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Neuroprotective effect of tetramethylpyrazine against all-trans-retinal toxicity in the differentiated Y-79 cells *via* upregulation of IRBP expression

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ABSTRACT

It is estimated that abnormal accumulation of all-trans-retinal (atRAL) is a leading cause of photoreceptor degeneration in retinal degenerative diseases. Deficiency of interphotoreceptor retinoid-binding protein (IRBP), a retinoid transporter in the visual cycle, is responsible for the impaired clearance of atRAL and results in atRAL toxicity in retina. Therefore, IRBP has been proposed to be a potent target in preventing atRAL-induced photoreceptor degeneration. In this study, the neuroprotective effect of tetramethylpyrazine (TMP) against atRAL toxicity in the differentiated Y-79 cells, a *in vitro* model of photoreceptor, was first investigated. Our findings showed that atRAL could induce cytotoxicity, oxidative/nitrosative stresses, apoptosis and leukostasis in the differentiated Y-79 cells; however, the pre-treatment of TMP significantly attenuated such effects in a dose-dependent manner. Furthermore, our results indicated that TMP exerted its neuroprotective effect mainly through upregulating IRBP expression. The present study significantly contributes to better understanding the important role of IRBP in retinal degenerative diseases and forms the basis of the therapeutic development of TMP in such diseases in the future.

1. Introduction

In the eye, photoreceptor (PR) is responsible for detecting light and initiating the biochemical signal that is eventually transmitted to the brain [1]. Degeneration of PR induced by genetic mutation or environmental factors is the main cause of many retinal degenerative disorders, which eventually results in human adult blindness [2,3]. All-trans-retinal (atRAL) is a major modulator in visual cycle, and impaired clearance of which can lead to retinal degeneration, particularly PR cell death [4,5]. Chen et al. have reported that high levels of atRAL are associated with photoreceptor degeneration in the mouse models of Stargardt disease and Age-related macular degeneration [6]. Cia et al. have shown that atRAL accumulation contributes to photoreceptor cell death *via* inducing oxidative and carbonyl stress [7]. Therefore, prevention of atRAL-induced retinal toxicity can potentially slow down the pathological process of PR degeneration and reduce the incidence of retinal degenerative diseases.

Existed evidence has estimated that deficiency of either ATP-binding cassette transporter (ABCA4) or all-trans-retinol dehydrogenase (RDH) in photoreceptor cells is the major cause of abnormal accumulation of atRAL [8–10]. ABCA4 transports atRAL from the disc lumen back into the cytoplasm [6]. RDH8 is one of the main enzymes that reduces atRAL in rod and cone outer segments [6]. Recently, several studies have indicated that mutations in interphotoreceptor retinoid-binding protein (IRBP), a retinoid transporter in the visual cycle, are associated with retinal diseases and vision impairment [11–13]. Interphotoreceptor retinoid-binding protein (IRBP) is the most abundant protein in the interphotoreceptor matrix (IPM) and is known to bind with 11cRAL, atRAL, 11cROL and atROL in IPM [14]. Lee et al. have demonstrated that IRBP is required for preventing the excessive accumulation of retinal atRAL in *in vitro* and *in vivo* models, and addition of IRBP can protect photoreceptors from tumor necrosis factor (TNF) activation, oxidative stress and nitrosative stress caused by atRAL [15]. These findings indicated the protective effect of IRBP against atRAL toxicity in photoreceptors, which is a potential target for the treatment of retinal degenerative diseases.

Tetramethylpyrazine (TMP) is a purified and chemically identified component of *Ligusticum wallichii Franchat* (ChuanXiong), a Chinese herb that is widely used in the treatment of neurovascular and cardiovascular diseases [16–18]. In recent years, the neuro-protective effect of such compound in retinal diseases has been reported by a number of *in vitro* and *in vivo* studies [19,20]. Yang et al. have suggested that TMP protects retinal cells through multiple pathways

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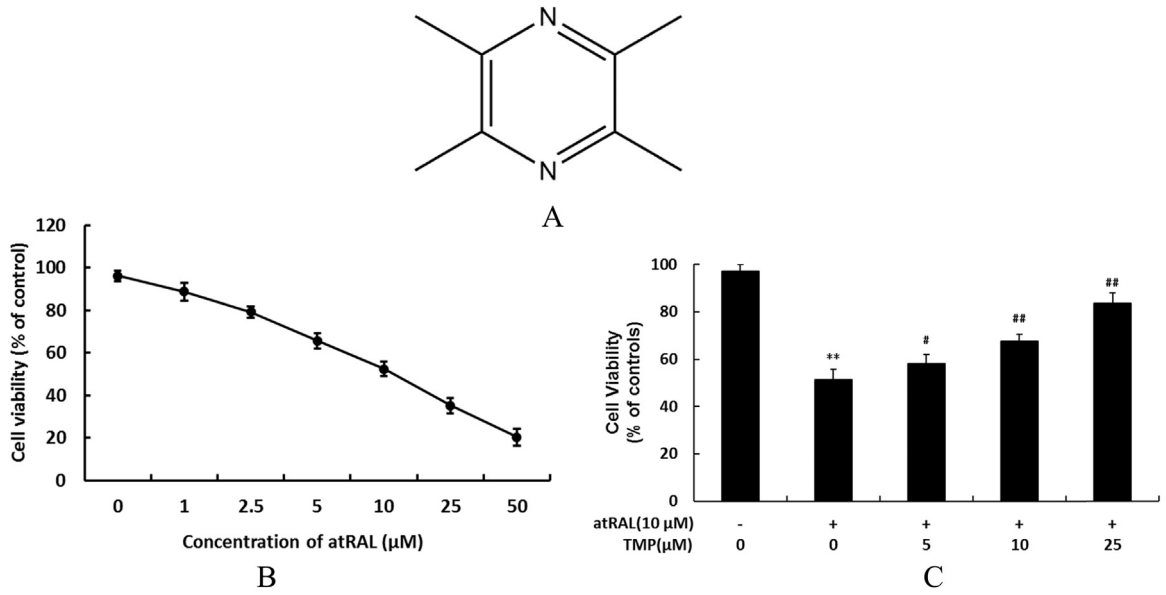

Fig. 1. The neuroprotective effect of TMP against atRAL-induced cytotoxicity in the differentiated Y-79 cells. (A) The chemical structure of TMP. (B) Cells were treated with atRAL (1–50 μM) for 24 h and cell viability was determined by MTT assay. (C) Cells were pre-treated with TMP (0, 5, 10 and 25 μM) for 4 h and then exposed to 10 μM atRAL for 24 h. After that, cell viability was determined by MTT assay. All data were expressed as mean ± SD of three experiments and each experiment included triplicate repeats. $^{**}p < 0.01$ *vs.* control group; $\#p < 0.05$, $\#\#p < 0.01$ *vs.* atRAL-treated group. TMP: tetramethylpyrazine.

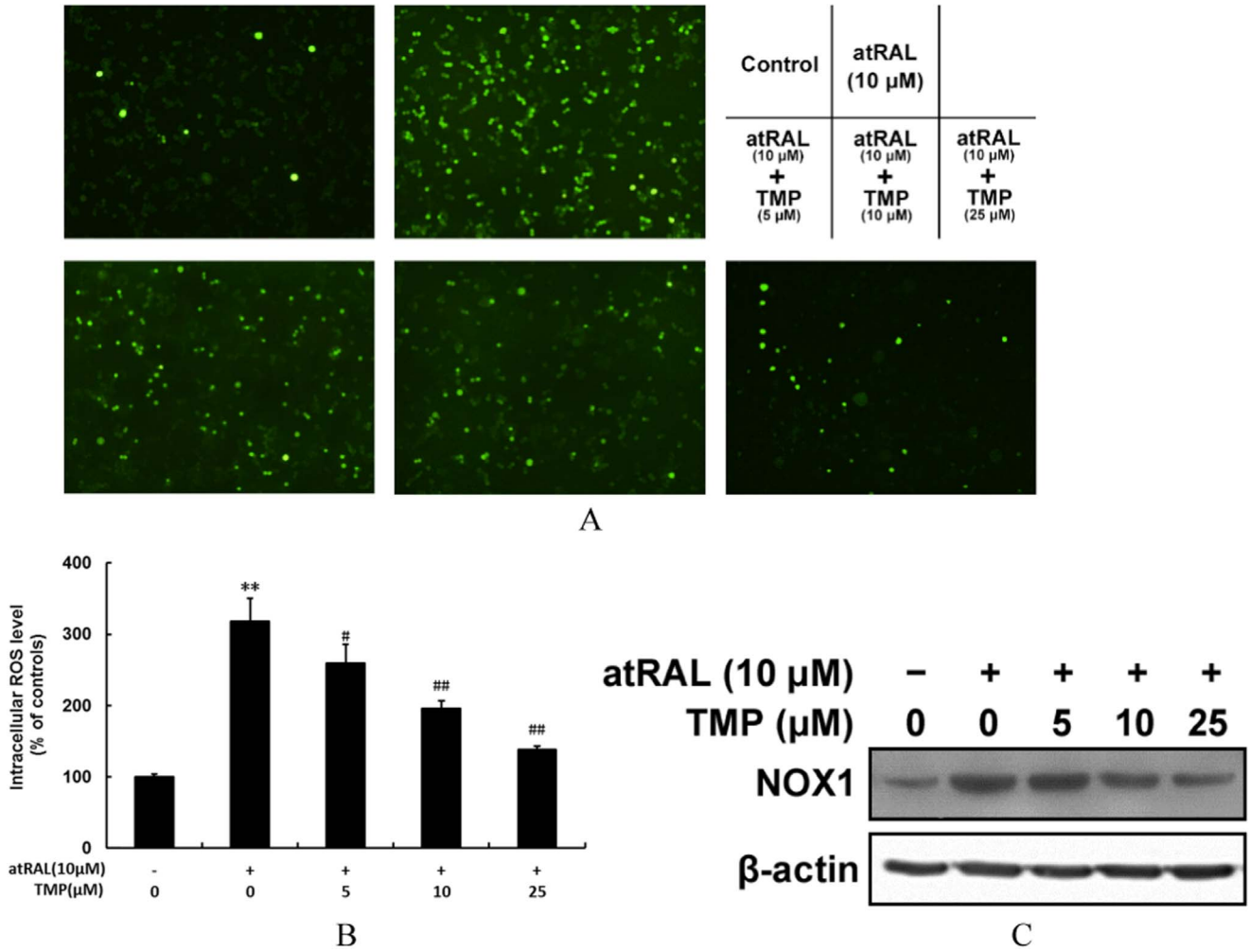

Fig. 2. The neuroprotective effect of TMP against atRAL-induced oxidative stress in the differentiated Y-79 cells. Cells were pre-treated with TMP (0, 5, 10 and 25 μM) for 4 h and then exposed to 10 μM atRAL for 6 h. (A) Representative images of fluorescence staining by DCFH-DA. (B) The relative fluorescence intensity was analyzed by flow cytometry. (C) The protein level of NOX1 was assessed by western blot analysis. All data were expressed as mean ± SD of three experiments and each experiment included triplicate repeats. $^{**}p < 0.01$ *vs.* control group; $\#p < 0.05$, $\#\#p < 0.01$ *vs.* atRAL-treated group. TMP: tetramethylpyrazine.