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Research Article

Increase Hsp90 in Rats' Lungs Exposed to Self-Cure Acrylic Methyl Methacrylate Monomer

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ABSTRACT
Introduction: Methyl Methacrylate (MMA) is a clear, highly volatile flammable- liquid monomer, with unpleasant strong fruity odor. It is an ester compound that results from the reaction between methanol and methacrylic acid. Aim: this study aims to investigate the expression of hsp90, a heat shock protein, in lung tissue of rats exposed to MMA, subsequent to the oral administration of MMA monomer in rats. Methods: 20 Sprague-Dawley rats were randomly selected and divided into two groups: control group, and experimental group. The experimental group was orally administered 120 mg/kg of MMA daily 5 times per week for four weeks. Changes in the expression of HSP90 in the lung tissue were investigated using Immunohistochemistry technique. Results: rat in the experimental group showed statistically significant ($p < 0.001$) increase, by approximately 15 folds, in the expression of HSP90 compared to that in the control group. Conclusion: exposure to MMA increased hsp90 expression in rat lungs, which may explain, at least in part, the inflammatory interactions that are associated with exposure to MMA.

Keywords: Methyl Methacrylate, hsp90, overexpression, lungs, exposure.

INTRODUCTION

Methyl Methacrylate (MMA) is a clear, highly volatile flammable liquid monomer, with unpleasant strong fruity odor. It is an ester compound that results from the reaction between methanol and methacrylic acid[1-4]. MMA is a widely used, high volume synthetic chemical, for example, it is used in the production of plastic, printing colors and paints, glues, floor polishes, synthetic finger nails, soft lenses, surface treatment of leather[5]. Furthermore, MMA is very commonly used in dental prosthetics, neurosurgical and orthopedic surgical procedure[6-9]. Clinical and experimental studies have shown that the exposure to MMA monomers may cause a wide range of adverse health problems such as irritation to eyes, irritation to skin and mucus membrane, allergic dermatitis, asthma, stomatitis, liver toxicity, disturbances of the central nervous system, neuropathy, and changes in blood parameters[10-14]. The primary source of exposure to MMA is occupational exposure, which may occur in chemical industry during manufactures and further processing of MMA and its polymers. Outside the chemical industry, orthopedic surgeons may be exposed to MMA upon direct contact with the MMA monomer, such as during the insertion of prostheses when using acrylic cement, and preparation of the appliances by theater technicians[15]. Furthermore, medical staff may be exposed to MMA fumes during the ultrasonic removal of MMA cement and a volatilized MMA monomer, which may contaminate the medical clinic, operating theater, or laboratory environment[16]. In dentistry, dental technicians become exposed to MMA during making, grinding and finishing acrylic dental prostheses[17]. Patients with acrylic dental prostheses or have an orthopedic surgery may be exposed to residual monomer, which is the unreacted MMA monomer released from the appliances as a consequence of incomplete conversion of MMA monomer to polymer[18,19]. Heat shock protein70 (HSP70) is a member of heat shock proteins (HSPs) family, whose expression is low in cells under normal conditions, and is significantly increased in cells exposed to biological stress such as heat, high pressure, and toxic compounds[20]. Such stresses act to damage proteins, causing their unfolding or aggregation[21]. The HSP90 plays a role in proteins folding by preventing their aggregation, promoting their folding, and refolding the aggregated proteins[22-24].

METHODS

Animals

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Table 1: The expression rates of hsp90 in control and study groups.

	Control group	Study group
	0.099	0.115
	0.010	0.252
	0.07	0.229
	0.12	0.405
	0.116	0.199
	0.123	0.287
	0.113	0.356
	0.142	0.432
	0.109	0.453
	0.097	0.343
Average	0.099	0.3071

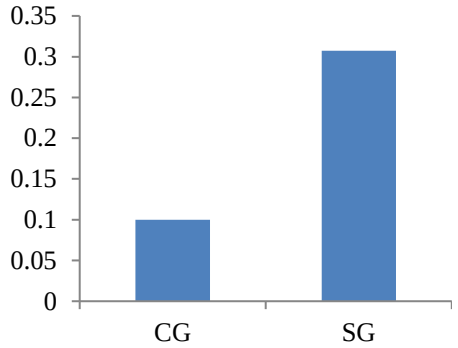

Figure 1: The expression of hsp90 is illustrated in study and control group.

Table 2: The relationship between the expression of hsp90 among study groups.

Variable	Mean	SD	P-Value
Control Group	0.099	0.037	0.000
Study Group	0.307	0.109	

The experimental procedures were approved by the Animal Care and Use Committee (ACUC) of Jordan University of Science and Technology that follow the National Institute of Health Guide for the use of laboratory animals (NIH No 8023). Female Sprague-Dawley rats weighing 230-280g were purchased from the Animal Care Unit at Jordan University of Science and Technology (Irbid, Jordan).The rats were maintained in a 12:12 light/dark cycle and at standard temperature and air moisture. The animals were insulated for 10 days prior to starting the experiment. Rats were given access to clean water and standard rodent food (Sahil-Huran Animal Food Company, Ramtha, Jordan). The rats (n=20) were randomly divided into two groups (n=10); Control Group (Ctr.) and Experimental Group (Exp.). Rats in the Experimental group had oral administration of MMA diluted with water (120mg/kg/day) for four weeks, five days a week.

Tissue preparation and immunohistochemistry staining

At the end of the treatment, animals were sacrificed, and their lung tissues were dissected and subsequently fixed in 10% neutral buffered formalin. Then, the tissue samples were processed and subsequently embedded in paraffin. A 5µm thick paraffin-embedded sections were prepared for immunostaining with anti-HSP90. So the sections were deparaffinized and rehydrated. After that, antigen retrieval was performed by processing the sections in the reveal solution for 2 minutes in the Decloaking chamber. After washing the sections in phosphate buffered saline (PBS), incubated with anti-HSP90 antibody (sc-32239, Santa Cruz Biotechnology, CA, USA), according to the dilutions recommended by the manufacturers. Then, the sections were rinsed off with PBS before and after being incubated with biotinylated secondary antibody. Next, samples were incubated with streptavidin horse radish peroxidase (sc2019, Santa Cruz Biotechnology, CA, USA) for 15 minutes at room temperature and washed with PBS. 3′-Diaminobenzidine[25] was applied until the desired staining intensity was reached. Finally, the slides were washed with tap water to stop the reaction. Negative control sections were processed without the primary antibody (phosphate buffered saline). All sections were then counterstained 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 sections per slide. Ten slides from each animal of all 10 animals in each group were analyzed by counting the total pixels area occupied by positive staining in the lung sections in relation to the total pixels in each field in the sections, using Adobe Photoshop software[26,27]. t-test was used to statistically compare the expression of hsp90 in lung sections among control and study groups. Differences in hsp90 expression were considered statistically significant at p-value <0.05.

RESULTS

The expression of hsp90 in lung tissue of control group and experimental group: Table 1 and Figure 1, the average expression of hsp90 was 0.099, and under the effect of exposure to MMA, the average expression increased in the study group to the level of 0.3071.

The relationship between the expression of hsp90 among study groups

Table 2, shows that the changes in the expression level of hsp90 among study groups are statistically significant (p=0.000).

DISCUSSION

HSP90 functions as part of a multichaperone complex via its association with cochaperones[28]. The main application of HSP90 inhibitors is related to cancer therapy, these drugs are potent inhibitors of certain proinflammatory mediators in different cell types[29]. This study has one major finding when investigating the mechanism of lung toxicity of MMA monomer when administrated orally to a sample of rats, an increased level of hsp90 expression. HSP90 is overexpressed in a wide variety of solid and hematologic malignancies and correlates with a poor prognosis[30]. Over expression of hsp90 is associated with inflammatory conditions[31]. There were no previous studies that associated the overexpression of hsp90 as a consequence of exposure to MMA in lung tissue.

Accordingly, we have unique findings. Our results demonstrated that the exposure to MMA overexpressed the expression of hsp90 significantly compared with control group (p=0.000).

CONCLUSION

The results of the present study demonstrated molecular impacts due to the exposure of MMA through overexpression of HSP90 in the lung tissues of exposed rats.

CONFLICT OF INTEREST

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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