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Modulation of paracetamol-induced hepatotoxicity by phosphodiesterase isozyme inhibition in rats: a preliminary study

Abstract

Background: Altered regulation of nitric oxide-cyclic guanosine monophosphate (NO-cGMP) is present in liver cirrhosis. Several experimental studies have shown that selective modulation of NO metabolism in the liver reduces intrahepatic resistance and portal pressure in cirrhosis. This preliminary study investigated whether selective inhibition of phosphodiesterase-5 (PDE-5), which prevents the conversion of cGMP to 5′-GMP, as well as non-selective inhibition of PDE isozymes could ameliorate hepatic toxicity induced by paracetamol (PCM).

Methods: PCM (250 mg/kg, i.p.) was administered to induce hepatotoxicity. Control rats received physiological saline (10 mL/kg, p.o.), while sildenafil (a selective PDE-5 inhibitor) and aminophylline (a non-selective PDE inhibitor) were administered separately at 10 mg/kg p.o. to PCM-treated rats.

Results: PCM hepatotoxicity, characterized by elevation of aspartate and alanine aminotransferases, hepatic degeneration, and centrilobular necrosis, was attenuated by both PDE inhibitors. Sildenafil and aminophylline significantly ($p<0.05$) reduced plasma aspartate aminotransferase activity by 49.6% and 39.8%, respectively, with moderate increase in alanine aminotransferase activity by 26.1% and 20.4%, respectively, in PCM-treated rats. Decreases in total protein and albumin induced by PCM were significantly ($p<0.05$) prevented by 30.0% and 22.2%, respectively, following sildenafil administration, while aminophylline decreased these proteins by 14.0% and 25.9%, respectively. Sildenafil and aminophylline significantly ($p<0.05$) reduced lipid peroxidation by 30.7% and 19.7%, respectively, while moderately increasing glutathione (GSH) in the PCM-treated rats. Both drugs did not significantly alter the total cholesterol and triglyceride levels.

Conclusions: These preliminary data suggest that pharmacological inhibition of PDE isozymes may be a useful strategy in protecting against PCM hepatic toxicity.

Keywords: aminophylline; hepatotoxicity; paracetamol; phosphodiesterase; sildenafil.

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Introduction

Phosphodiesterases (PDEs) include a large group of structurally related enzymes with different substrate specificities, kinetics, allosteric regulation, and subcellular localization achieved through modulation of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) signaling mechanisms in conjunction with adenylyl and guanylyl cyclases [1]. Both cAMP and cGMP act predominantly as key second messengers in regulating inflammatory cell function and can influence a large number of pharmacologic processes. This has been shown to contribute significantly to their distinct roles in specific physiological processes [2, 3]. PDEs terminate these processes by converting cGMP and cAMP to 5′-GMP and 5′-AMP, respectively [4–6].

Studies have shown that the nitric oxide-cyclic guanosine monophosphate (NO-cGMP) system is dysregulated in liver cirrhosis [7–9]. The disturbed liver architecture, perisinusoidal fibrosis, and cellular alterations of liver sinusoids as well as functional changes arising from this altered regulation result in increased intrahepatic vascular resistance and eventually portal hypertension [10]. The reduced activity of the endothelial NO synthase (eNOS) during these pathophysiological changes in liver endothelial cells decreases NO, and hepatic stellate cells transform to contractile myofibroblasts [8–11]. All these, together with an increased PDE-5 activity in liver cirrhosis,

result in the contraction of sinusoids [12, 13]. In contrast to the intrahepatic condition in the splanchnic vascular system, NO production increases, causing dilation of the mesenteric blood vessels and splanchnic hyperperfusion [7, 14]. Overproduction of NO in the liver, however, is also recognized as an important event in various models of hepatic inflammation and injury [15–18]. Although the mechanism underlying the actions of NO in the liver is unknown, this highly reactive oxidant has been reported to suppress liver protein and DNA synthesis [16, 19] and to induce apoptosis and necrosis [16, 20], and these activities have been suggested to contribute to hepatotoxicity.

Several animal studies have shown that selective modulation of NO metabolism in the liver reduces intrahepatic resistance and portal pressure in cirrhosis [21–24]. The ability of the PDE-5 inhibitor vardenafil to increase portal venous flow in normal and cirrhotic liver, and lower the portal pressure and hepatovenous pressure gradient in cirrhotics was demonstrated in a clinical pilot study [25]. Halverscheid et al. [26] demonstrated that the compromised NO bioavailability in the cirrhotic liver may lead to constriction of the sinusoids, which can contribute to the functional component of portal hypertension. They went on to propose that this effect may be reversed by application of PDE-5 inhibitors. Other clinical and experimental studies, in contrast, have suggested that PDE-5 inhibitors may even increase portal pressure [27–30]. Furthermore, there are experimental evidences suggesting that manipulations of different PDE isozymes by various pharmacological inhibitors could provide great therapeutic benefit in both immune-mediated and inflammatory conditions associated with liver pathology [12, 13]. In this preliminary investigation, we evaluated the possible protective benefit derivable from selective inhibition of PDE-5 isozyme with sildenafil alongside non-selective inhibition of PDEs with aminophylline in an experimental model of liver injury induced by paracetamol (PCM) in Wistar rats. PCM is an ideal hepatotoxin for this study because its administration has been reported to upregulate inducible NOS protein and NO production in hepatocytes [31].

Materials and methods

Chemicals and drugs

Sildenafil citrate, aminophylline, and PCM were obtained from Pfizer (Poce-sur-Cisse, France), Usan Pharmaceuticals Pvt. Ltd. (Mumbai, Maharashtra, India), and GVS Laboratories (Dombivli, India), respectively. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), and triglyceride (TG) assay kits were obtained from Randox Laboratories Ltd. (Crumlin, UK). Thiobarbituric acid and 5′,5′-dithiobis-2-nitrobenzoate (Ellman's reagent) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Reduced glutathione (GSH), metaphosphoric acid, and trichloroacetic acid were purchased from J.I. Baker Inc. (Phillipsburg, USA). Sodium hydroxide was obtained from E. Merck (Darmstadt, Germany), and all other chemicals and reagents used were of analytical grade.

Animals and treatment schedule

Male Wistar albino rats (120–200 g) were purchased from a commercial private colony in Ibadan, Oyo-State, Nigeria, and were kept within the experimental animal handling facility of the Department of Pharmacology, Olabisi Onabanjo University, Nigeria. They were maintained at ambient temperature and humidity with a 12 h light/12 h dark schedule and fed with standard pelleted rodent feeds (Bendel Feeds, Edo State, Nigeria) and water ad libitum during the acclimatization and experimental periods. This study was carried out in compliance with standard guidelines for the Care and Use of Laboratory Animals [32].

Thirty-one rats were divided into six experimental groups. Control rats (n=6) received physiological saline (10 mL/kg, p.o.). PCM (250 mg/kg) was administered intraperitoneally to a separate group (n=5) to induce hepatotoxicity. Sildenafil (10 mg/kg, p.o.) and aminophylline (10 mg/kg, p.o.) were administered [33, 34] to separate groups of PCM-treated rats (n=5 rats per group). Treatment was given for 7 days, and rats were killed 24 h after the last administration.

Necropsy

Animals were killed by cervical dislocation after an overnight fast (12 h), and blood samples were collected by cardiac puncture into lithium heparin bottles. Plasma was separated by centrifugation at 4200 rpm (1200 *g*) at room temperature for 5 min. The liver was immediately removed, cleared of adhering tissues, and weighed. A small portion of the liver was carefully excised, fixed in 10% formaldehyde, and processed for histopathology. The remaining portion of the liver was weighed and homogenized in four volumes of 0.1 M phosphate buffer (pH 7.4). Both plasma and liver homogenate were used for biochemical analysis.

Biochemical assessment

The activities of plasma aminotransferases (AST and ALT) were measured to assess liver function. The activities of these enzymes were determined according to the principle described by Reitman and Frankel [35]. The synthetic function of the liver was assessed by measuring plasma total protein (TP) and albumin (ALB) concentrations according to the principle based on Biuret and bromocresol green reactions, respectively [36, 37]. TC and TG concentrations were estimated following the principle described by Trinder [38] using commercial kits obtained from Randox Laboratories. GSH concentration was determined according to the method of Beutler et al. [39]. Lipid peroxidation (LPO) was estimated by the thiobarbituric acid reactive substance method as described by Varshney and Kale [40] and expressed in terms of malondialdehyde (MDA) formed per milligram of protein.

Data analysis

All data were expressed as mean±standard error of mean and analyzed by one-way analysis of variance using Statistical Package for Social Sciences software for Windows version 16 (SPSS Inc., Redmond, WA, USA). Post hoc testing was performed for intergroup comparisons using the least significant difference [41], and $p<0.05$ was considered significant.

Results

AST and ALT

The effect of various treatments on marker enzymes of liver function is shown in Figure 1. PCM (250 mg/kg, i.p) increased AST activity by 127.6% ($p<0.001$) and ALT activity by 20.8% ($p>0.05$) when compared with control. Both sildenafil (10 mg/kg, p.o.) and aminophylline (10 mg/kg, p.o.) significantly ($p<0.05$) lowered AST activity by 49.6% and 39.8% and ALT activity by 26.1% and 20.4% ($p>0.05$), respectively, in the PCM-treated rats when compared with control.

TP and ALB

PCM significantly ($p<0.001$) decreased TP and ALB concentrations by 24.2% and 25.0%, respectively, when compared with control. The PDE inhibitors, sildenafil and aminophylline, significantly ($p<0.05$) prevented the PCM-induced increase in TP by 30.0% and 22.2% and ALB by 14.0% and 25.9%, respectively, when compared with control (Figure 2).

TC and TG

Figure 3 shows the effect of sildenafil and aminophylline on PCM-induced changes in TC and TG. PCM hepatotoxicity was associated with a significant ($p<0.05$) elevation of serum TC by 55.2% with a modest increase of 19.3% in TG concentration ($p>0.05$) when compared with control (saline) group. The levels of these lipids in the PCM-treated rats were not significantly altered following treatment with sildenafil and aminophylline.

LPO and GSH

The effect of sildenafil and aminophylline on PCM-induced changes in biomarkers of oxidative stress is shown in Figure 4. Oxidative stress associated with PCM hepatic toxicity was ameliorated following treatment with both PDE inhibitors. PCM-induced increase in hepatic LPO was significantly reduced by 30.7% ($p<0.001$) in the sildenafil-treated rats and 19.7% ($p<0.05$) in rats treated with aminophylline. GSH was elevated by 18.4% and 20.4% in the PCM-treated rats following treatment with sildenafil and aminophylline, respectively.

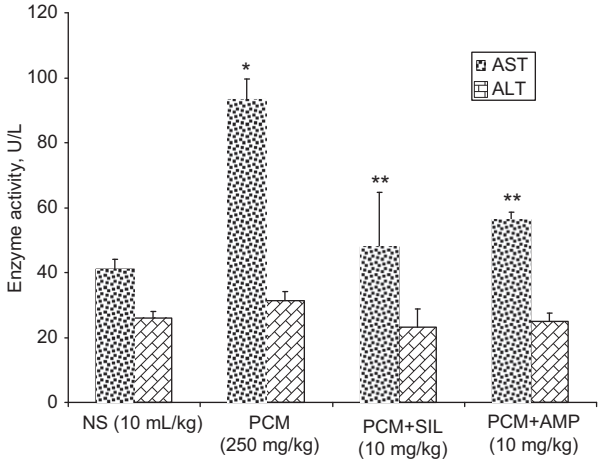

Figure 1 Effect of sildenafil and aminophylline on liver function of paracetamol-treated rats.
Values are mean±standard error of mean. NS, normal saline; PCM, paracetamol; SIL, sildenafil; AMP, aminophylline; AST, aspartate aminotransferase; ALT, alanine aminotransferase. *$p<0.001$ and **$p<0.05$ when compared with control and PCM, respectively.

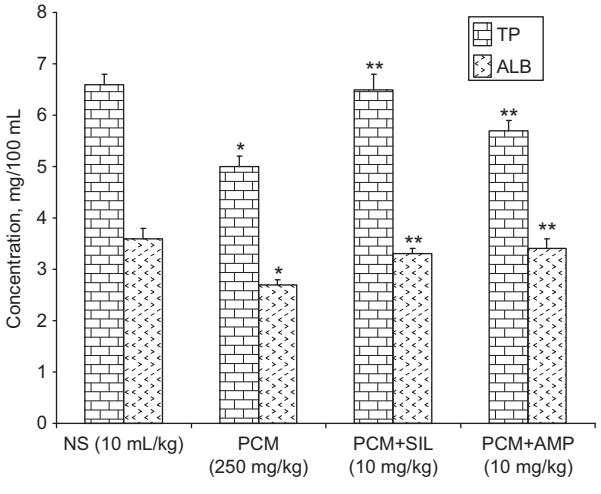

Figure 2 Effect of sildenafil and aminophylline on plasma total protein and albumin levels in paracetamol-treated rats.
Values are expressed as mean±standard error of mean. NS, normal saline; PCM, paracetamol; SIL, sildenafil; AMP, aminophylline; TP, total protein; ALB, albumin. *$p<0.001$ and **$p<0.05$ when compared with control and PCM, respectively.

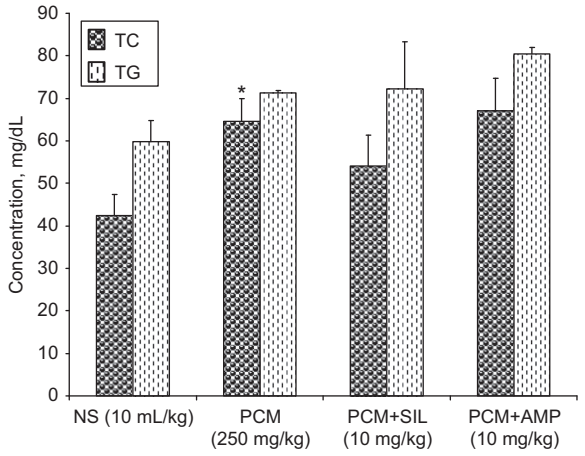

Figure 3 Effect of sildenafil and aminophylline on plasma total cholesterol and triglyceride levels in paracetamol-treated rats.
Values are mean±standard error of mean. NS, normal saline; PCM, paracetamol; SIL, sildenafil; AMP, aminophylline; TC, total cholesterol; TG, triglyceride. *p<0.05 when compared with control.

Histopathology

Representative photomicrographs of the liver histology of rats in the various treatment groups are shown in Figure 5. PCM hepatic toxicity was characterized by degeneration and centrilobular necrosis of the hepatic cells. PCM-intoxicated rats treated with sildenafil exhibited mild central venous congestion, while central venous and portal congestion with extensive periportal fibroplasia were seen in those that received aminophylline. No visible lesion was observed in control rats and liver histology was essentially normal.

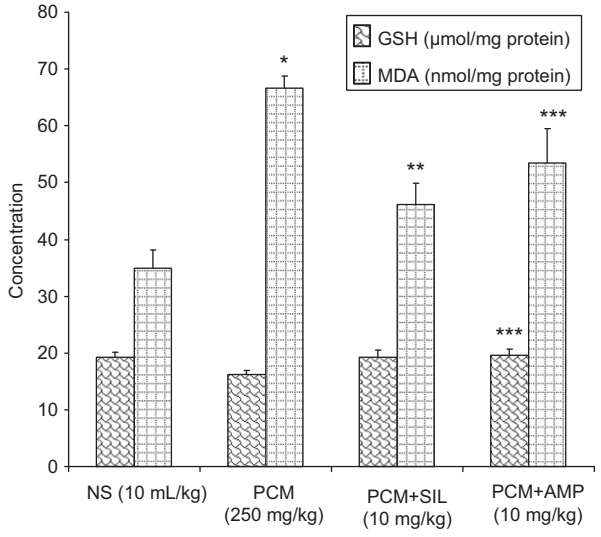

Figure 4 Effect of sildenafil and aminophylline on reduced glutathione and malondialdehyde levels in normal and paracetamol-treated rats.
Values are mean±standard error of mean. NS, normal saline; PCM, paracetamol; AMP, aminophylline; GSH, reduced glutathione; MDA, malondialdehyde. *p<0.001 when compared with control; **p<0.001 and ***p<0.05 when compared with PCM, respectively.

Discussion

There are conflicting reports on the exact nature of alteration of the NO-cGMP system in liver cirrhosis and other forms of liver pathology. While some observed decreased eNOS activity and NO together with an increased PDE-5 activity during pathophysiological changes in liver cirrhosis [8–13], others have reported overproduction of NO in endotoxin shock and other models of hepatic inflammation and injury [17, 18, 42]. Observation from this present preliminary investigation suggests that elevated PDE activity may be associated with PCM-mediated hepatotoxicity. Both sildenafil (a selective PDE-5 inhibitor) and aminophylline (a non-selective PDE inhibitor) demonstrated potentials as useful pharmacological agents in the prevention of PCM-associated liver injury. Sildenafil and aminophylline decreased the elevated serum AST and ALT activities in the PCM-treated rats. The decrease in synthetic function of the liver associated with PCM toxicity, demonstrated by the decreases in serum TP and ALB concentrations, was also prevented by both PDE inhibitors. The improvement in hepatic function following sildenafil and aminophylline administration underscores the role of PDE isozymes and by extension, cGMP and cAMP signaling mechanisms in physiological processes within the liver. PDE activities are generally modulated in coordination with adenylyl cyclase and guanylyl cyclase activities through direct effectors and feedback pathways, thereby maintaining cAMP and cGMP levels within optimum ranges for responsiveness to signals [6]. NO, synthesized by endothelial cells and also produced by liver parenchymal and non-parenchymal cells, plays a crucial role in hepatic microvascular blood flow under physiological conditions [7, 43, 44]. This reactive nitrogen species activates the soluble guanylate cyclase of stellate cells, resulting in the formation of cGMP, which regulates the tonus of stellate cells and sinusoids [45, 46], and PDE-5 terminates this action by converting cGMP to 5′-GMP [4, 5]. The improvement of liver function produced by sildenafil in this study can be related to its ability to prevent cGMP degradation through PDE-5 inhibition, and subsequently potentiating or enhancing the signaling function of this second messenger. As PCM hepatic toxicity is associated with elevated NO production in

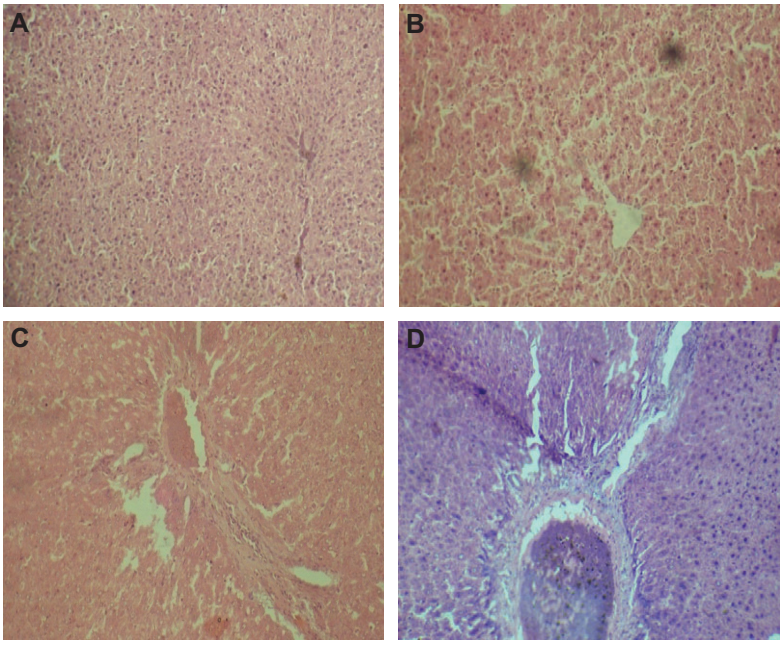

Figure 5 Liver section (×100) of rat treated with (A) normal saline (10 mL/kg) without visible lesion; (B) PCM (250 mg/kg) showing degeneration and centrilobular necrosis; (C) PCM+sildenafil (10 mg/kg) showing mild central venous congestion; and (D) PCM+aminophylline (10 mg/kg) showing central venous and portal congestion with extensive periportal fibroplasias.
PCM, paracetamol.

hepatocytes [31], it is also possible that accumulation of intracellular cGMP arising from PDE-5 inhibition may exert feedback inhibition on NO production and down-regulate the involvement of this reactive nitrogen species in PCM hepatotoxicity.

Our result also suggests that cAMP signaling may play an important role in the regulation of hepatic physiology. PDE-3 and PDE-4, which are among the most widely distributed and abundant PDEs in body tissues and within cells, preferentially hydrolyze cAMP [6]. The non-selective inhibition of these PDE isozymes by aminophylline, in this study, produced similar effects with sildenafil, which selectively inhibits PDE-5. Like sildenafil, aminophylline reduced PCM-mediated increases in plasma aminotransferases and improved synthetic function of the liver. Both drugs, however, did not attenuate the marked hypercholesterolemia and moderate TG elevation that characterize PCM hepatic toxicity in this study. Although the cholesterol-lowering effect of chronic PDE-5 inhibition with DA-8159 and its potential usefulness in treating erectile dysfunction secondary to hypercholesterolemia has been reported [47], short-term administration of sildenafil in this study only produced a non-significant decrease in PCM-associated elevation in serum cholesterol. Similarly, the decreased serum TG observed by Tani et al. [48] with cilostazol, a selective type PDE-3 inhibitor, in streptozotocin diabetic rats was not observed with aminophylline (a non-selective PDE-3 and PDE-4 inhibitor) in PCM-induced hepatotoxic rats.

Furthermore, sildenafil and aminophylline exhibited antioxidant potential in this study. Both PDE inhibitors significantly attenuated PCM-induced lipid peroxidation while producing modest increases in GSH levels. Recently, administration of low-dose sildenafil citrate was reported to prevent increases in MDA and NO levels and decreases in GSH induced by testicular torsion in Wistar rats [49]. The antioxidant potential of sildenafil has also been demonstrated in a clinical study [50]. Similarly, the ability of theophylline (the active component of aminophylline) to inhibit oxidative stress in the process of bronchopulmonary inflammation in asthmatics has been reported [51]. Our result corroborates these previous reports and suggests that the protective effects produced by sildenafil and aminophylline against PCM hepatic toxicity in this study may be related to this antioxidant action. It is, however, not clear if the antioxidant action is related to PDE isozyme inhibition or independent of it. In addition to improvement in some biochemical indices of hepatic function and oxidative stress, sildenafil and aminophylline also showed some potential to improve hepatic histological changes associated with PCM toxicity. While total protection against morphological injury was not observed, the marked hepatic degeneration and centrilobular necrosis that characterized PCM toxicity in this

study was ameliorated to some extent by both PDE inhibitors. This is evident in the mild central venous congestion observed in the sildenafil-treated rats and extensive fibroplasias in addition to the mild central venous and portal congestion seen in the group that received aminophylline.

In conclusion, data from the present preliminary investigation suggest that pharmacological inhibition of PDE isozymes may provide some chemopreventive benefit in liver injury associated with PCM toxicity.

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Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
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