

PARP-1 Inhibitor Attenuates Cocaine-Induced Hepatotoxicity

J.D. McCluskey, S.C. Harbison, D. Sava, G.T. Johnson* and R.D. Harbison

Department of Environmental and Occupational Health, College of Public Health, University of South Florida, Tampa, FL, USA

Abstract: Cocaine abuse is associated with multiple health problems including occasional hepatic failure and death. The mechanism of cocaine-induced hepatotoxicity (CIH) is not clear, although studies in mice have demonstrated that cocaine-induced liver injury may be mediated by nitric oxide and reactive oxygen species. Recently, we have found that cocaine increases poly (ADP-ribose) polymerase (PARP) activity in the liver. Therefore, inhibition of PARP may block CIH. A preliminary assessment of the PARP inhibitor 3,4-Dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone (DPQ) in ICR mice failed to attenuate CIH. However, the PARP inhibitor 1,5-dihydroxyisoquinoline (DIQ) successfully attenuated CIH. Mean ALT activity of 569 IU caused by cocaine treatment alone, was limited to 79 IU in ICR mice concomitantly treated with DIQ (10 mg/kg, ip). The protective effect of DIQ was also associated with prevention of development of lipid peroxidation in liver tissue, reduced depletion of glutathione (GSH), and a reduced production of nitrites. Cocaine-induced TBARS was significantly decreased by DIQ from a mean of 5.7 nmol/mg protein to a mean of 2.5 nmol/mg protein, similar to untreated mice. Hepatic GSH was reduced more than 2 fold following cocaine administration but treatment with DIQ conserved GSH at the level of untreated mice. The protective effect of DIQ in attenuating CIH can potentially be explained by the dual inhibitory effect of DIQ that reduces induction of PARP and inducible nitric oxide synthetase (iNOS). The DIQ attenuation of CIH provides evidence for a PARP and iNOS modulated mechanism of action for this hepatocellular pathology.

Keywords: PARP-1, Cocaine, Hepatotoxicity, PARP Inhibitors.

INTRODUCTION

Cocaine abuse is associated with multiple medical problems including occasional hepatic failure and consequent death. Cocaine-induced hepatotoxicity (CIH) has been demonstrated in both humans and experimental animals [1-4]. However, the mechanism of CIH has not been clearly elucidated, although it has been demonstrated that transformation of cocaine to norcocaine by cytochrome P-450 produces nitric oxide [5-7]. Free radicals produced in the liver during metabolic transformation of cocaine may cause mitochondrial impairment and nuclear DNA damage, leading to the activation of DNA repair mechanisms such as poly(ADP-ribose) polymerase 1 (PARP-1) induction [8]. Upon binding to damaged DNA, PARP-1 forms long branches of ADP-ribose polymers. Under normal cellular conditions, this activity provides protection of DNA; however the excessive activation of PARP-1 leads to rapid depletion of NAD⁺ and ATP that eventually ends with cell death [9].

Previously, we have observed the ability of the PARP inhibitor 6(5H)-phenanthridinone to render hepatoprotective effects in animal models [10, 11]. However, 6(5H)-phenanthridinone was required to be injected in dimethyl sulfoxide solution due to low water solubility, which potentially compromises bioavailability and possibly reduces

the hepatoprotective effect. Recently, several novel water-soluble PARP-1 inhibitors have been developed and tested in various models of toxic exposure [12]. We have previously demonstrated the ability of several water-soluble PARP-1 inhibitors including 3-aminobenzamide (ABA), 5-aminoisoquinolinone (AIQ), and *N*-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-*N,N*-dimethylacetamide HCl (PJ-34) to block or attenuate centrilobular hepatotoxicity in a carbon tetrachloride model of hepatotoxicity [13].

Cocaine has also been demonstrated to activate PARP-1 when producing hepatotoxicity [14, 15]. There is therapeutic potential for poly(ADP-ribose) polymerase inhibitors to protect the liver against the side effects of cocaine [16-18]. The aim of this study was to determine the efficacy of two water soluble PARP inhibitors 3,4-Dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone (DPQ) and 1,5-dihydroxyisoquinoline (DIQ) in attenuating cocaine induced hepatotoxicity, and to elucidate the possible mechanism(s) of their hepatoprotective effect in order to better understand the role of PARP-1 activation in cocaine induced hepatotoxicity.

MATERIALS AND METHODS

Materials

Cocaine was purchased from Fisher Scientific. Malondialdehyde bis (dimethyl acetal), thiobarbituric acid, and 5,5'-dithiobis-2-nitrobenzoic acid were obtained from Sigma Chemical Co. (St. Louis, MO). The Alanine Amino Transferase (ALT) kit was obtained from TECO Diagnostics (Anaheim, CA). The colorimetric assay for PARP activity was from Trevigen, Inc. (Gaithersburg, MD) All other

*Address correspondence to this author at the Department of Environmental and Occupational Health, College of Public Health, University of South Florida, Tampa, FL, USA; Tel: 813-974-3467; Fax: 813-905-9912; E-mail: rharbiso@health.usf.edu

chemicals were of analytical grade or higher from Merck (Darmstadt, Germany).

Animals and Treatment

Adult male ICR mice (30 ± 5 g) were used for all experiments. Animals were housed under controlled conditions ($25 \pm 2^\circ\text{C}$ and a 12 h light/dark cycle) and allowed free access to food and water. The animals were divided into the following groups: pure control group received no cocaine or PARP inhibitor, the positive control group received cocaine alone, and experimental group received cocaine and PARP inhibitor. Cocaine was dissolved in normal saline and administered intraperitoneally (ip) with a dose of 60 mg/kg. PARP inhibitors were dissolved in normal saline and administered intraperitoneally in the following time intervals after cocaine injection: 0, 1 and 2 hours. Each time interval represents an independent treatment group and each animal received a single dose. The injection sites for cocaine and PARP inhibitors were administered distant to one another to avoid chemical interaction. All the experiments were conducted under controlled conditions according to the Guide for the Care and Use of Experimental Animals and the University of South Florida Institutional Animal Care and Use Committee (IACUC). The mice were sacrificed after 24 h and blood samples to determine alanine transaminase (ALT) activity and nitrite content were withdrawn by cardiac puncture. Livers were isolated, perfused with normal saline, and dissected for histopathology and stored at -80°C for determination of total GSH, thiobarbituric acid-reactive substances (MDA), PARP activity.

Determination of PARP Activity

The colorimetric assay for PARP activity was performed in 96-well plates (Trevigen, Inc., Gaithersburg, MD) according to manufacturer's protocol. Serial dilutions of the PARP enzyme were distributed into wells to generate a standard curve. To determine cellular PARP activity, 10 μL /well of the tissue homogenates, prepared according to manufacturer recommendations, were distributed in triplicates. The reactions were allowed to proceed for 1 h at room temperature. The plate was washed 4 times with $1\times$ phosphate-buffered saline (PBS) and then incubated for 20 min with 50 μL /well Streptavidin-horseradish peroxidase (Strep-HRP) diluted 1:500 in $1\times$ Strep-Diluent (Trevigen). The plate was washed 4 times with $1\times$ PBS prior to the addition of the HRP substrate. For colorimetric readout, 50 μL of TACS-Sapphire (Trevigen) was added to each well and incubated in the dark, at room temperature, for 15 min. Development of the colorimetric reaction was stopped by the addition of an equal volume of 0.2 M HCl. This generated a yellow color that was read at 450 nm. The results were expressed as units of PARP activity calculated per milligram of protein and normalized to equal concentrations of protein. Protein determination in all assays described here were carried out with the bicinchoninic acid assay using bovine serum albumin as standard [19].

Alanine Transaminase Activity

ALT activity, expressed as IU/L, was determined by a previously described method using a commercially prepared reagent kit from TECO Diagnostics (Anaheim, CA) [20]. Serum (0.2 mL) was obtained by centrifugation of cardiac

puncture blood samples at 4000 rpm for 15 min. Each serum sample was then transferred to a new sterile microcentrifuge tube and stored at 4°C until time of assay. ALT activity was expressed as IU/L.

Glutathione Assay

Total GSH was determined as described previously by [21], Individual liver samples (100 mg) were homogenized in 1 mL of 0.2 M PBS (pH 8.0) and the mixture was centrifuged at 12,000 rpm for 30 min. The supernatant (0.5 mL) was mixed with 0.5 mL of 4% sulfosalicylic acid, allowed to stand for 5 min at 4°C , and centrifuged again at 3000 rpm for 10 min. The supernatant obtained (0.5 mL) was mixed with 2 mL of 0.2 M PBS (pH 8.0) and 10 μL of 10 mM 5,5'-dithiobis-2-nitrobenzoic acid [22]. The absorbance was measured at 412 nm and concentration of GSH was expressed as nmol/mg protein.

Lipid Peroxidation Assay

Formation of lipid peroxide derivatives was evaluated by measuring TBARS according to a method previously described by [23]. Liver samples were homogenized in ice-cold 1.15% KCl (w/v). Homogenates (0.4 mL) were mixed with 1 mL of 0.375% TBA, 15% trichloroacetic acid (TCA) (w/v), 0.25 N HCl, and 6.8 mM butylated-hydroxytoluene, placed in a boiling water bath for 10 min, removed and allowed to cool on ice. Following centrifugation at 3000 rpm for 10 min, the absorbance in the supernatants was measured at 532 nm. The amount of TBARS produced was expressed as nmol TBARS/mg protein using malondialdehyde bis(dimethylacetal) (MDA) for calibration.

Determination of Nitric Oxide (NO) Synthesis

The production of NO was assessed indirectly by measuring the nitrite levels in the plasma using Griess reaction [24]. Plasma samples were diluted four times with distilled water and deproteinized by adding zinc sulfate to a final concentration of 1.5%. After centrifugation at 10,000 g for 5 min at room temperature, 100 μL supernatant was applied to a microliter plate well, followed by 100 μL of Griess reagent consisting of 1% sulfanilamide and 0.1% N-1-naphthylethylenediaminedihydrochloride in 5% phosphoric acid). The absorbance was read after 10 min of color development at room temperature with a micro reader at 540nm. Sodium nitrite was used to produce a calibration curve for calculation of total nitrite levels [25].

Statistical Analysis

Summary descriptive analysis for all cytotoxicity outcome data is reported as the mean levels of biomarkers and the standard error of the mean (SEM). One way ANOVA was performed to evaluate the differences between all treatment groups and the cocaine control group, as well as the negative control group ($p < 0.05$ was considered to indicate a statistically significant difference).

RESULTS

PARP activity (Fig. 1) assessed in the livers of ICR mice intoxicated with cocaine (60 mg/kg, ip) ($n = 7$) was elevated 1.5 times as compared to untreated animals' level of 4.6 ± 0.8 U/mg protein ($n = 6$) with borderline statistical significance ($p = 0.0979$). Both DPQ ($n = 5$) and DIQ ($n = 5$) injected

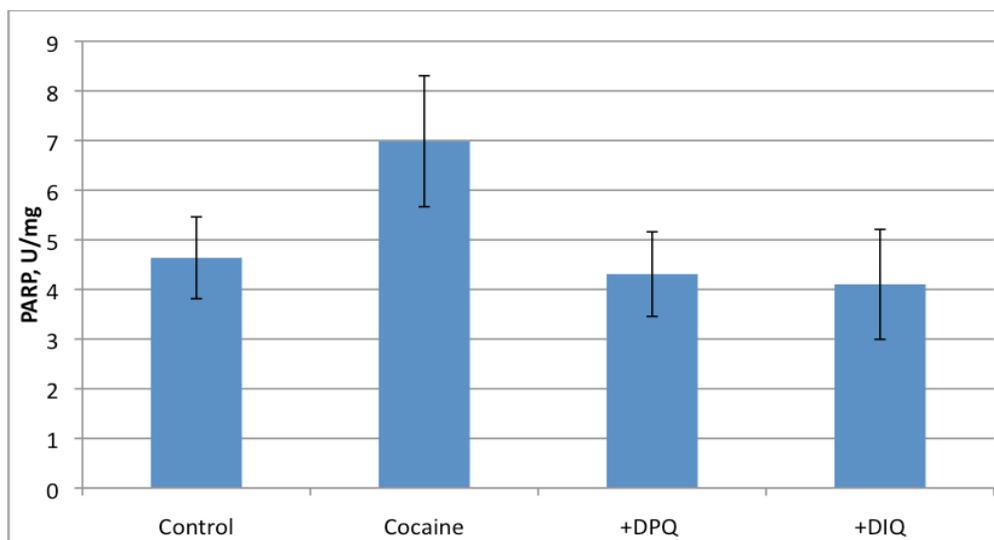


Fig. (1). PARP-1 activity in the liver of ICR mice untreated (Control) treated with cocaine (Cocaine) and treated concomitantly with cocaine and DPQ (+DPQ) or cocaine and DIQ (+DIQ). Measurements are mean PARP U/mg, \pm SEM.

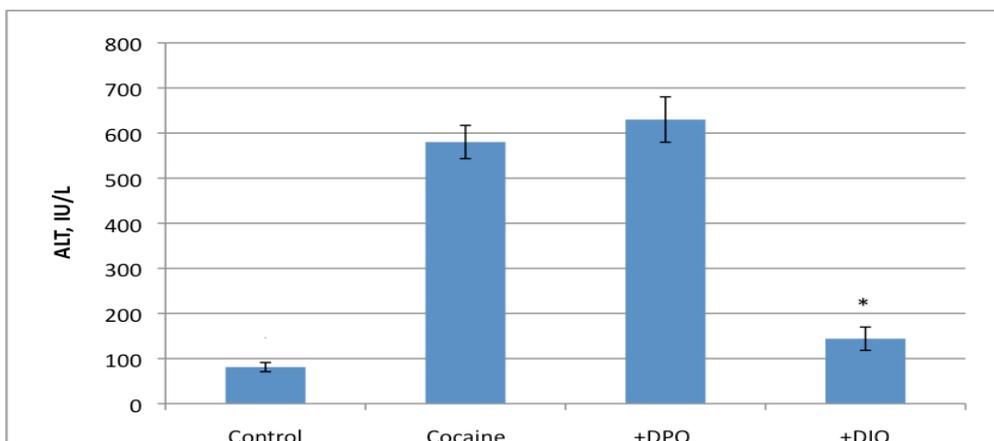


Fig. (2). Alanine transaminase (ALT) in ICR mice untreated (Control) treated with cocaine (Cocaine) and treated concomitantly with cocaine and DPQ (+DPQ) or cocaine and DIQ (+DIQ). Measurements are mean ALT IU/L, \pm SEM. An '*' indicates $p < 0.05$ compared to cocaine treatment alone.

concomitantly with cocaine lowered the mean elevation of PARP-1 activity in livers of observed mice approaching statistical significance compared to cocaine only treated mice ($p = 0.0744$ and $p = 0.0560$ respectively). At equal doses of 10 mg/kg, concomitant treatment of DPQ and DIQ with cocaine returned the activity of PARP-1 in the liver of ICR mice to the level of untreated controls ($p = 0.8264$ and $p = 0.7214$ respectively compared to untreated mice). Thus, the tested inhibitors demonstrated ability to hinder the elevation of PARP activity elicited by cocaine.

Intraperitoneal administration of 60 mg/kg cocaine ($n = 6$) elicited substantial elevation of serum ALT activity in ICR mice 24 hours post injection (Fig. 2). There was no statistically significant difference in serum ALT activity resulting from concomitant administration of cocaine and DPQ (10 mg/kg) ($n = 6$) as compared to administration of cocaine alone. Concomitant treatment with 10 mg/kg of DIQ ($n = 6$) and cocaine resulted in significant reduction in ALT, producing mean ALT levels 75% less than mice treated with cocaine alone.

As DIQ demonstrated preliminary efficacy in attenuating CIH from cocaine insult, this PARP inhibitor was selected

for dose response evaluation. Dose dependence for DIQ when administered concomitantly with cocaine demonstrated that the effect on ALT attenuation from cocaine insult plateaued at doses exceeding 10 mg/kg, without further substantial reduction at 20 mg/kg (Fig. 3). For all groups in this experiment ($n = 6$).

The time dependence of administration of DIQ was evaluated through administration of DIQ 10 mg/kg at 0, 1, and 2 hours post cocaine treatment (Fig 4). Concomitant administration of cocaine and DIQ ($n = 6$) resulted in a statistically significant reduction of ALT compared to cocaine alone. Administration of DIQ 1 ($n = 6$) and 2 hours ($n = 5$) after cocaine treatment modestly reduced mean serum ALT levels, but this effect was not statistically significant.

Total glutathione (reduced and oxidized) was measured in liver samples 24 hours after intoxication. Administration of cocaine alone ($n = 6$) resulted in 2.2-fold reduction in GSH, while concomitant administration of DIQ ($n = 6$) conserved GSH near the control level (Fig. 5). Delayed injection of DIQ decreased efficacy of DIQ with decreased GSH levels observed in mice treated with DIQ 1 hour after cocaine treatment ($n = 6$) compared to concomitantly treated mice,

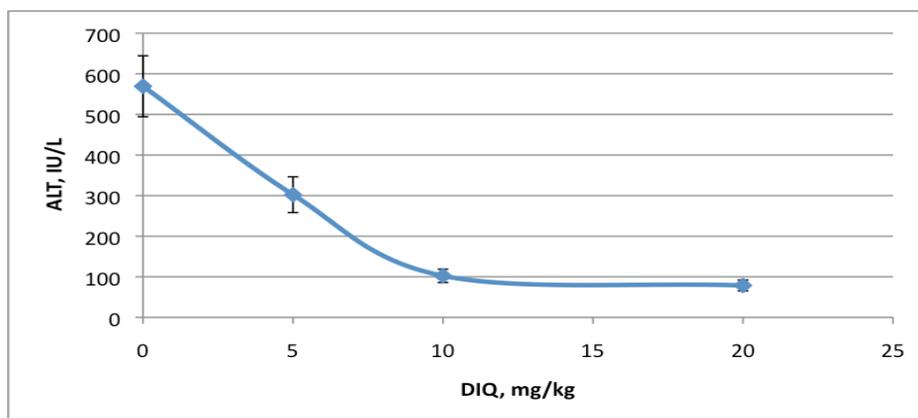


Fig. (3). Dose Dependence of DIQ on Alanine Transaminase

Reduction of elevation of ALT caused by concomitant administration of 0, 5, 10, and 20 mg/kg DIQ and 60 mg/kg of cocaine. Measurements are reported as the mean ALT IU/L \pm SEM.

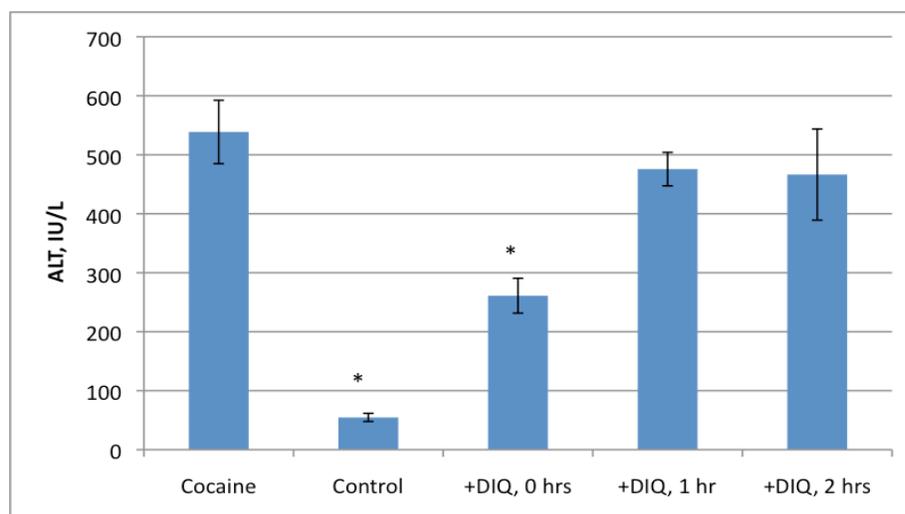


Fig. (4). Time Dependence of DIQ on Alanine Transaminase

Alanine transaminase levels in ICR mice treated with DIQ 10 mg/kg at 0, 1, and 2 hours after treatment with cocaine 60 mg/kg. Measurements are reported as the mean ALT IU/L \pm SEM. An '*' indicates $p < 0.05$ compared to cocaine treatment alone.

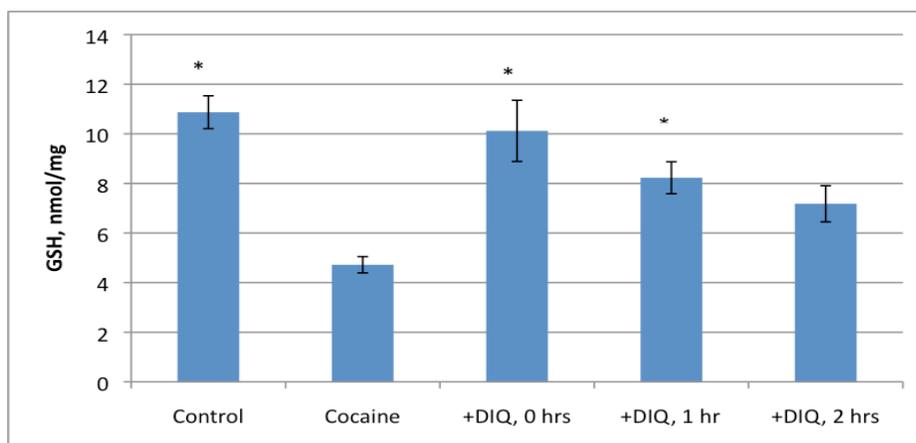


Fig. (5). Total liver glutathione concentration in ICR mice treated with DIQ 10 mg/kg at 0, 1, and 2 hours after treatment with cocaine 60 mg/kg. Measurements are reported as the mean GSH nmol/mg \pm SEM. An '*' indicates $p < 0.05$ compared to cocaine treatment alone.

though this group produced significantly higher GSH levels than the cocaine only treated mice. Mice treated with DIQ 2 hours after treatment with cocaine (n= 5) produced GSH levels 1.5 times higher as compared to the level of cocaine alone, though this effect was not statistically significant.

Changes in MDA, which reflects involvement of free radical oxidation in development of hepatotoxicity, were evaluated in ICR mice livers (Fig. 6). In mice treated with cocaine only (n= 7), the level of MDA was elevated 2.5 times as compared to untreated controls (n= 6) at 2.5

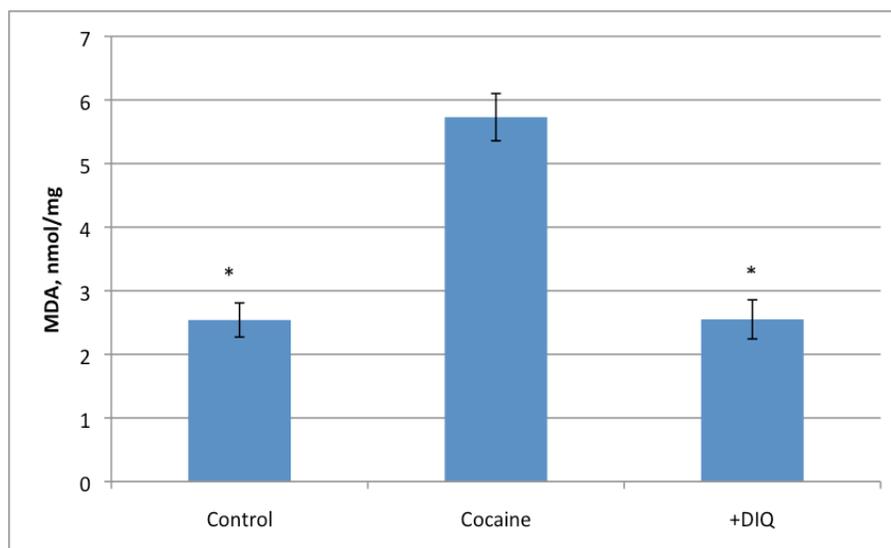


Fig. (6). Hepatic lipid peroxidation in ICR mice untreated (Control), treated with cocaine (Cocaine) and treated concomitantly with cocaine and DIQ (+DIQ). Measurements are mean MDA nmol/mg, \pm SEM. An ‘*’ indicates $p < 0.05$ compared to cocaine treatment alone.

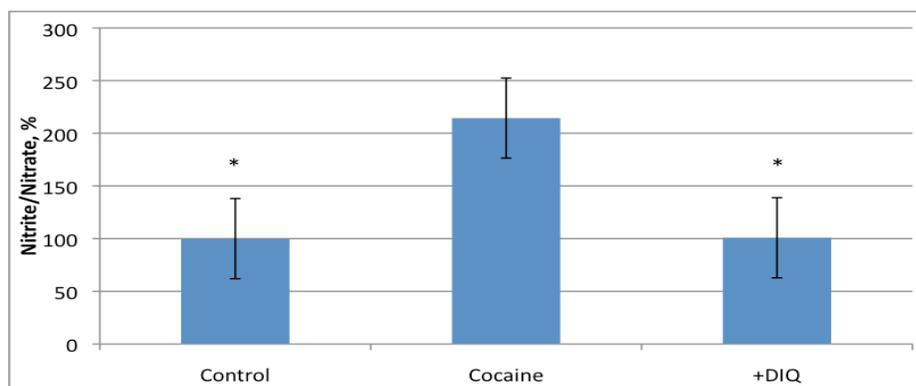


Fig. (7). Nitrite Index

Nitrite in the plasma of ICR mice untreated (Control) treated with cocaine (Cocaine) and treated concomitantly with cocaine and DIQ (+DIQ). Measurements are mean percentage of nitrite to nitrates, \pm SEM. An ‘*’ indicates $p < 0.05$ compared to cocaine treatment alone.

nmol/mg of protein. Cocaine-induced MDA values were significantly decreased by concomitant administration of DIQ with cocaine injection. DIQ treatment ($n = 5$) effectively reduced free radical activity to a similar level of the untreated control group, with no statistically significant difference between the mean MDA values of these two groups ($p = 1.0000$).

LIPID PEROXIDATION

DIQ also demonstrated the inhibition of the production of nitrogen oxides shown by the reduction of nitrite (Fig. 7). Cocaine treatment alone ($n = 6$) produced mean nitrite levels 2.1 times higher compared than controls ($n = 3$). Administration of DIQ concomitantly with cocaine ($n = 6$) attenuated the nitrite index to the level of control (i.e. differences between untreated mice and mice treated concomitantly with DIQ and cocaine were not statistically significant ($p = 1.0000$)). Nitric oxide production appears to be an important factor in the pathology of CIH.

In addition to the evaluation of CIH with biomarker analysis, a seven-day mortality study was conducted for mice treated with 60 mg/kg cocaine alone and mice treated concomitantly with DIQ 10 mg/kg and cocaine 60 mg/kg. It

was found that mice concomitantly treated with DIQ and cocaine experienced zero mortality, while 61% of mice administered only cocaine died within the 7 day period.

Macroscopic examination of liver samples was in accordance with the biochemical findings. Gross anatomical findings showed hemorrhagic and necrotic damage in mice treated with cocaine. When DIQ was administered subsequent to cocaine, no pathological changes were observed.

DISCUSSION

PARP-1 inhibitors have been shown to be of therapeutic value in a number of pathological conditions including liver diseases. Several classes of competitive PARP-1 inhibitors have been reported including benzamide [26], 3,4-dihydro-5-hydroxy-1(2H)-isoquinolinone (DIQ) [27], 3,4-dihydro-5-[4-1(1-piperidinyl)butoxy]-1(2H)-isoquinolinone (DPQ) [27], and 6(5H)-phenantridinone (PND) [26]. Among these, dihydroisoquinolinone derivatives, such as DPQ, have been of particular interest due to their high potency as PARP-1 inhibitors, with an IC_{50} of 2.2 μ M estimated in vitro [26, 28]. DIQ has been identified as a moderately potent PARP-1 inhibitor, with an IC_{50} of 17 μ M in vitro as observed by [27].

In this study we tested the ability of DPQ and DIQ to attenuate CIH and various biomarkers of toxicity that potentially modulate this process. Using serum ALT as a measure of damage to hepatic tissue we have demonstrated that administration of DIQ concomitantly and within 1 hour of cocaine treatment renders a protective effect against the hepatotoxicity of cocaine. DIQ was able to reduce serum ALT levels up to 75% as compared to cocaine treatment alone. These results were confirmed with a 7 day mortality study in which concomitant treatment with cocaine and DIQ prevented mortality from occurring in treated mice and prevented gross pathological changes associated with cocaine induced hepatotoxicity. While the specific mechanisms for this effect are not clear, evidence suggests CIH is partially modulated by PARP-1 activation in the cascade of reactive oxygen species produced by cocaine exposure.

Development of hepatotoxicity elicited by cocaine is also associated with reduction of GSH levels. Depletion of GSH causes liver cells to become more susceptible to lipid peroxidation and studies have shown that inhibition of oxidative metabolism attenuates cocaine mediated hepatotoxicity [29, 30]. Therefore, preservation of GSH should also play a role in modulating CIH.

The current study indicates that GSH levels are preserved to levels similar to that of untreated mice in the animals treated with DIQ concomitantly with cocaine. The prevention of the deleterious effects of reactive species with treatment of DIQ was also demonstrated by measurements of lipid peroxidation represented by MDA. Cocaine treated mice demonstrated elevation of MDA in the liver on average twice that of untreated controls. Animals treated simultaneously with cocaine and DIQ did not produce hepatotoxicity and demonstrated significantly reduced MDA levels in addition to reduced ALT, preserved GSH, and the absence of abnormal histopathology.

Overproduction of NO in the liver has been implicated as an important event in different models of hepatic inflammation and injury [31, 32]. NO is known to react with superoxide radical, forming peroxynitrite, which has been identified as a more powerful oxidizing agent. This may cause GSH depletion, free radical generation, and up-regulation of iNOS [33]. In the current study we demonstrated that under acute cocaine intoxication significant elevations of nitrite levels are observed in blood plasma.

This study has shown that DIQ, a specific PARP-1 inhibitor, also attenuates nitrite elevation and can effectively block cocaine induced hepatotoxicity. This observation in combination with the results of DPQ treatment, which did not effectively block CIH, indicate that modulation of this process requires not only PARP-1 inhibition, but also a combination of modulating properties potentially related to iNOS associated metabolic processes [34]. The results of this study could have important implications for pharmaceutical interventions in the treatment of cocaine users, particularly for issues of hepatotoxicity [35].

CONCLUSION

This study demonstrated that the PARP-1 inhibitor DIQ has the capacity to attenuate the toxic effects of cocaine in

the liver. A potential mechanism for this protective effect is thought to be due to its ability to control PARP activity within the physiological norm. The protective effect of DIQ is also linked with the ability to prevent lipid peroxidation in liver tissue and improve the antioxidant defenses as demonstrated by the amelioration of elevated MDA and maintenance of glutathione levels. However, the most important factor could be associated with involvement of the iNOS metabolic pathway in the development of CIH. Treatment with DIQ had an inhibitory effect on plasma nitrite levels, which suggests the involvement of inducible nitric oxide synthetase (iNOS) in cocaine toxicity. The mechanism of action for DIQ in the attenuation of CIH can be potentially explained by a dual inhibitory effect that limits PARP-1 induction and iNOS activity. Further detailed studies elucidating the biochemical mechanisms responsible for the hepatoprotective effect of DIQ are needed.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Declared none.

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