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AN OPIUM ALKALOID- PAPAVERINE AMELIORATES ETHANOL-INDUCED HEPATOTOXICITY: DIMINUTION OF OXIDATIVE STRESS

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ABSTRACT

In this communication, we show the modulatory potential of papaverine, an opium alkaloid and a well known vasodilator agent on the ethanol-induced hepatic oxidative stress in male Wistar rats. Ethanol treatment (50% v/v) enhanced lipid peroxidation significantly accompanied by a decline in the activities of glutathione peroxidase (G-Px), glutathione reductase (GR) and depletion in levels of hepatic glutathione (GSH). Ethanol administration increased hepatic glutathione-s-transferases (GST). Enhanced lipid peroxidation induced by ethanol was significantly reduced when papverine was coadministered ($P<0.05$). In addition, the depleted levels of glutathione and inhibited activities of G-Px and GR recovered significantly ($P<0.05$) levelling off to control values on co-exposure. Papaverine (200 mg/kg bw) effectively antagonised the ethanol-induced lipid peroxidation and impaired glutathione levels and glutathione dependent enzyme systems. Our results suggest that papaverine is an effective chemopreventive agent in the liver and may suppress the ethanol-induced hepatotoxicity.

KEYWORDS: Papaverine, Lipid Peroxidation, Glutathione, Ethanol, Oxidative Stress

INTRODUCTION

Lipid peroxidation mediated by free radicals is considered to be a primary mechanism of cell membrane destruction and cell damage (1). One of the most thoroughly investigated examples is the lipid peroxidation stimulated by the model hepatotoxin - ethanol. Alcoholic liver diseases may be caused by oxygen radicals such as superoxide and hydroxyl radicals, generated during the metabolism of ethanol by the microsomal oxidising system (2).

Several mechanisms against cellular lipoperoxidative injury have been outlined in which glutathione (GSH) plays an important role (3-5). In addition to being a direct free radical scavenger, GSH is known to function as a substrate of GSH-peroxidases (G-Px) and GSH-transferases (GST) (6,7). Its absence may lead to the accumulation of lipid hydroperoxides. Hepatic GSH has an especially important relationship with lipid peroxidation because of its ability to bind with free radicals that may initiate peroxidation (8,9). When xenobiotics form conjugates with hepatic GSH, the GSH level decreases and the liver cell is made more susceptible to lipid peroxidation.

Papaverine, an opium alkaloid of the Papaveraceae family, plays an important role in alteration of lipid peroxidation in rat liver. Papaverine is known for its vasodilative effect (10,11). Experiments in our laboratory have shown that

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papaverine administration caused an increase in hepatic and pulmonary GST activity and glutathione content in a dose dependent manner, although the enhancement was not very significant in the lung. Data suggests that papaverine could act as a chemopreventive agent against certain electrophilic chemical carcinogens in the liver (12).

As ethanol and papaverine are individually known to have their individual effects on lipid peroxidation, our attempt in the present study was to decipher if papaverine protects against or aggravates the ethanol – induced changes in hepatic lipid peroxidation and glutathione – dependent defense mechanism in rats. In this investigation, our results have documented that oral administration of papaverine along with ethanol diminishes the ethanol - mediated enhanced lipid peroxide measures. A significant recovery of the inhibited activities of glutathione reductase (GR) and GPx was also observed on simultaneous administration of papaverine and alcohol.

MATERIALS AND METHODS

Chemicals

Papaverine was purchased from Sigma (P3510). All other chemicals were of analytical grade purity.

Animals and Treatment

Male Wistar rats weighing 150–180 g were used as experimental models for study. They were fed on a standard diet and water ad libitum until sacrificed. The rats were divided into four groups of eight rats each. Group I: Animals (n=8) on normal diet were fed with vehicle and served as control. Group II: Animals (n=8) were administered 2 ml of 50% ethanol (v/v) per day for a period of 7 days. Group III: Animals (n=8) were administered papaverine (200 mg/kg bw) per day for a period of 7 days. Group IV: Animals (n=8) were administered 2 ml of 50% ethanol (v/v) + papaverine (200 mg/kg bw) per day for a period of 7 days.

Post mitochondrial supernatant (PMS) and microsomes preparation

Animals were killed by cervical dislocation and the liver was quickly removed, perfused immediately with ice-cold saline (0.85% sodium chloride) and homogenized in chilled phosphate buffer (0.1M, pH 7.4) containing KCl (1.17%) using Potter-Elvehjem homogenizer. The homogenate was filtered through a muslin cloth, and was centrifuged at 800 x g for 5 minutes at 4°C in an IEC-20 Refrigerated Centrifuge (Rotor No 894) to separate the nuclear debris. The supernatant obtained was centrifuged at 10,500 g for 20 minutes at 4°C to obtain post-mitochondrial supernatant (PMS) which was used as a source of glutathione S-transferase and glutathione reductase. A portion of PMS was centrifuged in a Beckman preparative ultracentrifuge (Model L2-65B) at 105,000 x g for 60 minutes at 4°C. The pellet was washed with phosphate buffer (0.1M, pH 7.4) containing potassium chloride (1.17%). This pellet was used as the microsomal fraction and was suspended in phosphate buffer (0.1M, pH 7.4) containing KCl (1.17%).

Protein concentrations were determined according to the method of Lowry et. al. (13) using bovine serum albumin as the standard.

Assay for microsomal lipid peroxidation was carried out by following the method of Wright et. al.. (1981) (14). The amount of malonaldehyde formed in each of the samples was assessed by measuring the optical density of the supernatant at 535 nm using a spectrophotometer against a reagent blank. The results were expressed as nmol MDA equivalents formed/h/g of tissue at 37°C using a molar extinction coefficient of $1.56 \times 10^5 M^{-1}cm^{-1}$. Reduced glutathione (GSH) in the liver was assayed by the method of Jollow et. al. (1974) (15). The yellow colour developed was read immediately at 412 nm in a spectrophotometer. Glutathione S-transferase (GST) activity was measured by the method of Habig etal. (1974) (16), as described by Athar et. al. (1989) (17). The changes in absorbance were recorded at 340 nm and enzyme activity was calculated as nmol

CDNB conjugate formed/min/mg protein using a molar extinction coefficient of 9.6 x 10^3 M^{-1} cm^{-1}. Glutathione reductase (GR) activity was assayed by the method of Carlberg and Mannerviek (1975) (18), as modified by Mohandas et. al. (19). The enzyme activity was quantified at 25° C by measuring the disappearance of NADPH at 340 nm and was calculated as nmol NADPH oxidized/min/mg/ protein using a molar extinction coefficient of 6.22 x 10^3 M^{-1} cm^{-1}. The activity of Glutathione Peroxidase (GPx) was measured according to the procedure described by Mohandas et. al. (19). The enzyme activity was calculated as nmol NADPH oxidized/min/mg protein using a molar extinction coefficient of 6.22 x 10^3 M^{1} cm^{-1}.

STATISTICAL ANALYSIS

Data are expressed as means ± SEM. Statistical comparison of the treatment group with its control was made using Student's t-test (20).

RESULTS

The Data in Table I depicts the effect of administration of ethanol, papaverine and papaverine along with ethanol on lipid peroxidation, GSH levels, GST, GR and GPx activity in the liver. There occurred a marked induction in the MDA content by ~77% on ethanol administration. Papaverine caused a decline (~52%) in the hepatic MDA levels at a dose of 200 mg/kg bw per day administered for 7 days. Papaverine given along with ethanol levelled off the MDA content to control levels.

Table I. Effect of differential dosing regimens of Papaverine on Hepatic Lipid Peroxidation, Glutathione Content (GSH), Glutathione S-transferase (GST) activity, Glutathione Reductase and Glutathione Peroxidase[a]

Group treatment	Lipid peroxidation nmol of MDA formed/hr/tissue		GSH Content µmol/g tissue		GST Activity nmol CDNB conjugate formed/min/mg protein		Glutathione Reductase NADPH oxidized/min/ mg protein		Glutathione Peroxidase NADPH oxidized/min/ mg protein	
		%		%		%		%		%
I Control	14.07 ±5.1		79.1 ±3.15		171.24 ±6.3		58.6 ± 3.5		102.01 ±8.15	
II Ethanol (50% v/v)	24.90 ±4.01	+77	22.93 ±2.7	-71	289.40 ±5.07	+69	38.09 ±4.17	-28	80.58 ±8.4	-21
III Papaverine (200mg/kg bw)	6.76 ±2.4	-52	197.75 ±10.2	+150	462.34 ±11.4	+170	46.88 ±3.21	-20	26.52 ±5.92	-74
IV. Ethanol (50% v/v)+ Papaverine (200mg/kg bw)	15.48 ±2.01	+10	144.75 ±12.0	+83	376.73 ±7.09	+120	55.08 ±3.8	-6	23.46 ±4.041	-77

a Values are mean ± S.E.M. from 8 animals
**P<0.05

Ethanol administration evoked a decrease in hepatic glutathione content by ~71%. Acute ethanol ingestion has been found to produce a marked decrease in hepatic reduced glutathione (GSH) levels (21,22). It has been suggested that GSH-depletion could be related to its role in ethanol-induced lipid peroxidation. GSH levels were found to be increased in papaverine and papaverine + alcohol treated rats by ~1.5 fold and ~83% respectively. The data suggests that the papaverine mediated induction was antagonised when papaverine was coadministered along with ethanol. We contemplate on the basis of our results that an interaction between papaverine and ethanol profoundly modifies the hepatic metabolism of glutathione and may thus have important effects on the detoxification of xenobiotics by the liver.

An induction pattern was observed in the hepatic GST activity in all the treated groups. Alcohol administration enhanced GST activity by ~69% as compared to the control. Papaverine induced a statistically significant increase in the GST activity by ~1.7 fold. The combination dosage of papaverine + ethanol again produced an increase in the enzyme activity by ~1.2 fold.

There occurred a diminution in glutathione reductase and glutathione peroxidase activity in all the treated groups. A decline of GR activity by ~20% was observed on papave ıe administration. Supplementing papaverine with ethanol resulted in the activity patterns as shown by the control group. The hepatic glutathione peroxidase activity wa: lowered in ethanol treated group by ~21%. The combination dosage of ethanol and papaverine resulted in an inhibition of the enzyme activity by ~77% as compared to control.

DISCUSSION

Ethanol induced depletion of hepatic glutathione has been construed as evidence supporting the hypothesis that reactive oxygen intermediates generated during the metabolism of ethanol lead to glutathione oxidation and lipid peroxidation and are responsible for the toxic effects of ethanol. This study provides evidence that administration of papaverine along with ethanol results in an antagonistic effect of the alkaloid on the ethanol induced oxidative stress which may be due to the associated action of ethanol and alkaloid.

Raised levels of GSH have been reported to elicit a protective response against the toxic manifestations of chemicals, particularly those involving oxidative stress (23). The tissue GSH concentration reflects its potential for detoxification. Our results are noteworthy with respect to the papaverine-mediated amelioration of the hepatic GSH content declined by ethanol.

The induction of lipid peroxidation and depletion of GSH are events that occur after the formation of ethanol metabolites. GSH is presumed to be an important endogenous defense against oxidative stress and, therefore, a defense against the peroxidative destruction of cellular membranes. GSH can act either to detoxify activated oxygen species such as H_2O_2 or to reduce lipid peroxides themselves. On the basis of our results, we may speculate that the alkaloid reacts with lipid radicals and this reaction spares the consumption of GSH thus restoring it to normal levels. Any interaction between the alkaloid and activated oxygen species would also be expected to preserve GSH content. The restored levels of GSH in simultaneously treated groups (papaverine + ethanol) with respect to those in which the treatment is independent show the effect of this alkaloid on the metabolic pathways resulting in lipid peroxidation that are normally dependent on GSH for their inhibition.

Another line of thought suggests that the decrease in hepatic GSH and GST upon acute ethanol administration could be due primarily to an increased loss of both GSH and GST from the liver into plasma.

Sies et. al. (24) reported that suppression of hepatic GSH content might be due to increased sinusoidal efflux of GSH from the perfused liver mediated by hormonal responses on ethanol treatment. The amelioration of the GSH content and GST activity on simultaneous treatment of papaverine and ethanol, thus might be, mediated by altered

hormonal responses including release of corticosteroids, glycogen and epinephrine.

Ethanol – induced liver injury may be linked, atleast, to an oxidative stress resulting from increased free radical production and/or decreased antioxidant defence. Oxidative stress has been shown not to be restricted to the liver, but also to affect, under some experimental conditions of ethanol administration, extrahepatic tissues, such as the central nervous system, the heart and the testes. This stress can be partly prevented by papaverine supplementation. Papaverine possesses significant antioxidant potential. The antioxidants could act by reducing free radical production (eg. chelators of redox-active iron derivatives), trapping free radicals themselves, interrupting the peroxidation process or reinforcing the natural antioxidant defense.

We have observed in the course of our study the ethanol – mediated depletion of GSH and subsequent amelioration by papaverine coadministration. Our results showed that lipid peroxidation was inhibited when ethanol and papaverine were given simultaneously. We speculate that this perhaps would have been due to the fact that the coexposure did not deplete GSH levels to a threshold, beyond which lipid peroxidation ensures. This is suggestive of the therapeutic action of papaverine in ethanol – hepatotoxicity.

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