Original Article

Co-Exposure Effects of LPS with Various Aflatoxin B1 Doses in Isolated Perfused Rat Liver Model

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ABSTRACT

Background: Activation of inflammatory cells can cause more chemicals induced-hepatotoxicity. Aflatoxin B1 (AFB1) is a fungal toxin that induces acute hepatotoxicity in humans and animals. This study was conducted to examine the effect of co-exposure LPS and various aflatoxin B1 doses on the damage hepatic parameters in isolated perfused rat liver.

Methods: Thirty-two male wistar rats (250-300g) were divided to eight groups including Control and LPS; three groups with various doses of AFB1 (0.01, 0.1 and 1 ppm) and three groups with various doses of AFB1 and Lipopolysaccharide (LPS) (300 ppm). Activity of Aspartate transaminase (AST) and Alanine transaminase (ALT) were determined in perfusate. Thiobarbituric acid reactive substances (TBARS) and Glutathione (GSH) concentrations were measured in homogenate liver.

Results: At two groups of AFB 1 (with LPS and without LPS) at AFB1 concentrations of 0.1 and 1 ppm, elevation of AST and ALT enzymes activity were indicated. Values of GSH in both of groups (AFB 1 with LPS and without LPS) had reduced at concentration of 1 ppm. TBARS concentrations were enhanced in AFB1 concentration of 1 ppm in both of groups (with LPS and without LPS), however in comparison between groups (with LPS and without LPS) in similar concentrations significant different did not observe (P<0.05).

Conclusion: Non-injurious dose of LPS did not enhance liver susceptibility to various doses of AFB1 in perfused rat liver. This may be in part of due to extrahepatic factors, which contribute, in more liver damage.

Keywords: Aflatoxin B-1, Isolated Perfused Rat Liver, Lps.

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INTRODUCTION

Some factors contribute to development and sensitivity of xenobiotics toxicity in organisms. One of them is inflammation that can induce by exposure to bacterial endotoxin [1, 2]. Endotoxin comprises proteins in association with lipopolysaccharide (LPS), a major component of the outer cell walls of Gram-negative bacteria. LPS can translocate from the GI lumen into the blood and therefore liver and others organ become exposed. Exposure to nontoxic doses of bacterial endotoxin (LPS) can cause the liver more sensitive to injury from hepatotoxic chemicals [3, 4].

As regard to production and effects of inflammatory mediators, liver is one of the most important organs. Liver Kupffer cells are a major source of production of these mediators release in response to inflammatory stimulants such as LPS [5, 6].

Therefore, studies using the isolated perfused rat liver for determination of LPS effects are acceptable because they can significantly improve our understanding of the effects of inflammation processes on various liver functions under of condition of exposure with hepatotoxicants [6].

Aflatoxins are mycotoxins and a hepatotoxicant that were produced by fungi *Aspergillus flavus* and *A. parasiticus*. Among Aflatoxins that produced by these two strain, aflatoxin B1 has more important than another

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aflatoxin with regard to toxicity and tissue damage [5, 7, 8].

The aim of this study was to determine the ability of LPS for enhancing of AFB1 – induced liver injury. AFB1 was given at various doses in presence or absence of a small dose of LPS. Biochemical markers for determination of liver injury were analyzed.

MATERIALS AND METHODS

Chemical

Aflatoxin B, LPS and glutathions were obtained from Sigma (St. Louis, MO, USA). A perfusion fluid was made of Krebs-Henseleit bicarbonate buffer. The perfusion medium consisted of 118 mmol/l NaCl, 6 mmol/l KCl, 1.1 mmol/l MgSo4, 24 mmol/l NaHCo3 and 1.25 mmol/l CaCl2.

Animals

Male Wistar rats (250-300g) were obtained from the vivarium section of the department of pharmacology, Tehran University of Medical Sciences, Tehran, Iran. Animals were housed in cages in temperature room. They had free access to standard diet and tap water until 2 h before surgery. Experimental study was based on standard guideline animal welfare of Faculty of Veterinary Medicine of Semnan University.

Perfusion Apparatus

The liver perfusion apparatus used in our laboratory is based on the description of Miller et al. but whit changes [9-11]. The perfusion apparatus consisted of a perfused reservoir maintained at 37 °C, that perfusion was carried out by a peristaltic pump at a constant flow rate of 20 ml/min for 120 min. Central vein pressure (C.V.P) linear was used for measurement H2O pressure and serum set was used for attachment elements of apparatus and degassing perfusate. The selected mode for liver perfusion is single-pass because AFB 1 is high hepatic extraction [9].

Preparation of Isolated Perfused Rat Liver (IPRL)

The rats were anesthetized by an intraperitoneal injection of mixture of ketamin/xylazine (60:8 mg/ml) [6, 11]. The anterior abdomen is cleaned with alcohol and a ventricle longitudinal midline incision is made extending from pubis to upper chest. The animal heparinized by injecting the solution into the inferior vena cava anterior to the renal vein and immediately vena cava is ligated. After that hepatic portal vein (inlet) and the thoracic inferior vena cava (outlet) were cannulated. The liver was perfused with Krebs-Henseleit bicarbonate buffer (KHBB) (pH 7.4±2) saturated by 95%O_2/5% CO_2 through with catheter cannulated into the portal vein. The perfusate was collected from a catheter into superior vena cava via the right atrium. Flow rat perfusion was 20 ml/min with 15- 20 cm H_2O. The flow rate was measured by fractionating the effluent.

Experimental Design

A total of 32 livers were used in our study. Before treatment was initiated, the livers were allowed to equilibrate for 15 min. Perfused liver from rat was divided into eight groups with four replicate in every group. The control livers were perfused with the perfusate only. The LPS group was perfused with the perfusate and LPS (300 ppm). Three groups of treatment were perfused with the perfusate and AFB 1 at differential concentration (0.01, 0.1 and 1 ppm). The other three groups were perfused with differential doses aflatoxin B1 (0.01, 0.1and 1 ppm) and LPS (300 ppm) at separated groups four.

Sample Collection

Samples (1.5 ml) were taken from the outlet at 0, 15, 30, 45, 60, 75, 90, 105 and 120 min and stored at -20 °C for measurement of ALT, AST levels. The perfused livers lobs were used for measurements MDA, GSH and totals proteins.

Sample Analysis

Enzymes levels were quantitated based on colorimetric methods using a commercial kit from Teb Gostaran Hayan (Tehran, Iran). Lipid peroxidation was determined in liver tissue homogenate according to the thiobarbituric acid (TBA) method [12]. GSH was estimated by Kuo and Hook standard method [13] and total proteins was estimated by Bradford method [14].

Statistical Analysis

Values are presented as mean ± SE. Data were analyzed by one-way ANOVA, followed by Tukey test for multiple comparisons. Differences were considered to be statistically significant when $P < 0.05$.

RESULTS

Enzyme Release

Damage to perfused rat liver was detected by measuring the outlet AST and ALT, as indexes of hepatic injury (Fig. 1-4). In control group and LPS group, levels of enzymes were a little. In groups AFB 1 (various doses (0.01, 0.1 and 1ppm) with and without LPS, increase in AST and ALT were observed. However, enzymes fluctuation and increase in groups with LPS was more than groups without LPS.

In without LPS groups, concentration of 0.01 ppm in perfusate did not affect viability parameters. In the high dose groups, concentrations of 0.1 and 1 ppm affected viability parameters and increased AST and ALT levels.

In with LPS groups, considerable increase was observed in various AFB1 concentrations (0.01, 0.1 and 1 ppm). Analysis comparisons between LPS groups and without LPS groups did not show significant difference viability parameters (ALT and AST), statistically.

Effects of Aflatoxin B1 on Lipid Peroxidation, GSH and Total Protein

The presence of AFB1 in perfusate led to dose –dependent increase in lipid peroxidation and dose – dependent decrease in GSH levels in groups of with LPS and without LPS in comparison with LPS and Control groups significantly (P<0.05) (Figure 5-7).

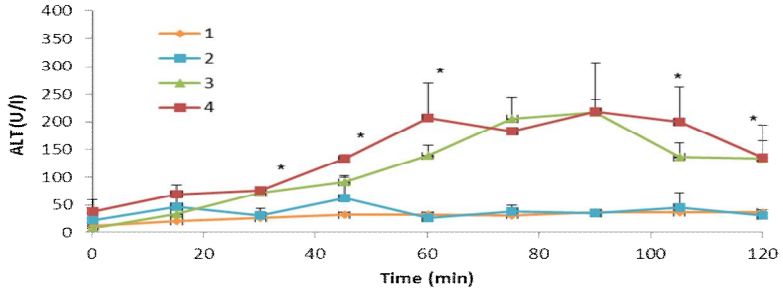

Figure 1. Time courses of alanine aminotransferase (ALT) release from the perfused liver rat for 2 h. The rat liver was exposured with various doses aflatoxin B1. (Group 1: control; group 2: 0.01 ppm; group 3: 0.1 ppm; group 4: 1 ppm). Values are means±S.E. of four experiments.

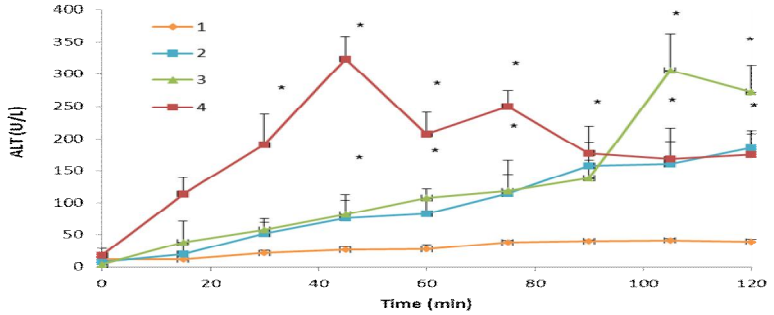

Figure 2. Time courses of alanine aminotransferase (ALT) release from the perfused liver rat for 2 h. The rat liver was coexposured for 2 h with LPS and various doses aflatoxin B1. (Group 1: LPS 300 ppm; group 2: 0.01 ppm; group 3: 0.1 ppm; group 4: 1 ppm). Values are means±S.E. of four experiments.

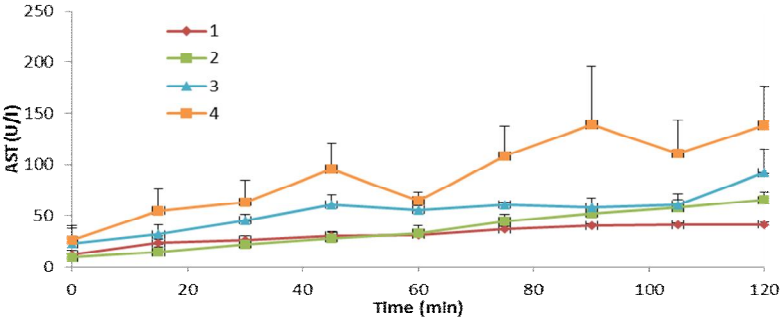

Figure 3. Time courses of alanine aminotransferase (ALT) release from the perfused liver rat for 2 h. The rat liver was exposured with various doses aflatoxin B1 (group 1: control; group 2: 0.01 ppm; group 3: 0.1 ppm; group 4: 1 ppm). Values are means±S.E. of four experiments.

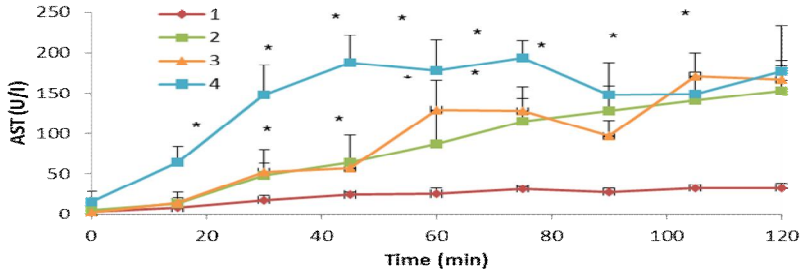

Figure 4. Time courses of AST release from the perfused liver rat for 2 h. The rat liver was coexposured for 2 h with LPS and various doses aflatoxin B1. (group 1: LPS 300 ppm; group 2: 0.01 ppm; group 3: 0.1 ppm; group 4: 1 ppm). Values are means±S.E. of four experiments.

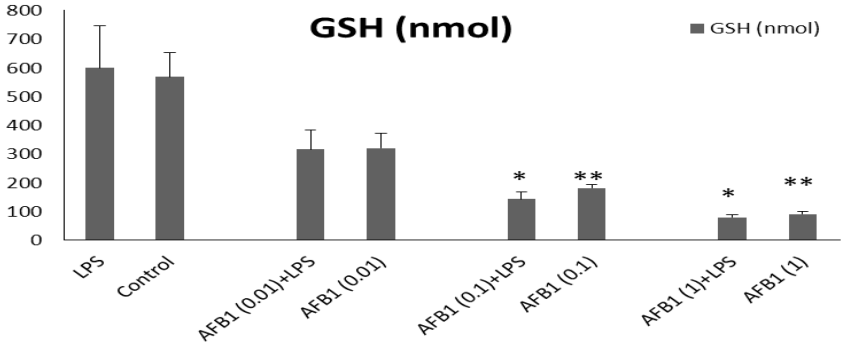

Figure 5. Concentration of glutathione (GSH), in the perfused rat liver at the end of the experiments. Values are mean ± SE. of four experiments.

* Statistically significant difference ($p < 0.05$) from LPS group

** Statistically significant difference ($p < 0.05$) from control group

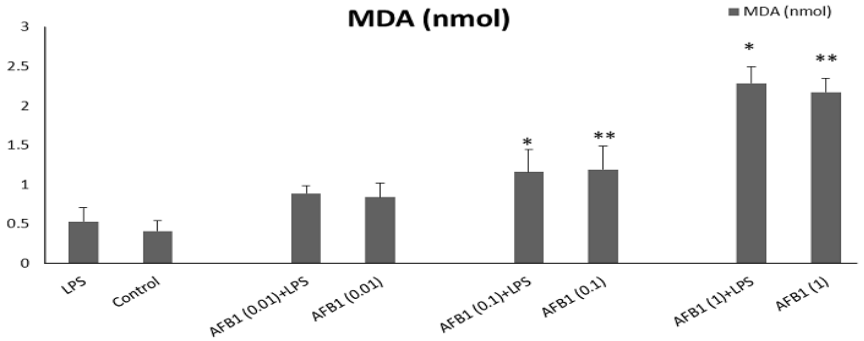

Figure 6. Concentration of malondialdehyde (MDA) in the perfused rat liver at the end of the experiments. Values are mean ± SE. of four experiments.

* Statistically significant difference ($p < 0.05$) from LPS group

** Statistically significant difference ($p < 0.05$) from control group

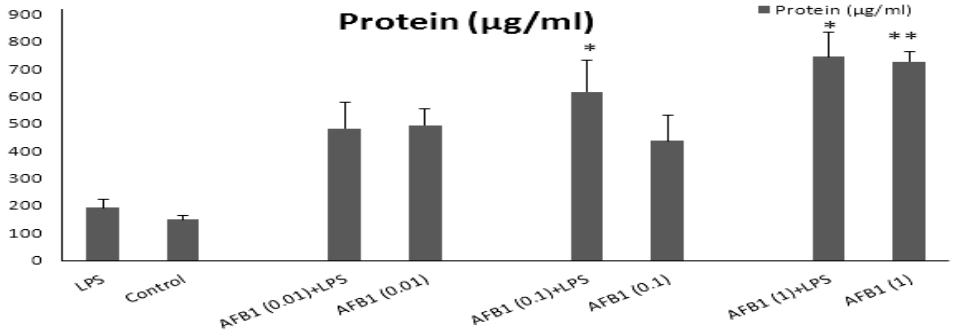

Figure 7. Concentration of protein and in the perfused rat liver at the end of the experiments. Values are mean ± SE. of four experiments.

* Statistically significant difference ($p < 0.05$) from LPS group

** Statistically significant difference ($p < 0.05$) from control group

DISCUSSION

The aim of the present study was to investigate hypothesis that whether non-injurious LPS dose can cause to increase hepatic susceptibility to various doses of aflatoxin B1. For this study, we used from perfused rat liver. Because of this model is considered as sensitive organ for chemical-induced hepatotoxicity. Several studies has been documented effects of co-exposure LPS with numbers of xenobiotics in vivo model including D-galactosamine, AFB1 and lead. Hepatocytes compromise the largest of liver cell mass. In events of injurious to hepatocytes, hepatocellular enzymes release into blood or perfusate. Although aspartate aminotransferase (AST) is not a liver specific enzyme, it is a damage marker in pefused rat liver. AST is present in both cytoplasm and mitochondria of hepatocytes and its release is elevated as severe damage of liver. Alanine aminotransferase (ALT) is present in cytoplasm of hepatocytes and its release is elevated as light liver damage. In this study, in groups without LPS, the levels of ALT and AST increased at AFB1concentrations of 0.1 and 1 ppm after15 min from injection of AFB1. AFB1 concentration of 0.01 ppm did not have statistically significant different with control group. In groups with LPS, similar to groups without LPS in time-dependent manner, levels of ALT and AST increased at AFB1 concentrations of 0.01, 0.1 and 1 ppm, that concentrations of 0.1 and 1ppm had statistically significant different with LPS group. Our finding is consistent with the most investigators finding about time course of enzyme changes in exposure with naproxen [15], copper and AFB1 [16-18], cadmium [19], vanadium [20] in perfused rat liver model.

MDA concentrations as index of lipid peroxidation increased in exposure with various concentrations of AFB1 both with and without LPS groups and significant different was observed at concentration of 1 ppm in AFB1/LPS and AFB. In addition, hepatic GSH levels as marker of stress oxidative decreased at AFB1 concentration of 0.1 and 1 ppm in groups of with and without LPS. Studies on oxidative stress and lipid peroxidation in perfused rat liver are frequently used some compound such as *t*-BuOOH and allyl alcohol [15, 16]. Lipid peroxidation is suggested in part as a possible mechanism of AFB1- induced hepatotoxicity and reactive oxygen species induced by AFB1 have been involved in the toxic actions of AFB1 [21-24].

In comparison of AFB 1 groups (with LPS and without LPS) we did not observe any significant difference for AST, ALT, MDA and GSH levels. Our results seem to differ with those of Roth et al. where concurrent inflammation render the liver considerably more sensitive to the hepatotoxic effects of AFB1 [4]. Enhancement of AFB1-induced hepatotoxicity by LPS depends on tumor necrosis factor α [3]. In addition, synergistic liver injury from LPS is suggested in rats of exposure with monocrotaline [25]. In all studies cited above examined as in vivo, it likely seems LPS increased hepatotoxicity AFB1 through activation of inflammatory cells and production of soluble inflammatory mediators. One of inflammation mediators is TNF-α that after administration of LPS resulted increase in circulating TNF-α and hepatic transcription factor for TNF-α. TNF-α depletion was associated with decrease of hepatic cell injury. Similarly, neutrophils (PMNs) accumulate early in liver of treatment with AFB1/LPS and depletion of PMNs resulted reduction in hepatocellular injury. TNF-α has the role in promoting PMNs accumulation. This cytokine can increase the expression of endothelial cells of adhesion molecules that cause PMNs accumulation. Furthermore, a rat neutrophil chemoattractant (CINC-1) contribute to the recruitment of hepatic PMNs [25, 26]. Thus, both LPS-induced TNF-α and CINC-1 that secret from hepatic parenchymal cells and sinusoidal endothelial cells contribute in accumulation of hepatic PMNs. At finally, TNF-α can stimulate the release of reactive oxygen and nitrogen species and toxic protease from PMNs that render liver more susceptible to AFB1 hepatotoxicity and result more liver injury.

Our results may seem to be due to used model. The liver is one of important organs with regard to kupffer cell as source of inflammatory cytokines production in response to immunostimulants such as LPS. Exposure of AFB1/LPS toward perfused rat liver seems to stimulate liver macrophages and release inflammatory cytokines. However, for liver generalized damage may need to be PMNs for release of reactive oxygen and nitrogen species and toxic protease. In fact, extrahepatic factors may contribute to increase of liver susceptibility during lipopolysaccharide exposure [27]. Platelets, neutrophils (PMNs) and several inflammatory mediators can cause liver injury during LPS exposure.

CONCLUSION

LPS in perfused rat liver cannot enhance liver susceptibility to AFB1 hepatotoxicity. It may

be in part of due to absence of extrahepatic factors that cannot render liver more damage.

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