novel biomarkers in drug-induced liver injury, fulfil all these criteria.[13] The balance of pro- and anti-inflammatory cytokines is discussed as modulating hepatic injury exacerbation or regeneration.[13] Several studies in patients suffering from ALF have reported elevated levels of inflammatory cytokines, such as interleukin (IL)-6, IL-8, IL-10, and tumor necrosis factor alpha (TNF-α).[14–16]

Objectives

The aim of this study was to analyze cytokine levels and ALF-related laboratory parameters before and over the course of acetaminophen-induced ALF in a porcine model in order to identify biomarkers for early prediction of the clinical outcome of acute acetaminophen poisoning.

Materials and methods

Animal care

The study was reviewed and approved by the Institutional Review Board for animal experiments of the Tübingen Regional Council, Germany (approval No. C3/09). National and institutional guidelines for the care and use of laboratory animals were used. All experiments were conducted in compliance with the standards outlined in the Guide for the Care and Use of Laboratory Animals and under the supervision of a veterinarian who set the guiding principles to minimize animal suffering.

Six female German Landrace pigs weighing 33.7 ±2.8 kg (range: 29.0–37.5 kg) and aged approx. 8 weeks underwent a laparotomy and consecutive acetaminophen administration.

During an adaptation period of 1 week, the animals were kept in cages in the Research Animal Care Facility at a temperature of approx. 20°C and relative humidity of approx. 60% with natural day–night cycles, in accordance with the European Union directive 2007/526/EG and the European Union guideline 2010/63/EU. They received a standard diet and tap water ad libitum. In the evening before the surgical procedure, the pigs were deprived of food (not water). Before the onset of the experiment, all animals were examined by a veterinarian, and no abnormalities were found.

Premedication, anesthesia protocols, intensive care treatment, and algorithms for standardized intensive care management have been reported in detail in previous studies.[17,18] Briefly, the animals received intramuscular premedication of atropine 0.1% (0.05 mg/kg), ketamine (14 mg/kg), azaperone (2 mg/kg), and midazolam (0.5 mg/kg). After oral intubation, ventilation was performed using a pressure-controlled ventilation machine. Continuous intravenous anesthesia was maintained with ketamine (15 mg/kg/h), fentanyl (0.02 mg/kg/h) and midazolam (0.9 mg/kg/h). The animals remained under general anesthesia for a total observation period of 40 h following acetaminophen administration.

Surgical procedures

Briefly, the internal carotid artery (Leadercath; Vygon, Écouen, France) and jugular veins (Multi-Lumen Central Venous Catheter; Arrow International, Reading, USA) were instrumented to measure mean arterial pressure (MAP) and central venous pressure (CVP), respectively. The abdominal cavity was entered through a midline incision. A jejunal catheter (Gentle-Flo™; Tyco Healthcare, Tullamore, Ireland) was implanted in the upper jejunum for acetaminophen administration, and a urinary catheter (Gentle-Flo™; Tyco Healthcare) was placed by cystostomy. The abdomen was then closed in a standard fashion.

Acetaminophen administration and acute liver failure

Immediately after the closure of the abdomen, the animals received a single bolus of acetaminophen of 1000 mg/kg through the jejunal catheter. For this purpose, commercially available acetaminophen tablets were crushed with a tablet mortar and dissolved in 10 mL of physiological sodium chloride solution. After the administration of the solution, the jejunal catheter was flushed with 50 mL of sodium chloride solution.

The onset of ALF syndrome was determined by the presence of coagulopathy, as shown by a decline in prothrombin time (PT, Quick) to below 30%.

Critical care management

Standard hemodynamic monitoring was used and recorded throughout the entire experiment, and included electrocardiography, oxygen saturation, MAP, CVP, and core body temperature. Arterial blood gas analysis (ABL 800; Radiometer, Copenhagen, Denmark), including the measurement of hemoglobin, methemoglobin, hematocrit, acid base balance, lactate, serum electrolytes, and blood glucose levels, was recorded hourly and directly corrected as required, while ventilation parameters were adjusted accordingly. General anesthesia was sustained with a continuous infusion of ketamine 15 mg/kg/h, fentanyl 0.02 mg/kg/h and midazolam 0.9 mg/kg/h. Urinary output was monitored, and volume resuscitation and vasopressor use were performed according to a standard protocol, as previously described.[17] After a total observation period of 40 h from the administration of acetaminophen, the surviving pigs were euthanized with a single intravenous bolus of 10 mL of T-61 (Intervet, Unterschleißheim, Germany). One mL of T-61 consists of 5 mg of tetracaine hydrochloride, 50 mg of mebezonium iodide and 200 mg of embutramide.

Biochemical analyses

All blood samples were collected through the internal carotid catheter and analyzed by the certified Central Laboratories of University Hospital Tübingen (Division of Endocrinology, Diabetology, Angiology, Nephrology, Pathobiochemistry, and Clinical Chemistry, Department of Internal Medicine), Germany. Laboratory parameters were obtained before and every 8 h after acetaminophen administration until death (or a maximum of 40 h). Acetaminophen levels were determined with an enzymatic assay performed on a fully automated biochemical analyzer (ACTM Flex® reagent cartridge, Cat. No. DF88; Dimension Xpand Plus; Siemens Healthcare Diagnostics, Eschborn, Germany) in an accredited laboratory. The certified assay has a range of 0.0–300.0 µg/mL (0.0–1985.4 µmol/L) and a total coefficient of variation of 4.44% (serum)/1.45% (plasma).

Samples with results in excess of 300.0 µg/mL (1985.4 µmol/L) were repeated following dilution. Albumin, total plasma protein, aspartate aminotransferase (AST) activity, and lactate concentration were measured on the ADVIA 1800 Clinical Chemistry Analyzer, and the ADVIA 2120 Hematology Analyzer was used for blood cell counts (both from Siemens Healthcare Diagnostics). Coagulation tests were performed on the ACL TOP 700 Hemostasis Testing System (Instrumentation Laboratory, Kirchheim, Germany). Tumor necrosis factor alpha and IL-6 (both R&D Systems, Minneapolis, USA), as well as hepatocyte growth factor (HGF) and epidermal growth factor (EGF) (both from Cusabio Biotech, Wuhan, China) plasma concentrations were analyzed using specific porcine enzyme-linked immunosorbent assay (ELISA) kits. The sample analysis was conducted within 1 h of blood withdrawal.

Pathological examinations

Immediately post mortem, a liver biopsy specimen of 1.0 × 1.0 × 0.5 cm from the lobus hepatis sinister lateralis was obtained from all 6 animals and fixed in 4% formaldehyde. Hematoxylin and eosin (H&E) were used to stain the specimens, and the POL HRP-006 Polymer Kit, monoclonal mouse antihuman Ki-67, antigen clone MIB-1 Code M7240, and Mouse IgGX 0931 for Ki-67 were used for immunostaining (Zytomed Systems GmbH, Berlin, Germany, and Agilent, Santa Clara, USA). Liver sections were examined and photographed with a Zeiss Axiovert 135 Microscope (Carl Zeiss AG, Jena, Germany).

Pharmacokinetic profile

The elimination half-life (t1/2el) was calculated. For this purpose, the acetaminophen concentration data were log transformed (logarithmus naturalis) and plotted against time. A trend line was calculated using linear regression. The slope in the equation shows the elimination rate constant (kel). To obtain the half-life of the elimination phase, the formula t1/2el = 0.693/kel was used.[19]

Statistical analyses

The mean values obtained before, during and after the administration of acetaminophen were compared using the Wilcoxon test (JMP® 12.0; SAS Institute, Cary, USA). A value of $p < 0.05$ was considered statistically significant. Results in the manuscript and figures are reported as mean and standard deviation (SD).

Results

Manifestation of acute liver failure

Three of the 6 animals developed ALF followed by multiple organ failure and subsequent death after 24 h, 25 h and 38 h. The other 3 of the 6 animals survived the administration of acetaminophen. A significant deterioration of MAP occurred 12 h after acetaminophen administration (51 ±5 mm Hg in nonsurviving animals compared to 72 ±1 mm Hg in surviving animals ($p < 0.05$, Wilcoxon test)). Changes in hemodynamic parameters and selected laboratory values during the experiment are presented in Table 1.

Histology of the liver

At autopsy, the H&E-stained liver biopsies in the nonsurviving animals demonstrated progressive centrilobular necrosis (Fig. 1A) in contrast to a small amount of necrosis around the central vein in an otherwise intact lobule of the liver in the surviving animals (Fig. 1B). Figure 1C shows necrotic cell nuclei and no evidence of Ki-67 expression in a representative nonsurviving animal specimen at the end of the experiment. In the surviving animals, a total of 35 ±15 Ki-67-positive cells/lobule of the liver were observed (Fig. 1D).

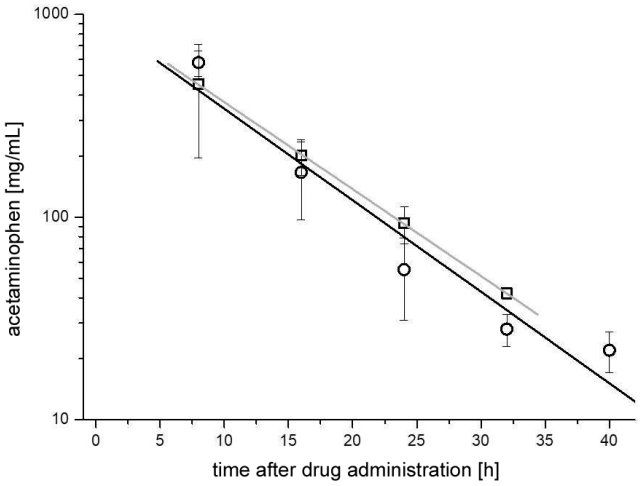

Fig. 2. Mean acetaminophen blood concentration in relation to time after drug administration

□ – nonsurviving animals; ○ – surviving animals.

Acetaminophen serum concentrations

The mean acetaminophen blood concentrations 8 h after receiving the bolus of 1000 mg/kg were 454 ±258 mg/L in the nonsurviving animals and 578 ±83 mg/L in the surviving animals; there was no statistically significant difference

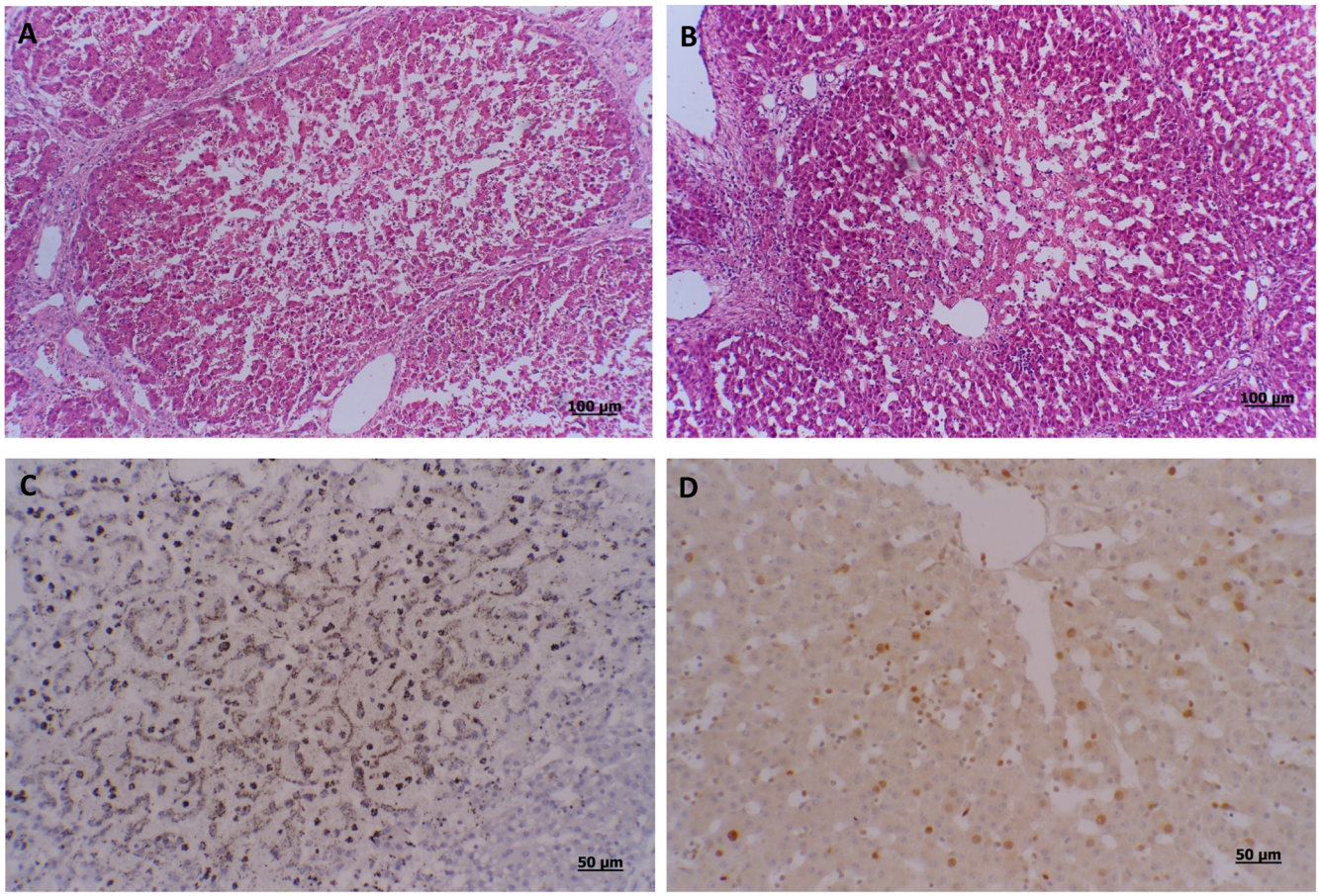

Fig. 1. Light microscope preparations (hematoxylin and eosin (H&E) staining) of post-mortem liver biopsies in nonsurviving (A) and surviving animals (B) and Ki-67 immunostaining in nonsurviving (C) and surviving animals (D)

between the groups in this regard (Fig. 2). The pharmacokinetic profiles were similar for both groups (kel in nonsurviving animals: −0.0989, resulting in a half-life of 7.0 h; kel in surviving animals: −0.10396, resulting in a half-life of 6.7 h).

Cytokines and growth factors

The baseline TNF-α levels before receiving the acetaminophen bolus were significantly higher in the group of nonsurviving animals (200 ±95 pg/mL compared to 28 ±26 pg/mL in the surviving animals; $p < 0.05$, Wilcoxon test, Fig. 3A). The concentration of TNF-α in the nonsurviving animals remained higher than in the surviving animals throughout the experiment. In the group of surviving animals, the TNF-α concentration increased to 120 ±60 pg/mL 8 h after drug administration, followed by a steady decline to 32 ±29 pg/mL at the end of the monitoring period.

The baseline IL-6 concentrations did not differ significantly between the groups (Fig. 3B). An increase in IL-6 levels to 1676 ±790 pg/mL was observed in the nonsurviving animals after 8 h, and IL-6 concentrations remained significantly elevated during the observation period. The surviving animals showed slightly elevated IL-6 values (443 ±388 pg/mL) at the time of drug administration, which decreased to below the initial values over the observed time (121 ±107 pg/mL after 32 h). The EGF concentrations oscillated from 0 ng/mL to 2 ng/mL and the HGF concentrations were approx. 1500 ng/mL in both groups.

Acute liver failure-related laboratory parameters

All biochemical parameters at baseline were within the physiological range reported earlier for German Landrace pigs.[20] The PT values in the nonsurviving animals

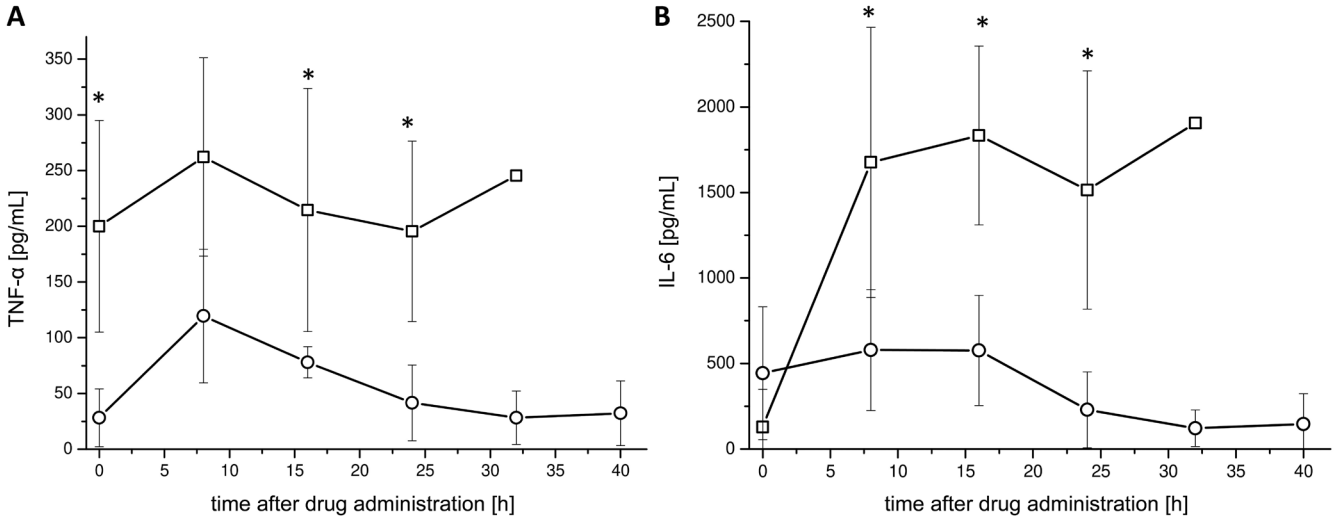

Fig. 3. Course of tumor necrosis factor alpha (TNF-α) (A) and interleukin (IL)-6 (B) in relation to time after drug administration

□ – nonsurviving animals (n = 3 at 24 h, n = 1 at 32 h); ○ – surviving animals (n = 3); * $p < 0.05$, Wilcoxon test.

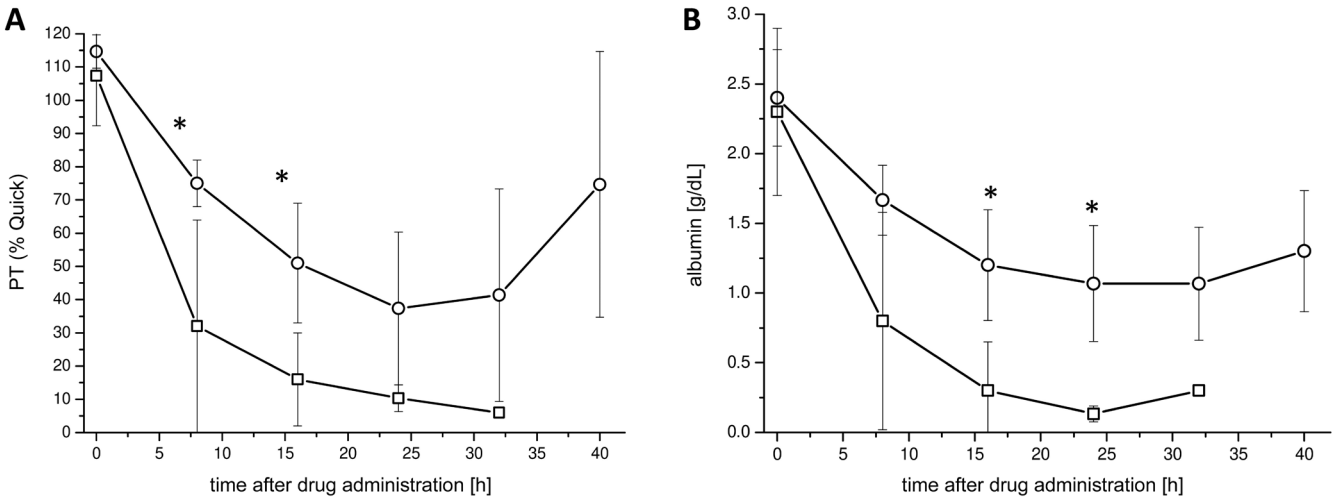

Fig. 4. Course of prothrombin time (A) and albumin (B) in relation to time after drug administration

□ – nonsurviving animals (n = 3 at 24 h, n = 1 at 32 h); ○ – surviving animals (n = 3); * $p < 0.05$, Wilcoxon test.

declined continuously, demonstrating the onset of ALF after 8 h of acetaminophen administration, and remained constantly below 30% during the observation period (Fig. 4A). The surviving animals showed a decline in PT values after the administration of acetaminophen, but their mean values remained above 30% and recovered to 75 ±40% after 40 h.

The baseline albumin concentrations ranged between 2.3 mg/dL and 2.4 mg/dL in both groups (Fig. 4B). Sixteen hours after drug administration, the nonsurviving animals showed significantly lower albumin concentrations than the surviving animals (0.3 ±0.4 mg/dL compared to 1.2 ±0.4 mg/dL; $p < 0.05$, Wilcoxon test).

Aspartate aminotransferase activity did not differ between the nonsurviving and surviving animals during the entire observation period and showed a gradual increase (before drug administration: 53 ±24 U/L; after 8 h: 52 ±21 U/L; after 16 h: 72 ±36 U/L; after 24 h: 191 ±175 U/L; after 32 h: 524 ±515 U/L; and after 40 h: 603 ±627 U/L).

Immediately before the administration of acetaminophen, the average lactate concentrations were 2.0 ±0.7 mmol/L in the surviving animals and 2.0 ±1.4 mmol/L in the nonsurviving animals. Eight hours after drug administration, the mean lactate concentration in the nonsurviving animals increased to 5.0 ±2.2 mmol/L, while it decreased to 1.3 ±0.9 mmol/L in the surviving animals ($p < 0.05$, Wilcoxon test). In the last 12 h before death, the lactate concentrations reached levels above 10 mmol/L in the nonsurviving animals.

Methemoglobinemia occurred during the first 4 h after acetaminophen administration (18.6 ±8.7% in the nonsurviving animals and 10.8 ±8.0% in the surviving animals; the difference was not statistically significant). Twenty-four hours after drug administration, the methemoglobin levels decreased to 2.8 ±3.4% in the surviving animals and 6.9 ±4.0% in the nonsurviving animals.

Discussion

Acetaminophen is a potentially harmful medication with pronounced variability in individual tolerance. The toxic metabolite of acetaminophen is N-acetyl-p-benzoquinone imine (NAPQI), which can be reduced to a nontoxic metabolite by glutathione in therapeutic doses.[21] However, NAPQI in higher doses can deplete glutathione and then covalently bind to mitochondrial proteins, leading to oxidative stress, inflammation, mitochondrial dysfunction, and cell death. Understanding of the mechanism involved in this procedure is essential for analyzing existing and prospective biomarkers.

The critical dose of acetaminophen for developing severe liver injury in humans is 4000 mg in 24 h.[21] In pigs, 1000 mg/kg body weight was identified as a critical dose by Artwohl et al. in 1988.[22] In their study, animals receiving 500–1000 mg/kg of acetaminophen survived, while animals receiving a dose of 1000–2000 mg/kg died within 6.5 h due to methemoglobinemia.

Thus, patients who are prone to developing ALF after acetaminophen intoxication should be identified as early as possible in order to ultimately prevent acetaminophen-induced ALF and increase their likelihood of survival. The purpose of this study was to measure blood cytokine levels and ALF-related laboratory parameters in a porcine model of acetaminophen overdose in order to guide the search for early biomarkers that could discriminate between susceptibility and tolerance to acetaminophen.

The prominent finding of this study is that an elevated baseline level of TNF-α seems to predict an increased susceptibility to acetaminophen-induced ALF. The TNF-α levels in the nonsurviving animals remained higher than in the surviving animals. It is known that TNF-α blood levels are increased in ALF[23–25] and correlate directly with disease activity and overall prognosis.[14,26] However, it is not clear why the animals differed in TNF-α before the treatment was initiated and what might have induced the expression of TNF-α. The animals were young (approx. 8 weeks old), healthy and obtained from the same farmer. Clinical examination by the veterinarian before commencing the experiments showed no clinical signs or symptoms of illness. All laboratory parameters, including liver enzymes and infection parameters at baseline, were within the physiological range for German Landrace pigs, which makes pre-existing subclinical or undetected liver damage or infection unlikely.

Our data suggest that elevated TNF-α blood concentrations at baseline might accelerate disease progression. Further investigation is needed to clarify whether an elevation of TNF-α at baseline directly affects the outcome by enhancing toxic acetaminophen effects or indicates an epiphenomenon. However, an elevated baseline TNF-α level could potentially serve as an early and easily obtainable prognostic marker for determining a risk–benefit ratio for the development of ALF after the administration of acetaminophen.

The nonsurviving animals had a significant increase in IL-6 after acetaminophen administration, and even though the IL-6 levels decreased steadily within 16 h after acute exposure, the IL-6 concentrations remained higher than in the surviving animals throughout the course of the experiment. In contrast, HGF and EGF levels did not differ between the surviving and nonsurviving animals.

To examine the histological signs of liver damage and determine regeneration potential, we performed liver biopsies at the end of the experiment. All of the surviving animals showed a large quantity of Ki-67-positive cells as a marker of regeneration, in contrast to the nonsurviving animals, that showed centrilobular necrosis and no signs of Ki-67 expression.

Bernal et al. identified an increased blood lactate concentration as an early predictor of outcome in acetaminophen-induced ALF and proposed a modification to the King's College Criteria in order to include blood lactate concentrations with cutoff values of 3.5 mmol/L measured early after admission and 3.0 mmol/L after

Table 1. Hemodynamic parameters and laboratory values of selected biochemical parameters before and after drug administration

Time after drug administration [h]	0		8		16		24		32		40	
Group	non-surv	surv	non-surv	surv	non-surv	surv	non-surv	surv	non-surv	surv	non-surv	surv
Number of animals	3	3	3	3	3	3	3	3	1	3	0	3
Heart rate [bpm]	79 ±27	62 ±17	126 ±18	93 ±113	116 ±25	87 ±15	145 ±31	75 ±11	126	69 ±8	–	66 ±9
Oxygen saturation [%]	100 ±0	100 ±1	95 ±5	98 ±1	98 ±3	100 ±0	95 ±7	100 ±0	100	99 ±1	–	99 ±2
Mean arterial pressure [mm Hg]	62 ±6	59 ±9	69 ±11	84 ±112	51 ±6	67 ±5	46 ±1	71 ±6	44	75 ±6	–	80 ±11
Central venous pressure [mm Hg]	8 ±2	8 ±3	11 ±5	10 ±2	12 ±2	10 ±4	14 ±3	11 ±5	11	12 ±6	–	10 ±4
Hemoglobin [g/dL]	9.1 ±0.6	9.7 ±0.8	8.7 ±1.6	8.8 ±1.8	8.2 ±1.2	9.4 ±0.8	7.0 ±1.4	8.8 ±0.2	7.5	8.6 ±0.6	–	9.1 ±0.4
Methemoglobin [%]	0.7 ±0.1	1.4 ±0.2	24.9 ±16.7	11.5 ±6.4	10.9 ±6.6	3.5 ±4.7	6.9 ±4.0	2.8 ±3.4	1.5	3.1 ±3.9	–	2.8 ±3.5
Hematocrit [%]	27 ±1	29 ±3	26 ±5	27 ±6	24 ±4	29 ±3	21 ±5	26 ±1	22	26 ±2	–	27 ±2
pH	7.47 ±0.02	7.47 ±0.02	7.33 ±0.07	7.37 ±0.05	7.31 ±0.03	7.39 ±0.06	7.33 ±0.06	7.42 ±0.04	7.27	7.40 ±0.02	–	7.42 ±0.07
Natrium [mmol/L]	140 ±1	140 ±3	148 ±4	146 ±4	155 ±3	146 ±3	161 ±6	151 ±7	158	156 ±1	–	153 ±6
Potassium [mmol/L]	4.1 ±0.3	3.9 ±0.8	3.2 ±0.9	3.5 ±0.5	3.5 ±0.4	3.8 ±0.3	3.9 ±0.8	3.6 ±0.1	5.5	3.3 ±0	–	3.7 ±0.1
Blood glucose [mmol/L]	6.5 ±2.5	7.0 ±1.7	7.3 ±1.5	6.2 ±1.5	5.8 ±1.0	6.1 ±1.3	6.7 ±1.7	5.7 ±1.1	5	5.8 ±0.1	–	4.8 ±0.6

Non-surv – nonsurviving animals; surv – surviving animals.

volume resuscitation.[27] In the current study, the lactate concentrations in the surviving animals remained between 0.9 mmol/L and 1.3 mmol/L, while the nonsurviving animals had peak lactate levels of 5.0 ±1.2 mmol/L as early as 8 h after acetaminophen administration. The French guidelines on the management of liver failure in general intensive care unit recommend monitoring lactate levels as well as the results of arterial blood gas analysis.[3]

Multiple animal studies with different species have linked the production of methemoglobin as a toxic by-product in acetaminophen-induced ALF with lethal outcomes.[22,28,29] Interestingly, in another porcine experiment, methemoglobinemia correlated directly with administered doses and blood levels of acetaminophen.[28] In a further porcine study, acetaminophen levels higher than 250 mg/L were seen to increase serum methemoglobin levels.[30] In our study, the concentration of acetaminophen rose to nearly 600 mg/L and methemoglobinemia occurred during the first 4 h after acute exposure. However, under standardized intensive care therapy, methemoglobin began to decrease continuously, even after the animals developed the typical hemodynamic and laboratory signs of ALF. The methemoglobin levels were slightly higher in the nonsurviving animals than in the surviving animals, but did not differ to a statistically significant degree. The presence of methemoglobinemia did not affect survival in this study.

After receiving an acetaminophen bolus of 1 g/kg body weight, the serum acetaminophen concentrations increased to 600 mg/L, which was previously described as a hepatotoxic concentration in humans.[31] The acetaminophen serum concentrations and elimination of acetaminophen did not differ between the surviving and nonsurviving animals. Even though acetaminophen is known to be a dose-dependent toxicant, it has been shown that acetaminophen dosing information in patients with ALF is an unreliable predictor of survival.[32] Furthermore, the total ingested dose did not influence survival in this study. Thus, it seems unlikely that acetaminophen serum concentration is the sole driving force that determines the clinical outcome.

Limitations

The small sample size and lack of a control group, which are factors of the pilot nature of the study, are clear limitations of this study. Therefore, larger investigations including controls are needed to validate the present data. In addition, the study did not analyze further acetaminophen metabolites, levels of NAPQI bound to proteins, or glutathione, and did not assess enzymes linked to oxidative stress.

Conclusions

In summary, TNF-α, IL-6, lactate, PT, and albumin blood concentration were identified as early predictors of outcome after acetaminophen intoxication. On the contrary, methemoglobinemia, acetaminophen serum

concentration, AST activity, and plasma HGF and EGF concentration did not differ between animals that were susceptible to acetaminophen and those who survived the acute phase of the poisoning.

An elevated TNF-α plasma concentration before the exposure to acetaminophen was the earliest prognostic plasma marker suggesting lethal outcome, and thus could possibly serve as a very early prognostic marker. Further studies in larger study groups and with human samples obtained from clinical cases are warranted.

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