International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2018.889.895

Research Article

Hepatic Upregulation of Tumor Necrosis Factor Alpha and Activation of Nuclear Factor Kappa B Following Methyl Methacrylate Administration in the Rat

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Abstract

Background and Objective: Tumor necrosis factor alpha (TNF-α) and nuclear factor kappa B (NF-κB) have been implicated in hepato-toxicity. Methyl methacrylate (MMA) has been shown to cause diverse health effects on the liver. Thus, the aim of this study was to illustrate the impact of MMA administration on the hepatic expression of TNF-α and activation of NF-κB. **Material and Methods:** Twenty sprague-dawley female rats were randomly selected and subsequently divided into two equal groups: control group and experimental group. Methyl methacrylate (120 mg kg^{-1}) was orally administered daily 5 days/week for 4 weeks in the experimental group. After that, liver samples were evaluated by immuno histochemistry to examine the expression of TNF-α and activation of NF-κB in the two groups of animals. **Results:** Hepatocytes displayed ballooning degeneration following the oral administration of MMA. Concomitantly, the hepatic expression of TNF-α and activation of NF-κB were significantly increased in the experimental rats compared with those in the control rats ($p<0.01$). **Conclusion:** Thus, the present data indicate a correlation of ballooning degeneration of the hepatocytes with the hepatic TNF-α up regulation and NF-κB activation, potentially promoting the adverse health effects of MMA on the liver.

Key words: Methyl methacrylate, TNF-α, NF-κB, immuno-histochemistry, ballooning degeneration

Received: March 04, 2018 Accepted: March 26, 2018 Published: July 15, 2018

Citation: N.S. Erekat, A.A. Al-Jarrah, A.M. Shotar and Z.A. Al-Hourani, 2018. Hepatic upregulation of tumor necrosis factor alpha and activation of nuclear factor kappa B following methyl methacrylate administration in the rat. Int. J. Pharmacol., 14: 889-895.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Methyl methacrylate is a colorless fluid acrylic resin monomer that is widely used in medical and dental fields as well as in industry[1-3]. For instance, MMA is used medically as bone cement in total hip and total knee replacements[4]. It is also used in manufacturing intraocular lenses[5-7]. Dentally, MMA is used as a major component of restorative and prosthetic resins[2]. In industry, MMA is very important in making plastics besides many other materials[3]. Methyl methacrylate has been reported to cause many adverse health effects, such as dermatitis, conjunctivitis and hepatotoxicity[8,9].

Methyl methacrylate has been shown to be cytotoxic causing reduction in the hepatic levels of reduced glutathione, which is an antioxidant that protects the cell against cytotoxicity, potentiating the activation of the transcription factor NF-κB[10-14]. Age-induced reduction in the hepatic reduced glutathione has also been reported to be associated with high levels of NF-κB activity[15]. NF-κB has been implicated in hepatotoxicity[16,17]. Upon its activation, NF-κB is released and translocated to the nucleus, where it affects gene expression causing the production of many proteins including pro-inflammatory cytokines, such as TNF-α[18]. Furthermore, both TNF-α up regulation and NF-κB activation have been shown to be involved in the pathogenesis of alcoholic liver disease[19,20].

Although MMA has been shown to be toxic to the liver[9], its impact on TNF-α and NF-κB has never been investigated before. Thus, in this study, the liver was studied histo-pathologically in order to illustrate the hepatotoxic effect of MMA. Additionally, immuno-histochemistry and light microscopy were used in order to examine any alterations in TNF-α expression and NF-κB activation in the liver following the oral administration of MMA in rats.

MATERIALS AND METHODS

The study was conducted at Jordan University of Science and Technology, Faculty of Medicine in 2016.

Animals: Twenty female sprague-dawley rats weighing 230-280 g were randomly selected and subsequently divided into two equal groups, which were: Control (Cr) and experimental (Exp) groups. Rats in the experimental group had oral administration of MMA diluted with water (120 mg kg^{-1}/day) 5 days a week for 4 weeks as described previously[21]. The rats were maintained in a 12:12 light/dark cycle and at standard temperature and air moisture. Rats had access to clean water and standard rodent food. All the experimental procedures were approved by the Animal Care and Use Committee (ACUC) at Jordan University of Science and Technology.

Histo-pathological examination of the liver: After sacrificing the animals, their livers were dissected and subsequently fixed in 10% neutral buffered formalin. Then, the tissue samples were dehydrated, cleared, infiltrated and subsequently embedded in paraffin. Subsequently, 5 μm thick paraffin-embedded sections of the liver were prepared, deparaffinized, rehydrated and stained with Hematoxylin and Eosin (H and E). Ten slides from each animal in each of the 2 groups were examined for histopathological alterations microscopically. Ten random areas from each section were photographed using digital camera.

Tissue preparation and immuno-staining: The animals were sacrificed at the end of the treatment and their livers were dissected and subsequently fixed in 10% neutral buffered formalin. Then, the tissue samples were dehydrated, cleared, infiltrated and subsequently embedded in paraffin. After that, 5 μm thick paraffin-embedded sections were sliced and prepared for processing with anti TNF-α and anti NF-κB antibodies via immuno-histochemistry, according to the protocol described previously[22-28]. Thus, after rehydrating and deparaffinizing the sections, they were processed to retrieve their antigen. Next, sections were cooled down to room temperature. Then, the endogenous peroxidase activity was blocked by incubating the sections with 3% hydrogen peroxide in methanol, for 5 min. After that, the sections were rinsed off in phosphate buffered saline (PBS). Subsequently, some of the sections were incubated with anti-TNF-α antibody (Abcam, Cambridge, MA, USA), while others were incubated with anti-NF-κB antibody (Abcam, Cambridge, MA, USA), according to the dilutions recommended by the vendors. Then, the sections were washed with PBS before and after being incubated with biotinylated secondary antibody (LSAB kit, Dako, Carpinteria, CA, USA). Then, samples were incubated with streptavidin horse radish peroxidase (LSAB kit, Dako, Carpinteria, CA, USA) for 15 min at room temperature and successively washed with PBS. 3-Diaminobenzidine was applied until the desired staining intensity was observed. Finally, the sections were rinsed off with tap water to stop the reaction. Negative control sections were processed without the primary antibody.

All sections were then counter stained with hematoxylin and viewed under the light microscope (BIO2T, BEL, Engineering, Italy).

Data collection and statistical analysis: Digital camera (Video Head, BEL, Engineering, Italy) was used to photograph three randomly selected regions per liver section. Ten sections from each animal of all 10 animals in each group were analyzed by counting the total pixels area occupied by positive staining in each of the selected regions in each liver section and computing it as a proportion of the total pixels in each field in the sections, using Adobe Photoshop software, as described previously[22-28]. Independent samples t-test was used to statistically compare TNF-α and NF-κB expression in liver sections between the 2 different groups. Differences in TNF-α and NF-κB expression were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Hepatocytes from experimental rats manifested ballooning degeneration, since they appeared enlarged with wispy cleared cytoplasm and centrally located nuclei (Fig. 1b), indicating the toxic effect of MMA administration on the liver. Immuno-histochemical staining revealed low level of TNF-α expression and NF-κB nuclear localization (Fig. 2a and c, respectively) in livers from the control group. However, TNF-α (Fig. 2d and 3) and nuclear NF-κB (Fig. 2b and 4) immunoreactivities were obviously increased in the livers following the administration of MMA, indicating their potential involvement in the hepatotoxicity, which was indicated by the ballooning degeneration of hepatocytes, following MMA administration.

Consistent with current finding of ballooning degeneration of hepatocytes following MMA administration (Fig. 1b), previous studies illustrated hepatotoxicity in the form of ballooning degeneration of hepatocytes due to toxic substances, such as alcohol, iron oxide and carbon tetrachloride[29-31]. Methyl methacrylate has been illustrated to induce liver toxicity and histopathological alterations in the liver[9,32].

The present study was the first to illustrate the impact of methyl methacrylate on the hepatic expression of TNF-α and activation of NF-κB. The consequent analysis revealed increased TNF-α expression and NF-κB activation in the liver subsequent to the administration of MMA in the rats.

The administration of MMA has statistically significantly elevated the levels of TNF-α ($p<0.01$, Fig. 3) and nuclear localization of NF-κB ($p<0.01$, Fig. 4) in the liver when compared with that in the control liver.

Low levels of TNF-α were expressed in normal non-stressed tissues[33]. This supports current results that revealed hardly detected TNF-α in the control liver (Fig. 2a). Methyl methacrylate has been reported to induce liver toxicity and histopathological alterations in the liver[9,32]. It has also been shown to cause inflammation in different tissues such as the olfactory epithelium[34]. TNF-α is a cytokine that is involved in inflammation[35]. Previous studies have demonstrated MMA-induced TNF-α overexpression in osteoclast precursor cells[36]. Additionally, elevated hepatic levels of TNF-α have

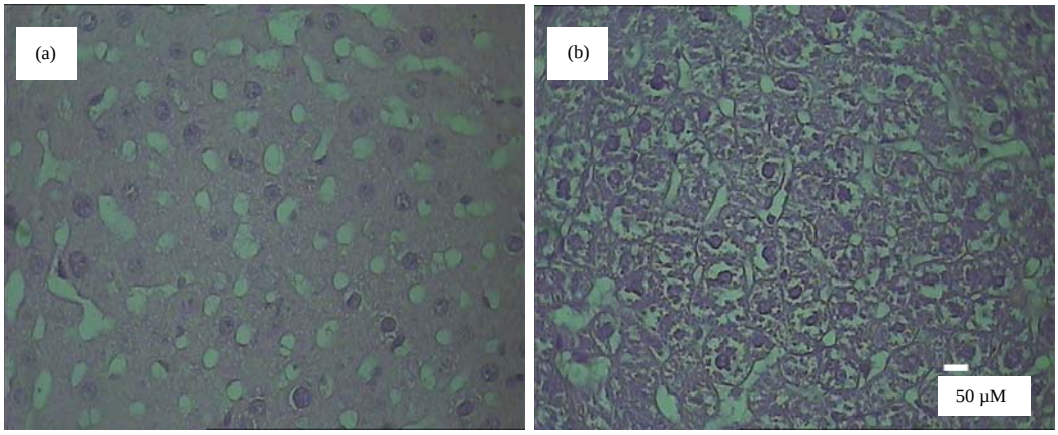

Fig. 1(a-b): Histopathological alterations in 5 µm thick paraffin-embedded liver sections. (a) Control hepatocytes show eosinophilic cytoplasm with round nuclei that have dispersed chromatin and prominent nucleoli. (b) Hepatocytes from experimental rats appeared enlarged with wispy cleared cytoplasm and centrally located nuclei indicative of ballooning degeneration. Scale bar in (b) applies to both images in the figure

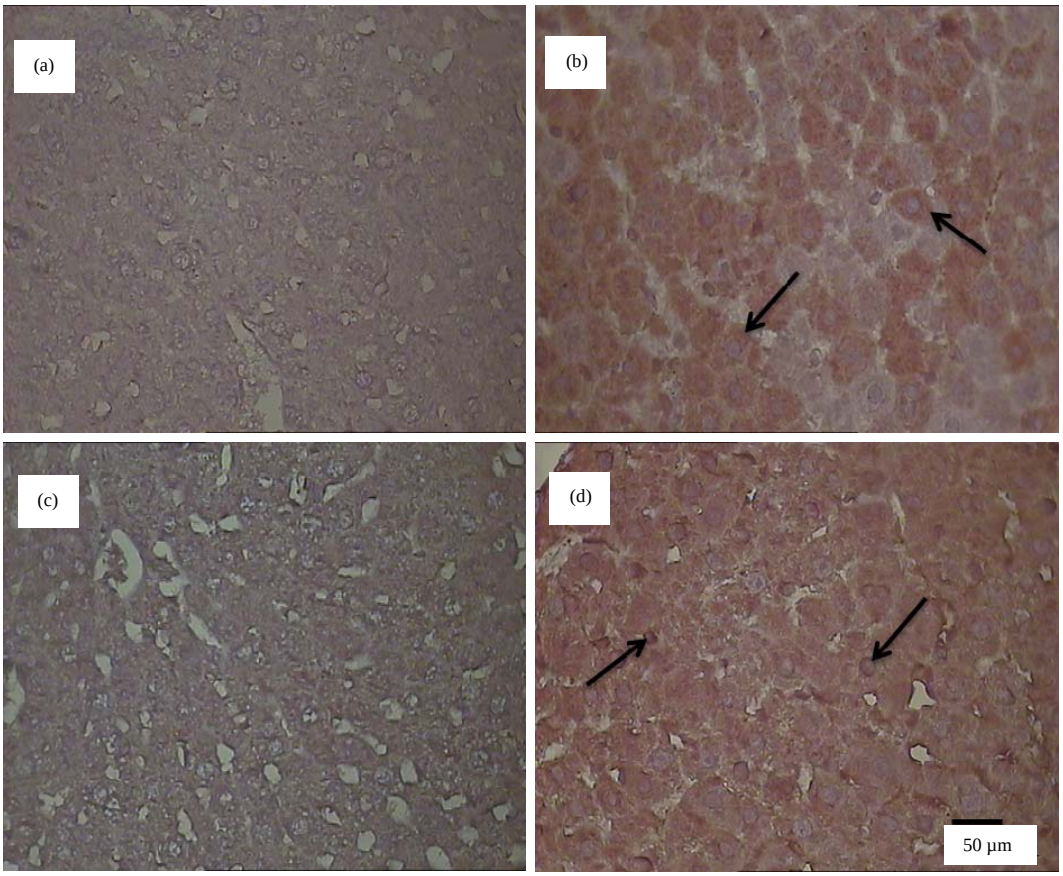

Fig. 2(a-d): Immunohistochemical staining of TNF-α and NF-κB in 5 µm thick paraffin-embedded liver sections. a and c: From control rats, b and d: From experimental rats, (a) TNF-α and (c) NF-κB, Immunostaining is hardly observed in the control liver. However, (b) TNF-α and (d) NF-κB immunoreactivities are very strong in the liver (at the tip of the arrows) following the administration of MMA in the experimental rats
Scale bar in (d) applies to all images in the figure

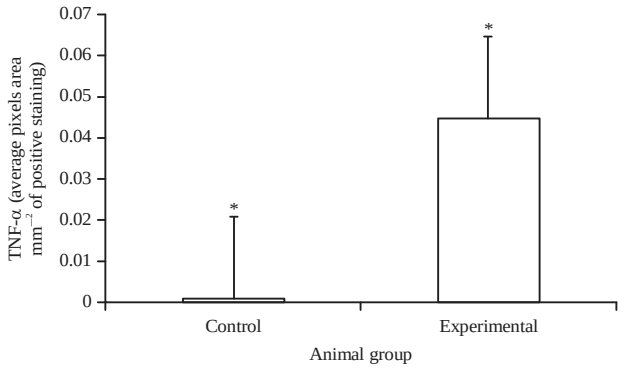

Fig. 3: Expression of TNF-α in the liver. The level of TNF-α expression increased significantly in the experimental group following the administration of MMA compared to control group (*p<0.01)

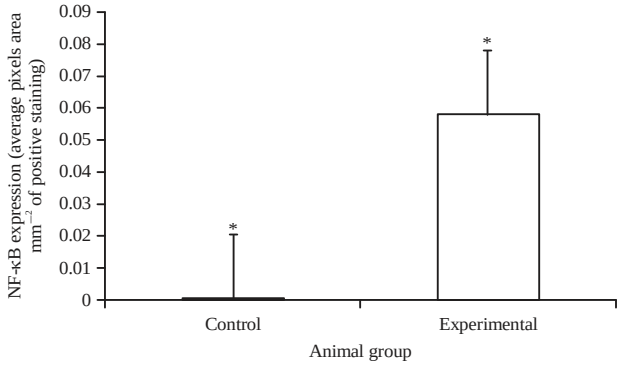

Fig. 4: Expression of NF-κB in the liver. The level of NF-κB expression increased significantly in the experimental group following the administration of MMA compared to control group (*p<0.01)

been suggested to be involved in the pathogenesis of alcoholic liver disease[37]. These previous reports were consistent with current results that revealed TNF-α upregulation in the liver subsequent to MMA administration

in the rat (Fig. 2b). Hence, these are the first data to demonstrate the MMA-induced alterations in TNF-α expression in the liver. Therefore, it can be postulated that MMA might have induced hepatotoxicity via stimulating the production of TNF-α, indicative of occurrence of inflammation, in the liver.

Thus, to further investigate the potential occurrence of inflammation in the liver, we tested the alterations in NF-κB activation following the administration of MMA in the rat. NF-κB can be activated by different stimuli including pro-inflammatory cytokines, such as TNF-α[38-42]. Thus, NF-κB is released and translocated to the nucleus upon its activation[43,44]. Accordingly, the nuclear localization of NF-κB is believed to be equivalent to its activation[44]. This is in agreement with current finding of hardly detected NF-κB in the hepatic nuclei of control rats (Fig. 2c and 4).

Previous studies have demonstrated MMA-induced NF-κB activation in osteoclast precursor cells[36]. Additionally, NF-κB activation has been suggested to be involved in the pathogenesis of alcoholic liver disease[37]. Thus, in agreement with those previous reports, these current results reveal hepatic NF-κB activation, as indicated by its increased nuclear expression, following MMA administration (Fig. 2d and 4).

Previous studies have suggested the upregulation of TNF-α and activation of NF-κB mediating inflammation that leads to the pathogenesis of alcoholic liver disease[37,45]. NF-κB is activated by pro-inflammatory cytokines including TNF-α[38]. On the other hand, NF-κB activation induced by stimuli contributes to the production of the pro-inflammatory cytokine TNF-α[18]. Consequently, a positive feedback loop involving TNF-α overproduction and NF-κB activation has been suggested[46]. Consistently, this analysis reveals the hepatic upregulation of TNF-α and activation of NF-κB (Fig. 2, 3 and 4), which indicate the potential occurrence of inflammation subsequent to MMA administration.

Previous reports indicated that MMA caused depletion of reduced glutathione that was associated with increased reactive oxygen species leading to cytotoxicity[9,11]. Additionally, MMA has been reported to be interacting with the mitochondrial membrane leading to structural and functional damage[9]. Subsequently, the oxidant stress in mitochondria can promote extramitochondrial activation of NF-κB that may affect nuclear gene expression leading to the production of many proinflammatory cytokines including TNF-α[12,18]. Furthermore, depleted levels of glutathione were shown to be associated with increased TNF-α production and NF-κB activation[47]. Thus, TNF-α upregulation and NF-κB activation, indicative of the potential occurrence of inflammation, following MMA administration, revealed by these current results (Fig. 2, 3 and 4), were in line with those previous reports[9,11,18,47]. On the other hand, enhancing glutathione synthesis by glutamine was shown to reduce TNF-α production and NF-κB activation in the liver[48].

CONCLUSION

The present data are indicative of an association of MMA administration with TNF-α upregulation and NF-κB activation in the liver, potentially promoting the hepatotoxicity, which is indicated by the ballooning degeneration of hepatocytes.

SIGNIFICANCE STATEMENT

Methyl methacrylate (MMA) has been shown to cause diverse health effects on the liver. The present study is the first to illustrate the association of MMA administration with the upregulation of TNF-α and activation of NF-κB in the liver potentially promoting hepatotoxicity, which was indicated by ballooning degeneration of hepatocytes. Accordingly, the data indicate TNF-α upregulation and NF-κB activation as a potential mechanism that might be involved in the toxic effects of MMA on the liver. Hence, the present data suggest that precautions should be undertaken while handling MMA. Additionally, the present study suggests the need for developing alternative materials to MMA."

ACKNOWLEDGMENT

This study was financially supported by The Deanship of Research at Jordan University of Science and Technology, Irbid, Jordan. Research grant no. 20160079.

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