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Original research article

Trimethylamine-N-oxide, as a risk factor for atherosclerosis, induces stress in J774A.1 murine macrophages

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ABSTRACT

Purpose: Trimethylamine N-oxide (TMAO) is a biomarker for kidney problems. It has also been introduced as a risk factor for atherosclerosis. The classic risk factors for atherosclerosis trigger cellular and humeral immunoreaction in macrophages through induction of heat shock protein expressions and increased levels of GRP94 and HSP70 are associated with increased atherosclerosis risk. The present study evaluated the possible effect(s) of TMAO on the expression of GRP94 and HSP70 at protein levels.
Methods: J774A.1 murine macrophages were treated with different micromolar concentrations of TMAO and 4-phenylbutyric acid (PBA), a chemical chaperone, for 8, 18, 24, and 48 h intervals. Tunicamycin was also used as a control for induction of endoplasmic reticulum stress. Western blotting was used to evaluate the expression of GRP94 and HSP70 in macrophages at protein levels.
Result: Tunicamycin greatly increased protein levels of GRP94. Similarly, but to a lesser extent compared to tunicamycin, TMAO also increased GRP94. In 24 h treated cells, only 300 μM of TMAO, and in cells treated for 48 h, all doses of TMAO produced a significant increase in relative HSP70 protein levels compared to the control. PBA failed to induce any changes in GRP94 or HSP70 protein levels.
Conclusion: GRP94 and HSP70 are stress-inducible heat shock protein, so the elevation in J774A.1 murine macrophages can clearly define cells under stress and elucidate the contribution of stress induced by TMAO that may have a part in the abnormal activation of macrophages involved in foam cell formation.

1. Introduction

Atherosclerotic vascular disease, a major cause of death worldwide, is governed by many complex etiological factors that can be classified into three major categories: genetic factors, environmental factors, and another determinant called the “microbiome” [1,2]. The relationship between genetic and environmental determinants and cardiovascular risk have been previously established. However, the study of the latter category of CVD determinants is increasing in magnitude across the globe and has attracted the attention of health care systems of worldwide for the use of new preventive therapeutic strategies [2]. Recently, the research has been focused on its role as commensal intestinal microorganisms (gut flora) in the metabolism of food containing precursors of trimethylamine N-oxide (TMAO) to produce proatherogenic metabolites and the potential consequences for the pathogenesis of atherosclerosis.

In a metaorganismal pathway, TMAO is produced from trimethylamine during liver flavin-containing monooxygenase reactions in many species, including fish, animals, and humans. In this pathway, trimethylamine is derived from dietary choline, phosphatidylcholines (lecithin), or carnitine by gut microbial flora [3,4]. In Wang et al, for the first time, TMAO was introduced as an independent risk factor for CVD [3]. Subsequent studies have also confirmed the role of this metabolite as a risk factor for atherosclerosis and clinical prognostic significance of its circulating level for cardiovascular diseases and any other complications [5–7]. Other studies have also demonstrated that TMAO appears to alter the reverse cholesterol transport (RCT) system and/or other aspects of the cellular metabolism of cholesterol to help with foam cell formation and the promotion of atherosclerosis and other vascular events [6]. Warrier et al have suggested that the gut microbiota-driven TMA/FMO3/TMAO pathway plays a key role in regulating lipid metabolism and inflammation [8]. TMAO circulating levels can also be used as a biomarker for kidney problems as its levels become elevated in the plasma of subjects with end-stage

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renal disease [9]. Moreover, TMAO is known as an important natural osmolyte to upregulate and/or induce a conformational change in heat shock proteins 70 and 90 [10,11].

Heat shock proteins (HSPs) belong to a group of highly conserved proteins in prokaryotes and eukaryotes. They are based on molecular weights as follows: HSP10, HSP40, HSP60, HSP70, HSP90, and HSP110 [12–14]. These proteins play a protective role under physiological conditions, but extracellular HSPs act as a "danger signal" for atherosclerosis and through some cell surface receptors that activate immunoinflammatory reactions [12,13,15,16]. Stress-inducible HSP70 is produced by all the cell types susceptible to atherosclerotic lesions including, aortic endothelial cells, macrophages, and smooth muscle cells [17]. GRP94 or Endoplasmin also known as gp96, ERp99, and TRA-1 are endoplasmic reticulum (ER) HSPs that have been implicated in the folding of proteins in the secretory pathway such as Toll-like receptors, integrins, and facilitates ER-associated degradation for misfolded proteins [18]. Its elevation is used as a marker for induction of ER stress and unfolded protein response activation [19,20].

Induced ER stress due to different risk factors is believed to correlate with all stages of atherosclerosis and is characteristic of lipid-rich macrophages in the atherosclerotic lesions [21–24]. Moreover, ER stress induction has been identified as an important inducer of abnormal macrophage activation, pathological inflammatory response, and, eventually, macrophage apoptosis, which all play important roles in atherosclerosis [25]. Therefore, considering the proatherogenic potential of TMAO as a risk factor, in this study, we measured GRP94 and HSP70 at protein level in response to trimethylamine N-oxide treatment in J774A.1 murine macrophage cell line to evaluate possible TMAO-induced stress.

2. Methods

2.1. Cell culture and treatments

Cell culture and treatments were performed as previously described [2]. J774A.1, a tumoral murine macrophage cell line (Pasture Institute, Tehran, Iran) were cultured in DMEM (Sigma) containing 10% fetal bovine serum (FBS, Sigma), 1% penicillin–

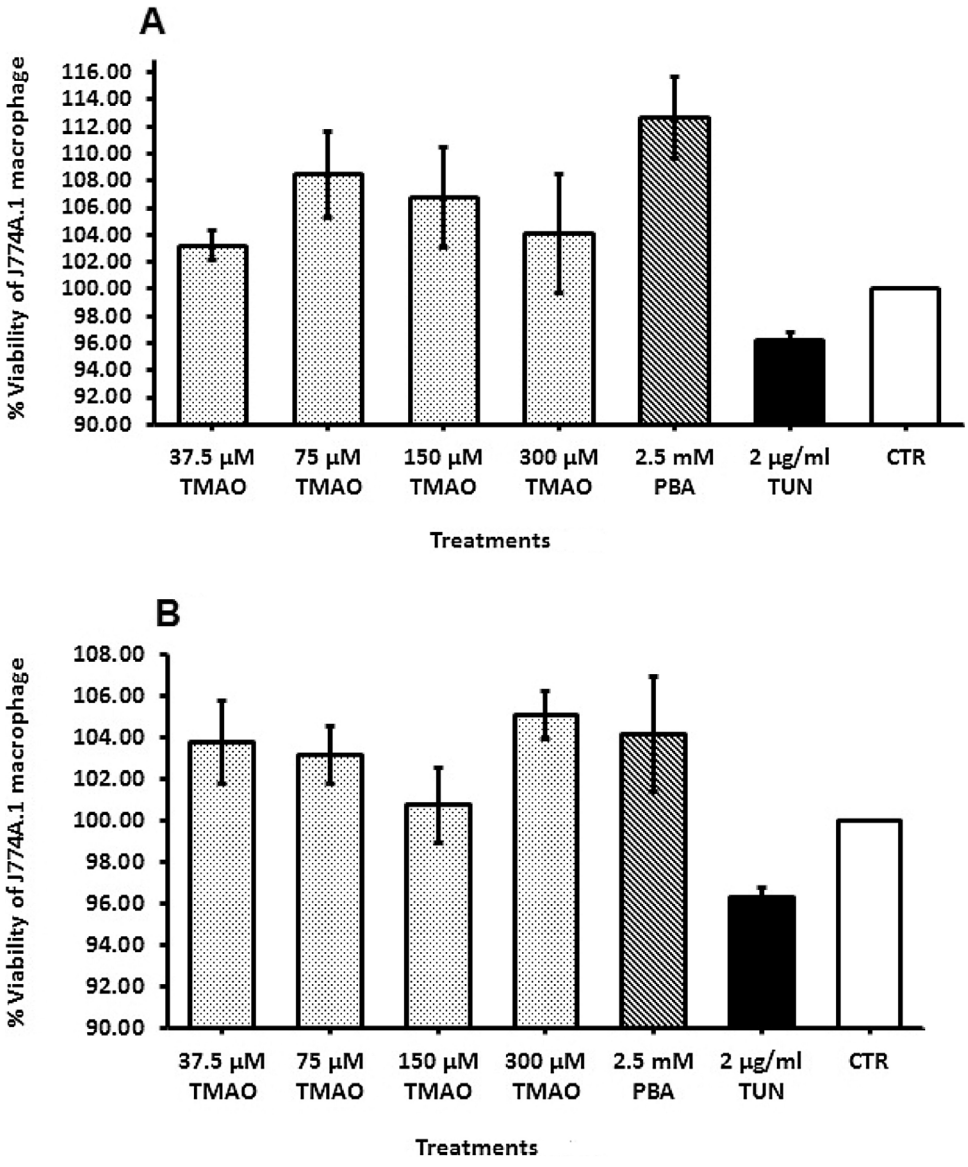

Fig. 1. The result of the MTT test for cell viability of J774A.1 murine macrophage cell line in different treatment and time intervals. A: Treatment 8 h. B: Treatment 18 h C: Treatment 24 h. D: Treatment 48 h. Values are mean ± standard error of six separate measurements.