

# Influence of geranylgeranylacetone on the expression of HSP70 in retina of rats with chronic IOP elevation

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## Abstract

• **AIM:** To study the effects of geranylgeranylacetone (GGA) on the expression of heat shock protein70(HSP70) on retinal ganglion cells (RGC) in rats with chronic intraocular pressure(IOP) elevation.

• **METHODS:** Seventy Wistars were divided into blank control group (10 rats), chronic hypertension group (30 rats) and GGA group(30 rats). Chronic hypertension was created by cauterizing the superficial scleral veins. 800mg/(kg·d)GGA was given by oral daily after cauterization. Immunohistochemistry was used respectively to observe the changes of expression of HSP70 in the model rats and GGA interference rats at different time points during the course of chronic IOP elevation.

• **RESULTS:** The successful model was identified as the IOP over 40% of normal rats. The retinal thickness was significantly reduced in model group and model + GGA group compared with normal rats from 21 days through 28 days after cauterization( $P < 0.05$ ), and that of model rats was obviously decreased in comparison with model + GGA rats( $P < 0.05$ ). The number of ganglion cells was significantly decreased in model rats and model + GGA rats compared with normal rats from 21 days and 28 days. The stronger expression intensity (IOD) value was seen for HSP70 in the model + GGA rats by immunochemistry ( $P < 0.01$ ).

• **CONCLUSION:** Systemic administration of GGA protects retina from chronic IOP elevation by regulating the expression of HSP70.

• **KEYWORDS:** retina; chronic hypertention; geranylgeranylacetone; heat shock protein70; retinal ganglion cell

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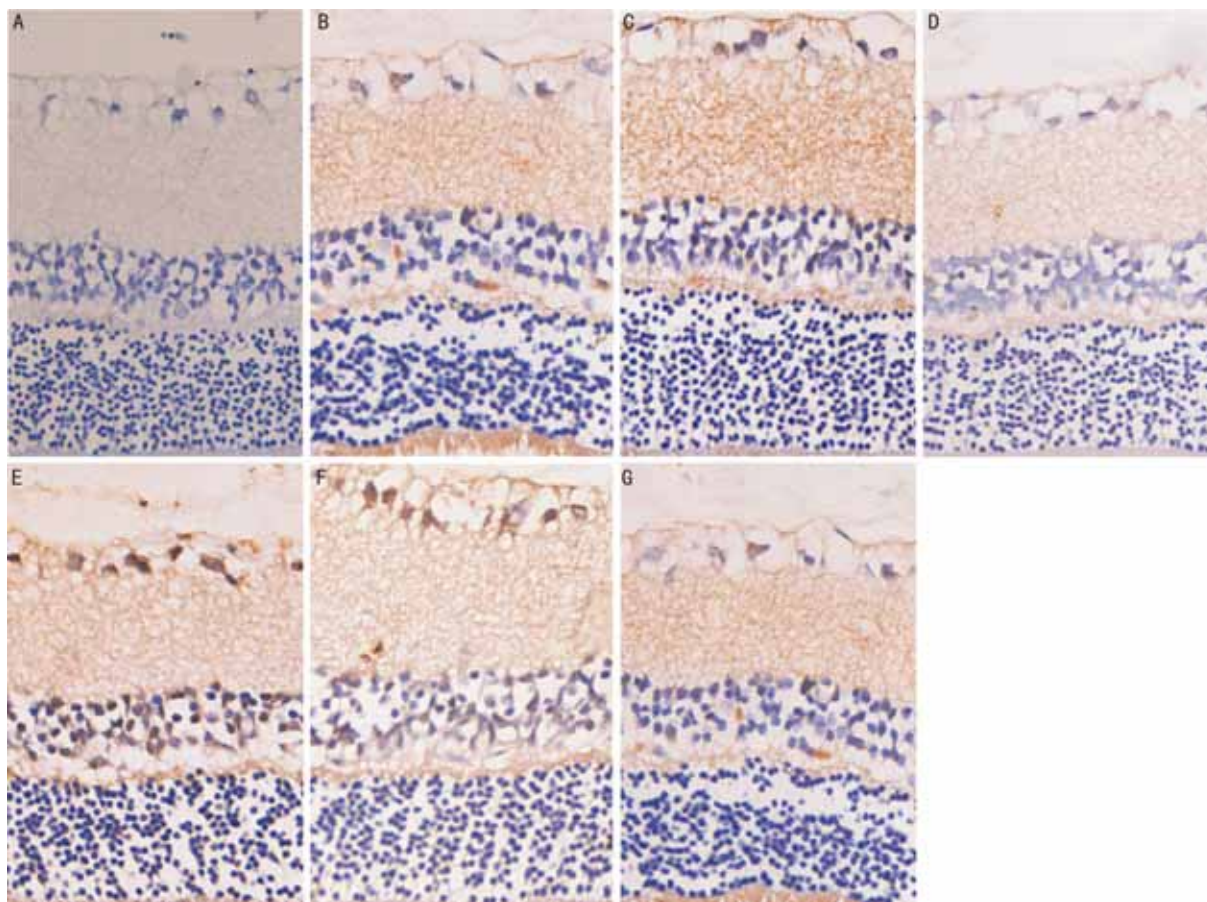
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## INTRODUCTION

Glaucoma, one of the world's leading causes of blindness, is characterized by progressive optic nerve damage with selective loss of retinal ganglion cells (RGC). It has been postulated that apoptosis, a highly regulated process of cell death, is the final common pathway for RGC in glaucoma which is not yet known. Therefore, it is very important to investigate how these apoptosis-related genes are involved in the apoptosis of RGC at different levels and how they are regulated, so that we can provide better treatment for patients. The heat shock protein70 (HSP70) family, classified according to molecular mass (70kDa), comprises several members, such as the inducible, the constitutive, the mitochondrial, and the endoplasmic reticular forms. Intracellular expression of HSP70 has been demonstrated to protect cerebral neurons against heat shock, oxidative stress, apoptotic stimuli, excitotoxic insults<sup>[1,2]</sup>, and ischemia-like conditions<sup>[3]</sup>. Neurons of transgenic mice expressing HSP70<sup>[4]</sup> or those of rats injected with the herpes virus containing HSP70 genes<sup>[5]</sup> also have been shown to be resistant to ischemia and seizures, which suggests that HSP70 is essential for neuroprotection. Geranylgeranylacetone (GGA) is a unique antiulcer drug that protects gastric mucosa without affecting gastric acid or pepsin secretion. Its cytoprotective effects have been found to correlate with the expression of HSPs in gastric mucosal cells induced by the systemic administration of GGA<sup>[6]</sup>. GGA induces HSPs in numerous tissues including rat small intestine, liver, lung, kidney, and heart<sup>[7]</sup>. We investigate in the current study whether HSP70 is induced in rat RGC with systemic administration of GGA, whether the induction of HSP70 by GGA enhances RGC survival of a rat glaucoma model. We performed this experiment to reveal the protection of glaucoma to some degree, and we look forward to applying our findings to clinical treatment.

## MATERIALS AND METHODS

**Materials** Seventy male Wistar rats, weighing between 200-300g were supplied by experiment animal department of China Medical University. The animals and experimental conditions followed laboratory animal regulations of State Science and Technology Commission. The animals were randomly divided



**Figure 1 HSP70 expression on retina of rats with chronic IOP elevation** A: Control; B: 3 days after IOP elevation; C: 7 days after IOP elevation; D: 21 days after IOP elevation; E: GGA + 3 days of high IOP; F: GGA + 7 days group of high IOP; G: GGA + ocular hypertension for 21 days

into 3 groups, which were 10 in blank control group (20 eyes), 30 in chronic IOP elevated group (60 eyes), and 30 in chronic IOP elevated with GGA application group (60 eyes). Mouse anti-rat HSP70 (Santa Cruz Biotechnology, INC.), and SP kit (Fuzhou Maixin Biotechnology) were pure.

**Methods** Rat model of chronic IOP elevation. The rats were anesthetized by intraperitoneal injection of 100g/L chloral hydrate 3mL/kg, bulbar conjunctiva was cut and two superficial venous tributaries were burnt (signs of successful burn showed episclera venous blood flow disappeared on the distal end of the burnt point, distension and darkness of the vessels near comeoscleral limbus); bulbar conjunctiva was reset with Tobradex drops and pasted as eyedrop application. IOP was also measured with Tonopen XL, and the measurement time points were before the operation, half an hour after the operation, the 1<sup>st</sup> day, the 3<sup>rd</sup> day, the 7<sup>th</sup> day, the 14<sup>th</sup> day, the 21<sup>st</sup> day and the 28<sup>th</sup> day. IOP that is 40% beyond preoperative value (9-16mmHg) means the modeling was successful. To group with GGA application GGA (Tokyo, Japan) was given after modeling with 800mg/(kg · d). The modeling procedure was all the same.

**Immunohistochemistry** After sampling fixation, dehydration and paraffin imbedding were performed according to the

instruction of the kit. Positive cells were those with yellow or brownish-yellow granules deposited in cytoplasm or nuclei. We selected 5 discontinued high power fields from each section to assess the expression intensity with etaMorph/BX51 microgram analytical system through data analysis of the determination of integrated absorbance of positive cells.

**Statistical Analysis** Analysis was done with SPSS 13.0. The data were expressed as the mean ± SEM. Mean data among groups were compared with one-way ANOVA, and data between groups were compared with the unpaired Student's *t*-test. Statistical significance was set at  $P < 0.05$ .

## RESULTS

**RGC Change and Thickness of Nerve Fiber Layer** On 21<sup>st</sup> day after modeling the retina became thinner and the number of RGC decreased, however, the change of medication group was relatively mild. Among three groups at least two of them had distinctive difference according to the analysis by Levene homogeneity test for variance. Inter-group multiple comparison test indicated that, in terms of thickness comparison, compared with control group the variances of other two groups both had statistical significance, thickness comparison between model group and model + GGA group had statistical significance ( $P < 0.05$ , Table 1).

**HSP70 Expression** It is illustrated in Figure 1. Control

**Table 1 Retinal thickness of rats with chronic IOP elevation** ( $\bar{x} \pm s, \mu\text{m}, n = 10$ )

Group	Thickness of RGCL and NFL in respective time	
	21d	28d
Control	27.5 ± 9.7	25.7 ± 6.4
Model	17.4 ± 5.8 <sup>a,c</sup>	16.6 ± 7.1 <sup>a,c</sup>
Model + GGA	22.3 ± 7.3 <sup>a</sup>	20.4 ± 5.5 <sup>a</sup>

<sup>a</sup>*P* < 0.05 vs control; <sup>c</sup>*P* < 0.05 vs model + GGA

**Table 2 HSP70 expression in rat retina** ( $\bar{x} \pm s, IA, n = 10$ )

Group	IOD					
	1d	3d	7d	14d	21d	28d
Model	31.7 ± 4.7	26.5 ± 3.7	16.8 ± 1.8	16.0 ± 2.8	14.5 ± 1.0	15.0 ± 0.5
Model + GGA	36.0 ± 5.2	48.5 ± 2.7 <sup>b</sup>	67.9 ± 6.3 <sup>b</sup>	40.7 ± 3.2 <sup>b</sup>	32.5 ± 2.4 <sup>b</sup>	16.9 ± 1.0

<sup>b</sup>*P* < 0.01 vs 3d

group with only trace quantity of positive expression was merely detected in layer of ganglion cells in the retina of normal rats. HSP70 was expressed in the ganglion cell layer in ocular hypertension 3 days group. The positive immune cells for HSP70 seemed to be reduced in retina of high IOP for 7 days group. In ocular hypertension for 21 days group, the expression of HSP70 in retina was significantly reduced. In GGA + 3 days group of high IOP, the number of round syncytium big positive cells for HSP70 in ganglion cell layer began to increase. HSP70 was positively expressed in the ganglion cell layer in GGA + 7 days group of high IOP. In GGA + ocular hypertension for 21 days, the positive ganglion cells for HSP70 reduced (Figure 1, Table 2).

## DISCUSSION

Beere *et al*<sup>[8]</sup> have proposed that HSP70 is an antiapoptotic chaperone protein that may interfere with multiple stages of the apoptotic pathway. These stages include suppression of c-Jun N-terminal kinase (JNK) activation, prevention of cytochrome c release, disruption of apoptosome formation, inhibition of apoptotic protease activating factor (Apaf)-1 oligomerization, and suppression of procaspase recruitment<sup>[8,9]</sup>. More recently Ikeyama *et al* demonstrated that GGA induces a rapid accumulation of HSP70 mRNA and HAP70, suppresses hydrogen peroxide- and ethanol-induced phosphorylation of JNK, and interferes with caspase-9 and subsequent activation of caspase-3-like proteases in rat hepatocytes. It appears likely that HSP70 plays an important role in promoting the survival of RGC.

In this study, HSP70 expression was induced in rat RGC by systemic administration of GGA, a heat shock protein inducer. We further demonstrated the protective effects of GGA on RGC survival in a rat glaucoma model. This is the first report to demonstrate that GGA induces HSP70 in retinal neurons in China, and provides evidence of a neuroprotective effect for RGC in a rat glaucoma model. GGA is used clinically for the treatment of gastric ulcers and gastritis. There are several published reports that show GGA to be

cytoprotective in other organs such as lung, heart, liver, and kidney. Our results indicate that the neuroprotective effects of GGA may be related to the expression of HSP70.

We administered GGA orally to the rats daily and demonstrated that there was an increased expression of HSP70 in RGC after 3 days, with no observable side effects. And HSP70 is positively expressed in the ganglion cell layer after 7 days. The mechanism of HSP induction by GGA is not clearly understood, but it is likely that GGA activates HSF1, a transcription factor that stimulates synthesis of mRNA for HSP70<sup>[10]</sup>. In our study, with the application of GGA, the degree of retina thinning diminished compared with model group (*P* < 0.05) was significantly inhibited; and the expression of HSP70 in retina of rat with chronic IOP elevation was positively induced. Immunohistochemical method showed that HSP70 IOD was induced and through statistical analysis the difference was significant (*P* < 0.05). Our study confirms that the systemic administration of GGA positively induces the expression of HSP70 in RGC and confers protection to RGC in rats with chronic IOP elevation.

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## 替普瑞酮对大鼠慢性高眼压视网膜 HSP70 表达的影响

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### 摘要

**目的:**研究慢性高眼压大鼠视网膜 HSP70 表达的变化及替普瑞酮的影响。

**方法:**Wistar 大鼠 70 只随机分为 3 组:空白对照组 10 只;慢性高眼压模型组 30 只;慢性高眼压模型 + GGA 组 30 只。采用烧灼巩膜浅层静脉的方法制备大鼠慢性高眼压模型。模型成功后给予替普瑞酮 800mg/(kg·d) 每日灌胃。应用免疫组织化学方法观察应用及未应用替普瑞酮的慢性高眼压大鼠视网膜不同时点的差异及 HSP70 表达的变化。

**结果:**平均眼压高于正常眼压 40% 的手术眼为造模成功。与正常对照组相比,慢性高眼压大鼠应用与未应用替普瑞酮者,视网膜均随着高眼压时间的延长逐渐出现形态学变化,于高眼压的 21d 和 28d 视网膜变薄,节细胞数量减少;在此过程中,HSP70 表达增多。慢性高眼压大鼠视网膜应用替普瑞酮者与未应用替普瑞酮者相比,其形态学变化较小,而 HSP70 表达则明显增多,两者差异具有统计学意义( $P < 0.01$ )。

**结论:**替普瑞酮通过上调 HSP70 表达发挥对慢性高眼压视网膜的保护作用。

**关键词:**视网膜;慢性高眼压;替普瑞酮;热休克蛋白 70;视网膜神经节细胞