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Indocyanine Green Clearance Varies as a Function of N-Acetylcysteine Treatment in a Murine Model of Acetaminophen Toxicity

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Abstract

Standard assays to assess acetaminophen (APAP) toxicity in animal models include determination of ALT (alanine aminotransferase) levels and examination of histopathology of liver sections. However, these assays do not reflect the functional capacity of the injured liver. To examine a functional marker of liver injury, the pharmacokinetics of indocyanine green (ICG) were examined in mice treated with APAP, saline, or APAP followed by N-acetylcysteine (NAC) treatment. Male B6C3F1 mice were administered APAP (200 mg/kg IP) or saline. Two additional groups of mice received APAP followed by NAC at 1 or 4 h after APAP. At 24 h, mice were injected with ICG (10 mg/kg IV) and serial blood samples (0, 2, 10, 30, 50 and 75 min) were obtained for determination of serum ICG concentrations and ALT. Mouse livers were removed for measurement of APAP protein adducts and examination of histopathology. Toxicity (ALT values and histology) was significantly increased above saline treated mice in the APAP and APAP/NAC 4 h mice. Mice treated with APAP/NAC 1 h had complete protection from toxicity. APAP protein adducts were increased in all APAP treated groups and were highest in the APAP/NAC 1 h group. Pharmacokinetic analysis of ICG demonstrated that the total body clearance (Cl_T) of ICG was significantly decreased and the mean residence time (MRT) was significantly increased in the APAP mice compared to the saline mice. Mice treated with NAC at 1 h had Cl_T and MRT values similar to those of saline treated mice. Conversely, mice that received NAC at 4 h had a similar ICG pharmacokinetic profile to that of the APAP only mice. Prompt treatment with NAC prevented loss of functional activity while late treatment with NAC offered no improvement in

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Conflict of Interest Statement

None.

ICG clearance at 24 h. ICG clearance in mice with APAP toxicity can be utilized in future studies testing the effects of novel treatments for APAP toxicity.

Keywords

Acetaminophen; N-Acetylcysteine; Pharmacokinetics; Hepatocellular Necrosis

1. Introduction

Acetaminophen (paracetamol, N-acetyl-*p*-aminophenol, APAP) is the most commonly used antipyretic and analgesic agent in the world today. Despite its safety at therapeutic doses, APAP is a major cause of acute liver failure in the United States and worldwide [1,2].

The toxicity of APAP is characterized by the pathologic appearance of centrilobular necrosis. Determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in serum and histopathologic examination of liver tissues are the common measures used to assess APAP toxicity in the experimental setting. Elevated hepatic transaminase levels are used as a measure of hepatocyte lysis and thus do not reflect the functional capacity of the liver. Because structurally injured hepatocytes may be partially functional [3,4], it is important to distinguish liver cell injury from loss of functional capacity. Assays that characterize liver function are critical in studies that assess hepatic recovery following toxin-mediated and other causes of liver injury. For example, a useful endpoint in the design of potential new therapies for liver toxicity would be demonstration of return to baseline liver function.

Indocyanine green (ICG), a water-soluble dye that does not undergo metabolism or enterohepatic circulation, serves as a measure of hepatic function because of its exclusive elimination via biliary excretion [5]. In addition, ICG distributes into the plasma volume without extravascular distribution [6]. The clearance of ICG has been used as a measurement of hepatic blood flow and liver function in clinical studies [7–9]. In addition, the clearance of ICG is used to evaluate hepatic function pre- and post-liver transplantation [10] and recent studies have found ICG to be a more reliable indicator of liver function post-operatively than other conventional endogenous markers such as bilirubin and prothrombin time [11]. Several studies performed in experimental models have examined the clearance of ICG as a functional marker of liver injury [6,12,13]. However, the clearance of ICG has not been previously studied as an endpoint to assess the effectiveness of treatments for drug-induced toxicity.

N-Acetylcysteine (NAC) is a thiol-containing compound that has been used for over 30 years as the antidote for APAP toxicity in man [14]. NAC acts as a glutathione (GSH) precursor, promoting GSH synthesis and increasing hepatic GSH stores in order to detoxify N-acetyl-*p*-benzoquinone imine (NAPQI), the reactive intermediate metabolite of APAP [15,16]. In addition, NAC may react directly with NAPQI and acts as a scavenger of the reactive oxygen/nitrogen species peroxynitrite. Treatment with NAC has been shown to reduce APAP-induced liver toxicity in humans if administered within 10 h of an APAP overdose [17]. NAC has an intermediate hepatoprotective effect if administered after 10 but put prior to 24 h of APAP overdose [17]. Additional studies have shown that NAC treatment improves patient outcome even when administered late (*e.g.*, more than 24 h post-overdose [18,19]), despite having minimal effects on the hepatic injury, *per se*.

We hypothesized that ICG could be used as a functional marker of APAP toxicity in the mouse and may represent a potential assay to assess the efficacy of various novel

interventions for the treatment of APAP toxicity. To this end, we characterized the pharmacokinetics of ICG in APAP-treated mice and compared these parameters to those of saline-treated mice. In addition, since NAC is the current cornerstone of management of APAP toxicity, the pharmacokinetics of ICG were examined in mice following NAC treatment. Two treatment schedules for NAC were tested to mimic the clinical setting of prompt and delayed treatment with NAC [17]. The data presented herein demonstrate that the pharmacokinetics of ICG differ among saline treated mice, APAP treated mice, and APAP treated mice that receive early or late NAC treatment.

2. Materials and Methods

2.1. Drugs and Reagents

APAP, ICG (Cardiogreen) and NAC were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals were of reagent grade or better.

2.2. Animals

B6C3F1 mice (six-week old males; average weight of 25.1 grams) were purchased from Harlan Sprague Dawley (Indianapolis, IN). One week before the initiation of experiments, mice were acclimatized to the facility and placed on a 12 h light/dark cycle under controlled ambient temperature. Animals were housed 3 per cage (APAP/ICG studies) or in individual cages (APAP/NAC/ICG studies); they were fed *ad libitum* and fasted overnight prior to all studies. All animal experimentation was in agreement with the criteria of the “Guide for the Care and Use of Laboratory Animals” as per the guidelines of the National Academy of Sciences. Experimental protocols were reviewed and approved by University of Arkansas for Medical Sciences Animal Care and Use Committee.

2.3. Experimental Protocol

Animals were segregated into four treatment groups as follows: 1) saline (0.9% NaCl; n=6), 2) APAP (200 mg/kg in saline; n=8), 3) APAP (200 mg/kg in saline) followed by NAC (1200 mg/kg IP in saline, pH 7.4) at 1 h (n=6), and 4) APAP (200 mg/kg in saline) followed by NAC at 4 h (n=7) given via IP injection. At 24 h after APAP or saline, mice received ICG (10 mg/kg) as a 0.2 ml bolus via tail vein injection. Mice were then anesthetized in a CO₂ chamber before blood was drawn from the retro-orbital vein. Blood samples for determination of ICG concentrations in serum were obtained at baseline and at 2, 10 and 30 min (control group) or at 2, 10, 30, 50 and 75 min (APAP groups) after ICG administration. The total amount of blood sampled from each mouse was less than 15% of the total circulating blood volume [20] to avoid hemodynamic changes that could potentially affect the pharmacokinetics of ICG. Blood samples were allowed to coagulate at room temperature and then centrifuged to obtain serum. Euthanasia was conducted by cervical dislocation and livers were quickly removed for histopathological analyses. All samples were stored at -80° C until analysis.

2.4. Toxicity assays and liver histopathology

Serum ALT levels were determined using an Ace Alera Chemistry Analyzer (Alfa Wassermann Inc., West Caldwell, NJ). Hematoxylin and eosin staining was performed for histological examination of mouse livers. The extent of necrosis in liver sections was quantified by outlining the necrotic areas with the interactive spline measuring tool in the AxioVision 4.6.3 program (Carl Zeiss Inc., Germany). Three images were obtained from each section at 10X magnification. Quantification of the extent of necrosis was expressed as a percentage of the entire histological field [21].

2.5. Measurement of ICG in serum by high performance liquid chromatography

Analysis of ICG concentrations in mouse serum was performed using a Waters Alliance 2695 Separations Module with a photodiode array detector set at 720 nm. The method was adapted from Kulkarni et al. [12] and validated in our laboratory. A Waters C₁₈ symmetry column equipped with a guard column (Waters Associates, Milford, MA) was used for reverse-phase chromatography at a temperature of 35° C. The mobile phase consisted of 50 mM potassium phosphate buffer (pH 5.52): acetonitrile (55:45), at a flow rate of 1.0 ml/min and 17-minute run time. A volume of 20 µl was injected following precipitation with acetonitrile. The retention time of ICG was approximately 12 min.

2.6. Pharmacokinetic analysis

ICG plasma concentration vs. time data were evaluated using a model independent approach. The pharmacokinetic profiles were curve fit using a peeling algorithm to generate initial polyexponential parameter estimates. Final estimates of the terminal elimination rate constant (λ_z) were determined from an iterative, nonlinear least squares regression algorithm. The area under the plasma concentration versus time curve (AUC) was determined using the log-linear trapezoidal rule. Extrapolation of the AUC to infinity ($AUC_{0-\infty}$) calculated by summation of $AUC_{0-n} + C_n/\lambda_z$, where λ_z is the apparent terminal elimination rate constant and C_n represents the final plasma concentration predicted from the fitted apparent terminal elimination phase. Mean residence time is calculated by dividing the area under the moment curve (AUMC) by the area under the curve [$MRT=AUMC/AUC$]. Model-independent (non-compartmental) pharmacokinetic parameters were calculated using standard (i.e., statistical moment theory) techniques. For the smaller number of samples wherein a sufficient number of post-peak observations permitted exploratory model-dependent analysis, pharmacokinetic parameters were calculated from final polyexponential parameter estimates. Final model selection was performed after application of goodness of fit criteria (e.g. objective function, Akaike and Schwartz criteria) and examination of the coefficients of variation for the polyexponential parameters estimated from a given model. Pharmacokinetic analyses were conducted using Kinetica software version 5.0 (Thermo Scientific, Waltham, MA).

2.7. Determination of APAP protein adducts

APAP protein adducts in the liver were quantified by assaying supernatants of liver homogenates using a high performance liquid chromatography with electrochemical (HPLC-EC) detection method developed by our laboratory [22,23]. Since the retention times of APAP-CYS and APAP-NAC on the chromatogram differ, the HPLC-EC assay was able to differentiate between APAP-CYS and APAP-NAC in mice that received NAC after APAP.

2.8. Statistical analysis

Toxicity and ICG pharmacokinetic data were examined using standard descriptive statistics. One-way analysis of variance was used to compare calculated pharmacokinetic parameters between treatment groups. Univariate analysis of variance and nonlinear regression were used to evaluate the relationship between transaminase values and pharmacokinetic parameter estimates. All analyses were performed in SPSS version 12.0 (SPSS, Chicago, IL) and p-values less than 0.05 were considered to indicate statistical significance.

3. Results

3.1. Biochemical and histopathological determination of APAP toxicity

B6C3F1 male mice were treated with either APAP (200 mg/kg IP in saline) or saline and sacrificed at 24 h. Other groups of mice were treated with APAP (200 mg/kg IP) followed

by NAC (1200 mg/kg IP) at 1 h or NAC at 4 h and were sacrificed at 24 h. Serum ALT levels were significantly increased in mice treated with APAP compared to the saline mice (* $p < 0.05$, Fig 1A). In addition, histopathological examination of livers from mice treated with APAP showed significant necrosis (Fig 1B) whereas the livers of saline treated mice were normal. Mice that received NAC 4 h after APAP had ALT levels that were significantly increased above the saline treated mice (* $p < 0.05$, Fig 1A). In contrast, mice that received NAC 1 h after APAP had ALT levels that were comparable to the saline mice. Likewise, histopathological analysis of livers indicated that mice treated with APAP followed by NAC at 1 h had necrosis scores similar to control mice, whereas mice treated with NAC at 4 h had necrosis scores similar to APAP only treated mice (Fig 1B). Thus, mice treated with NAC at 1 h had relative protection from APAP toxicity, while the mice that received NAC 4 h after APAP had similar amounts of necrosis as the APAP only mice (Figs 2A–2D). Concentrations of hepatic APAP protein adducts were measured at 24 h after APAP and were increased above saline in all the APAP treated groups (Fig 3; $p < 0.05$). Mice treated with NAC at 1 h had APAP protein adducts that were higher than those in the APAP/veh group ($p < 0.05$). In contrast, mice treated with NAC at 4 h had APAP protein adducts that were comparable to the levels of the APAP/veh mice. The higher concentration of adducts in the APAP/NAC 1 h mice compared to the APAP/veh mice represents adducts formed in the liver by 1 h, but not released into the serum because the hepatocytes did not rupture (Fig 1A). In contrast, the relatively lower adduct levels in the APAP and APAP/NAC at 4 h mice represent adducts that remained in hepatocytes that did not undergo lysis and release contents into the blood. Overall, the results were consistent with previous data from our laboratory showing that NAC administered 1 h after APAP prevented APAP-induced liver toxicity but did not appreciably decrease APAP-protein adduct formation, [24] whereas NAC administered 4 hr after APAP had no protective effects.

3.2. Pharmacokinetics of ICG in APAP toxicity: comparison of APAP treated mice to saline controls

The mean ($\pm$ SD) plasma ICG concentration-versus-time profiles by treatment group are illustrated in Figure 4. With the exception of the immediate post-bolus concentration (i.e. 2 min), ICG concentrations were higher at all time points in the mice that received APAP (Fig 4A) compared to the saline controls (Fig 4B). Summary data for the pharmacokinetic parameters for the APAP and the saline mice are presented in Table 1. Marked differences in pharmacokinetic parameters were observed between the APAP only mice and the saline control mice. Notably, the mean residence time (MRT), a measure of the average time that ICG remains in the body, was 4-fold longer and the total body clearance (Cl_T) was reduced by 3-fold in APAP mice compared to the saline mice (Table 1). A graphical depiction of pharmacokinetic parameters (C_{max} , Cl_T , MRT, and AUC) is depicted in Figure 5. As demonstrated, the pharmacokinetics of ICG clearly differed in the mice that received APAP and developed toxicity and the saline controls.

Despite the minimal sampling scheme, the pharmacokinetic profiles of ICG for the APAP only mice and the saline mice appeared to exhibit a pattern of bi-exponential decay (Fig 4). Consequently, an exploratory analysis was conducted to derive compartmental estimates for those mice in which a sufficient number of ICG concentrations were available. Although the coefficients of variation were large, compartmental parameter estimates could be derived with reasonable goodness-of-fit criteria for the majority of mice. As expected, based on visual inspection of the mean composite profiles, both the alpha (α ; Fig 6) and beta (β) rate constants differed more than 2-fold between the APAP only and the saline mice (Table 2). Consequently, the impact of APAP exposure on the clearance of ICG was more pronounced than reflected by the singular parameter λ_z derived from the non-compartmental analysis (Table 1).

3.3. Effect of NAC treatment on ICG pharmacokinetics in APAP treated mice

To further examine the pharmacokinetics of ICG in APAP toxicity, the same parameters were determined in mice that were treated with NAC 1 h or 4 h following APAP (Tables 1 and 2). Mice that received NAC 1 h after APAP had a biodisposition profile for ICG that was similar to that of saline mice (Fig 4). In particular, the values for Cl_T and MRT were very similar between the two groups of mice (Fig 5B, Fig, 5C). In contrast, mice that received NAC 4 h after the administration of APAP had a pharmacokinetic profile that was statistically similar to that of the APAP treated mice (Fig 4). The values for Cl_T , $t_{1/2}$, MRT, and λ_z in the APAP/NAC at 4 h group were similar to the values observed for the same pharmacokinetic parameters in the APAP only treated mice. Thus, the data indicated that late treatment with the antidote NAC failed to provide any functional hepatic benefits. Summary pharmacokinetic data for all four treatment groups are graphically depicted in Figure 5. In addition, the compartmental analysis showed that mice treated with NAC at 4 h had similar α and β rate constants to those of the APAP only treated mice. Thus, the data clearly demonstrate that the disposition of ICG is altered in APAP treated mice and that the timing of treatment with the antidote NAC affected the clearance of ICG.

4. Discussion

The toxicity of APAP has been extensively characterized in mouse models and in humans. ALT measurements are commonly used indicators of toxicity in experimental and clinical studies of APAP injury. However, measurement of these serum markers does not *per se* quantify liver function [6]. The current study was designed to examine ICG as a functional marker of APAP toxicity in the mouse model and to examine the effect of treatment with NAC on ICG clearance. Our data demonstrate that the pharmacokinetic profiles of ICG varied significantly between the APAP only and saline treated mice. The most striking findings were the changes in MRT and Cl_T observed between the APAP only treated mice and the saline treated controls (Table 1 and Fig 5). Moreover, the pharmacokinetics of ICG in APAP toxicity in the mouse were significantly altered by the administration of NAC, the common clinical antidote used in the management of APAP overdose. Remarkably, mice treated with NAC 1 h after APAP had a pharmacokinetic profile for ICG (eg., Cl_T and MRT) that was similar to that of the saline treated mice. However, mice treated with NAC 4 h after APAP had a pharmacokinetic profile for ICG (eg., Cl_T and MRT) that was similar to the APAP only treated mice.

ICG is used in the clinical setting to evaluate hepatosplanchnic circulation and as a marker for graft viability in liver transplantation [25,26]. The clearance of ICG has been previously examined as a functional marker in experimental models of liver injury or toxicity [12,13,27–29]. Inage *et al.* [6] examined the pharmacokinetics of ICG in rats with partial hepatectomy and in rats with fibrosis secondary to carbon tetrachloride toxicity and found that the clearance of ICG correlated with the functional reserve and liver mass [6].

The clearance of ICG is regarded to be a strong correlate of hepatic blood flow [30]. Hepatic blood flow has been shown to be altered in APAP toxicity [31,32] and very early changes occur in the microcirculation of the liver in APAP toxicity in the mouse. Electron microscopy studies have demonstrated a number of ultrastructural changes in the hepatic sinusoids [33]. For example, swelling of sinusoidal endothelial cells occurs *before* there is evidence of biochemical injury as indicated by ALT elevation at 2 h. In addition, others have observed hepatic sinusoidal endothelium swelling at 30 min with subsequent reduced blood flow as early as 2 h after the oral administration of APAP [33]. Our laboratory recently reported the initial elevation of plasma hyaluronic acid, a marker of endothelial cell dysfunction, at 2 h in mice treated with APAP [21]. Alterations in blood flow in APAP toxicity are also thought to occur in the later phases of toxicity. For example, elevation of

hemoglobin in mouse liver has been demonstrated at 24 h, indicating venous congestion [34] and the onset of hypoxia in mouse liver in APAP toxicity is a relatively late event (Chaudhuri, in submission). In the present study, we chose to examine the pharmacokinetics of ICG at 24 h because of our interest in late events in the toxicity and in particular the recovery stages of APAP toxicity. In previous work, we have reported that the proliferation of hepatocytes (ie., hepatocyte regeneration) following APAP injury occurs at 24 h after the administration of APAP [21,24,35].

Of interest, ICG administered to mice immediately prior to APAP was previously shown to reduce the extent of tissue necrosis at 24 h, but not at earlier time points, in APAP toxicity in mice [36]. It was postulated that competition between APAP-GSH and ICG for excretion via the biliary route may underlie the hepatoprotection of ICG. Thus, it is possible that the higher accumulation of oxidized GSH in the ICG treated mice led to increased regeneration of reduced GSH, potentially limiting the effects of the toxic metabolite NAPQI. In the present study, ICG was administered late in the toxicity (24 h) at a point in time when ICG would not be expected to interfere with the biliary elimination of APAP and its conjugates [37].

A primary finding of the present study was the preservation of hepatic function in APAP treated mice that received NAC treatment 1 h after APAP. The clearance of ICG was normal in these mice (Fig 4). Interestingly, hepatic APAP protein adducts were elevated in the mice. Previous data from our laboratory utilizing immunohistochemical approaches for detection of APAP protein adducts (22) suggests that the timely administration of NAC prevented the rupture of hepatocytes and the release of both adducts and ALT into the blood (Figs 1–3). However, a 4 h delay in NAC treatment offered no protection from toxicity and no functional benefits. The time course for the development of toxicity in mice [24] is highly compressed compared to the time course for the development of toxicity observed in humans in which evidence of hepatic injury may not be apparent until 24 h after the APAP ingestion [38]. Thus, identifying time points in the progression of hepatic injury in the mouse model that reflect toxicity versus those that reflect liver function may be difficult due to the compressed time course of APAP toxicity in the mouse. Examination of ICG clearance at alternative NAC treatment times may be able to segregate the effect of NAC on parameters that represent injury versus function. Nevertheless, the data presented in this study may have relevance for future investigations testing novel therapies for APAP toxicity. Despite the diminishing efficacy of NAC at later stages of APAP toxicity in humans [17], NAC is being utilized more widely in the clinical setting for non-APAP related causes of hepatotoxicity [1]. Some data support the hypothesis that NAC has beneficial effects that extend beyond replacement of GSH. For example, Devlin *et al* [37] found that NAC treatment substantially increased oxygen consumption and delivery, as well as the clearance of ICG, in human subjects with hepatic failure of diverse etiologies [37]. In the present study, we noted that mice treated with NAC 1 h after APAP had a clearance for ICG that was comparable to that of the saline treated mice. Thus, the findings of the present study would be consistent with the hypothesis offered by Devlin *et al* [37] that NAC may affect oxygen consumption and delivery.

We conclude that the clearance of ICG is a sensitive indicator of hepatic dysfunction resulting from APAP toxicity. Furthermore, the clearance of ICG is affected by the timing of NAC administration. Future studies of novel therapies for APAP toxicity could utilize ICG clearance to test the effect of these therapies on the restoration or maintenance of hepatic function.

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List of abbreviations

ALT	Alanine Aminotransferase
APAP	Acetaminophen, paracetamol, N-acetyl- <i>p</i> -aminophenol
AST	Aspartate Aminotransferase
AUC	Area under the curve
AUC_{0-∞}	AUC to infinity
Cl_T	total body clearance
C_n	Final plasma concentration
Az	Apparent terminal elimination rate constant
ICG	Indocyanine Green
GSH	Glutathione
MRT	Mean residence time
NAC	N-acetylcysteine
NAPQI	<i>N</i> -acetyl- <i>p</i> -benzo-quinone imine

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Figure 1. Biochemical and histological determination of APAP toxicity in mice treated with APAP (200 mg/kg IP) or saline and sacrificed at 24 h
Some mice received NAC at 1 h or 4 h after APAP. **A.** Serum ALT values. **B.** Hepatic necrosis scores. * $p < 0.05$ compared to saline group; # $p < 0.05$ compared to APAP/NAC 1 h. Median (bold line), inter-quartile range (boxes) and extremes (whiskers) of necrosis score values by treatment group. Open circles depict outliers.

Figure 2. Representative hematoxylin and eosin-stained liver sections

Liver sections from mice treated with **A.** Saline, **B.** APAP, **C.** APAP/NAC at 1 h and **D.** APAP/NAC at 4 h. All mice were sacrificed at 24 h. Mice treated with NAC at 1 h had reduced amounts of necrosis compared to the APAP treated mice. Mice that received NAC at 4 h had necrosis comparable to that of the APAP mice.

Figure 3. APAP protein adducts in liver homogenates

Median (bold line), inter-quartile range (boxes) and extremes (whiskers) of adduct values by treatment group. Open circles depict outliers. Adducts were increased in all APAP treated groups (* $p < 0.05$). APAP/NAC 1 h mice had adduct levels that were significantly higher than all treatment groups (# $p < 0.05$).

Figure 4. Mean ($\pm$ SD) ICG concentration versus time profile for mice treated with **A.** APAP, **B** saline, **C.** APAP/NAC 1 h or **D.** APAP/ NAC 4 h. All mice were sacrificed at 24 h.

Figure 5.

Pharmacokinetic parameters for indocyanine (ICG) disposition in mice treated with APAP (200 mg/kg IP), saline, APAP/NAC 1 h or APAP/NAC 4 h. Median (bold line), inter-quartile range (boxes) and extremes (whiskers) of maximum plasma concentrations values by treatment group. Open circles depict outliers.

B. Cmax (ug/mL). The APAP groups of mice had Cmax values that were greater than the saline mice (*p<0.05). **B.** Clearance (ml/min/kg). Clearance (Cl_T) was lower in the APAP mice compared to the saline mice (*p<0.05) and in the APAP/NAC 4 h mice compared to the saline mice (*p<0.05). Cl_T was also lower in the APAP mice and the APAP/NAC 4 h mice compared to the APAP/NAC 1 h mice (#p<0.05). **C.** Mean Residence Time (MRT, min). The MRT was significantly higher in the APAP mice and the APAP/NAC 4 h compared to the saline mice (*p<0.05). The MRT was also higher in the APAP mice and the APAP/NAC 4 h mice compared to the APAP/NAC 1 h mice (#p<0.05). **D.** Area under the curve analysis (AUC). Values for AUC for the APAP/NAC 4 h were significantly greater than the AUC in the saline (*p<0.05) and the APAP/NAC 1 h mice (#p<0.05).

Figure 6.

Elimination rate constant (α) for ICG in mice treated with APAP, saline, APAP/NAC at 1 h, APAP/NAC at 4 h. Median (bold line), inter-quartile range (boxes) and extremes (whiskers) of maximum plasma concentrations values by treatment group. Open circles depict outliers. α was significantly reduced in the APAP and the APAP/NAC 4 h mice compared to the saline mice (* $p < 0.05$) and compared to the APAP/NAC 1 h mice (# $p < 0.05$).

Table 1

Model-independent pharmacokinetic parameters for indocyanine green (ICG) in mice treated with acetaminophen (APAP), APAP and N-acetylcysteine (NAC) and saline controls, presented as mean ($\pm$ SD).

Parameter	Saline (n=6)	APAP (N=8)	APAP + NAC 1 hr (n=6)	APAP + NAC 4 hr (n=7)
C _{max} (μ g/ml)	101.8 $\pm$ 20.0	147.5 $\pm$ 31.1 *	149.6 $\pm$ 17.4 *	156.1 $\pm$ 24.3 *
AUC _{0-∞} (μ g.min/ml)	683.5 $\pm$ 191.7	2083.9 $\pm$ 988.9	798.3 $\pm$ 163.5	3221.4 $\pm$ 1932.6 *#
MRT (min)	6.2 $\pm$ 2.0	23.8 $\pm$ 14.1 *#	6.2 $\pm$ 0.7	31.5 $\pm$ 16.9 *#
λ_z (1/min)	0.081 $\pm$ 0.024	0.030 $\pm$ 0.012 *	0.038 $\pm$ 0.012 *	0.026 $\pm$ 0.010 *
t _{1/2} (min)	9.2 $\pm$ 2.5	28.7 $\pm$ 16.4 *	19.8 $\pm$ 6.3	30.4 $\pm$ 12.1 *
Cl _T (ml/min/kg)	15.4 $\pm$ 3.5	5.7 $\pm$ 2.4 *#	13.1 $\pm$ 3.4	4.0 $\pm$ 1.9 *#
V _{ss} (ml/kg)	90.6 $\pm$ 24.1	120.5 $\pm$ 58.9	79.5 $\pm$ 16.0	102.5 $\pm$ 26.1
ALT (IU/L)	31 $\pm$ 16.3	2431 $\pm$ 1061 *#	190 $\pm$ 217	2949 $\pm$ 965 *#

* P<0.05 vs, saline;

p<0.05 vs. APAP + NAC 1 hr;

C_{max} calc - maximum plasma concentration calculated from the fitted data, AUC - area under the plasma concentration versus time curve, MRT - mean residence time, λ_z - terminal rate constant, t_{1/2} - half-life, Cl_T - total body clearance, V_{ss} - apparent total body volume of distribution at steady-state.

Table 2

Model-dependent ICG pharmacokinetic rate constants derived from mice treated with acetaminophen (APAP), APAP and N-acetylcysteine (NAC) and saline controls, presented as mean ($\pm$ SD).

Parameter	Saline (n=3)	APAP (N=7)	APAP + NAC 1 hr (n=6)	APAP + NAC 4 hr (n=6)
A	191.3 $\pm$ 8.1	196.6 $\pm$ 39.2	291.8 $\pm$ 28.8 ^{*†§}	182.4 $\pm$ 50.9
α (1/min)	0.347 $\pm$ 0.022	0.184 $\pm$ 0.053 ^{*#}	0.345 $\pm$ 0.037	0.154 $\pm$ 0.070 ^{*#}
$t_{1/2} \alpha$ (min)	2.0 $\pm$ 0.1	4.1 $\pm$ 1.3	2.0 $\pm$ 0.2	5.9 $\pm$ 4.0 [#]
B	2.3 $\pm$ 1.1	13.4 $\pm$ 3.5	2.5 $\pm$ 1.8	30.2 $\pm$ 15.7 ^{*#†}
β (1/min)	0.051 $\pm$ 0.017	0.024 $\pm$ 0.013 [*]	0.034 $\pm$ 0.010	0.020 $\pm$ 0.012 [*]
$t_{1/2} \beta$ (min)	14.3 $\pm$ 3.9	41.7 $\pm$ 34.0	21.9 $\pm$ 7.2	47.7 $\pm$ 30.8

* p<0.05 vs. saline;

p<0.05 vs. APAP + NAC 1 hr;

† p<0.05 vs. APAP;

§ p<0.05 vs. APAP + NAC 4 h.

A - coefficient of the exponent in the initial phase, α - rate constant for the initial phase, $t_{1/2} \alpha$ - half-life during the initial phase, B - coefficient of the exponent in the terminal phase, β - terminal rate constant, $t_{1/2} \beta$ - half-life during the terminal phase.