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# N-acetylcysteine and Taurine inhibit hyperoxia-induced cataract in rabbit lens

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## Abstract

• AIM: To investigate the efficacy of N-acetylcysteine (NAC) and taurine (Tau) in preventing hyperoxia-induced the lens opacification and the changes of biochemical parameters on organ cultured rabbit lenses.

• METHODS: Twenty-four lenses from adult rabbits were divided into the control group, the hyperoxia-exposed group, the hyperoxia-exposed group containing 20mmol/L of NAC, the hyperoxia-exposed group containing 80mmol/L of Tau, respectively. The treated groups incubated with hyperoxia ( $pO_2 > 80\%$ ) for 4 hours per day throughout a 7-day period. Lens transparency, histology and enzymatic activities measurements were determined after this incubation.

• RESULTS: Gross morphological examination of these lenses revealed some severe cortical opacification in the hyperoxia-exposed group, moderate cortical opacification in the control group and the Tau treated group. There was minimal cortical opacification in the NAC treated group. The glutathione (GSH) content and the activity of Na, K-ATPase were significantly decreased in the hyperoxia-exposed group than that of the control group, by 37.8% ($P<0.05$) and 53.5% ($P<0.05$), respectively. However, they were increased in the two treated groups, especially in the NAC treated group. There were no significant differences in the water-soluble protein content and the catalase and GSH reductase activities in all group lenses.

• CONCLUSION: Hyperoxia can induce the cortical opacification in the lens. The role of NAC in the prevention of hyperoxia-induced cataract is superior to Tau.

• KEWORDS: N-acetylcysteine; taurine; hyperoxia; lens; rabbit

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## INTRODUCTION

Oxidative stress is thought to be one of the underlying factors of most cataracts [1,2]. Loss of protein sulfhydryl groups, oxidation of methionine residues and damaged of enzymatic antioxidant defence system were induced by oxidative stress [3]. N-acetylcysteine (NAC) and Taurine (Tau) were known as antioxygen agents, which had potential biological and therapeutic significance against cataract. Based on our previous studies of NAC [4-6] and Tau [7], the aim of present study was to further investigate whether NAC and Tau can prevent the lens damages induced by hyperoxia.

## MATERIALS AND METHODS

**Materials** NAC and Tau were purchased from Sigma. Rabbits were provided by Animal Laboratories of the Fourth Military Medical University, Xi'an, China. Protein and enzyme quantification kits were obtained from Jiancheng Biol Co (Nanjing). All other chemicals and solvents were analytic grade and obtained commol/Lercially from local companies.

**Methods**

**Hyperoxia treatment** All the lenses isolated from adult rabbits were immol/Lersed in Dulbecco's Modified Eagle Medium (DMEM, Sigma) and incubated for 8 hours. Only the intact clear lenses were chosen for the further experiment. In the experiment, each lens was cultured in 5mL of medium having a liquid-gas interphase surface area of 3.6cm$^2$ under 37℃, 50mL/L $CO_2$. The selected clear lenses were divided into four groups: the control group; the hyperoxia-exposed group; the 20mmol/L NAC treated group and the 80mmol/L Tau treated group. Except the control group was cultured conventionally, other groups were incubated with hyperoxia (more than 80%) for 4 hours per day throughout a 7-day period. All lenses were subjected to gross morphological examination daily. In addition, after the 7-day incubation period, quantitative analyses of enzyme activities and water-soluble protein were performed for the lenses of all groups.

**Morphological examination of the lenses** This was performed by gross examination as well as under the magnification of a dissecting microscope against a background of black gridlines. The degree of opacification was graded based on Geraldine described as follows: grade 0, absence of opacification (gridlines clearly visible); grade 1, slight degree of opacification (minimal clouding of gridlines and gridlines still visible); grade 2, diffuse opacification involving almost the entire lens (moderate clouding of gridlines and gridlines faintly visible); grade 3, extensive thick opacification involving the entire lens (total clouding of gridlines and gridlines not seen at all) [8].

**Protein determination** Protein concentration was determined by Coomassie brilliant blue method using protein assay kit. All lenses were ground in 9g/L physiological saline (1∶9) and homogenized by a hand-held homogenizer for 15 minutes over ice and then centrifuged (3 000r/min, 10minutes) in Eppendorf tubes. The clear supernatant was used for water-soluble protein determination, which was according to the method described with the kits (Jiancheng, China).

**Glutathione determination** The content of GSH in each lens was determined with 5-5' dithio-bis-(2-nitrobenzoic acid) following centrifugation (3 500r/min) in 2mL of 100g/L trichloroacetic acid for 10 minutes and measured using the colorimetric method at 25℃ and 412nm.

**Assay of Na, K-ATPase activity** Na, K-ATPase activity was measured by adding the clear supernatant of homogenate to a buffer containing 100mmol/L NaCl, 20mmol/L KCl, 5mmol/L $MgCl_2$, 3mmol/L ATP and 50mmol/L Tris, pH 7.4. After 15 minutes preincubation at 37℃, ATP was used as the substrate and the liberated inorganic phosphate was estimated by spectrophotometric estimation. Ouabain was used as a specific blocker of Na, K-ATPase activity and the ouabain sensitive ATPase activity was estimated and expressed as micromoles of inorganic phosphate released per mg protein per hour.

**Assay of catalase activity** Catalase (CAT) activity was determined by the method of Beers *et al* with modification by spectrophotometric recording of the cleavage of $H_2O_2$ at 240nm. The activity of catalase was expressed as U/mg protein.

**Assay of glutathione reductase activity** Glutathione reductase (GR) activity was measured according to the procedure of Bergmeyer. The activity of GR was expressed as U/g protein.

**Statistical Analysis** One-way ANOVA was used for testing statistical significance between groups. The median calculation of the lens opacity for each group was analyzed by using the Wilcoxon rank sum test. $P<0.05$ was considered significant. All the data were dealt by the SPSS 13.0 statistical package.

## RESULTS

**The Grading of Lenses** After seven days, all 6 lenses in the hyperoxia-exposed group exhibited total cortical opacification (Grade 3). In contrast, only few lenses revealed less opacification in the control group and the Tau treated group, and minimal opacification in the NAC treated group.

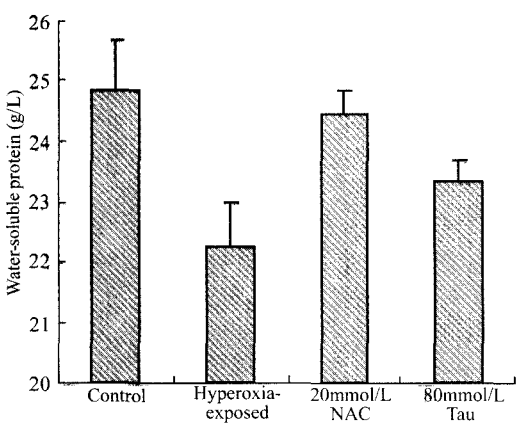


**Figure 1 The water-soluble protein in rabbit lenses**

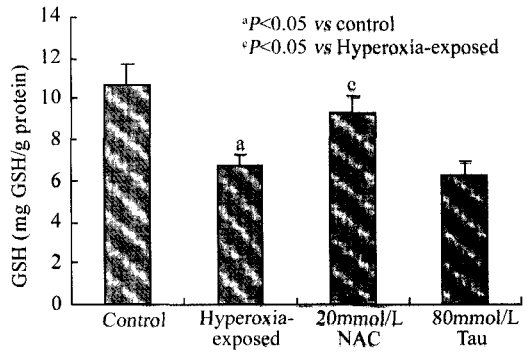


**Figure 2 The GSH content in rabbit lenses**

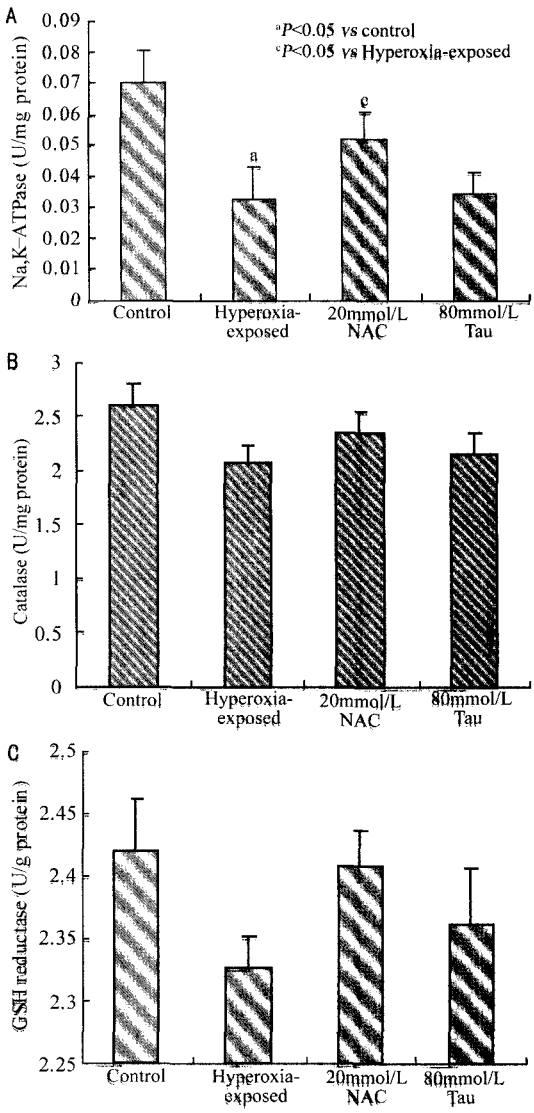


**Figure 3 The activities of enzymes in rabbit lenses**

**Protein Determination** There was a reduction of the water-soluble protein concentration in the lenses of the hyperoxia-exposed group than that in the control group, about decreased by 10.4%. It increased in the NAC treated group by 9.9% and in the Tau treated group by 5.0%, respectively (Figure 1).

**Glutathione determination** The level of GSH was significantly lower in the hyperoxia-exposed group than that in the control group, by 37.8% ($P<0.05$). Surprisingly, NAC enhanced glutathione (GSH) levels about 38.1% ($P<0.05$, Figure 2).

**The activities of enzymes** The activity of Na, K-ATPase was significantly lower in the hyperoxia-exposed group than that in the control group, by 53.5% ($P < 0.05$). It was increased in the NAC treated group and the Tau treated group, by 40.9% ($P < 0.05$) and 5.6%, respectively (Figure 3A). There was a reduction in Cat activities in the lenses exposed to the hyperoxia without NAC treatment when compared to the control lenses, about decreased 21.0%. Compared with the hyperoxia-exposed group, the activities of Cat were increased in the two treated groups, by 13.7% and 6.0%, respectively. However, there were no statistically significant differences in all groups ($P > 0.05$, Figure 3B). The activity of GR was not decreased dramatically in the hyperoxia-exposed group, and not increased in the two treated groups ($P > 0.05$, Figure 3C).

## DISCUSSION

The current results presented here provided evidence to support that NAC and Tau can protect the damages induced by hyperoxia. It's well known that the GSH system plays a key role in the protection against oxidative stress, which is quantitatively the most important endogenous rechargeable antioxidant and functions as an essential antioxidant vital for maintenance of the tissue's transparency [9]. Decreased GSH is found in many cataractous lenses.

NAC, a precursor of glutathione, has been used effectively to replenish intracellular glutathione stores directly and conveniently. It is an excellent source of sulfhydryl (-SH) groups, and is converted in the body into metabolites capable of stimulating GSH synthesis. Tau is an important non-enzymatic system antioxidant in the lens. It plays a critical role in maintaining the normal metabolism of lens and maintaining its transparence [7]. The mechanism of its anti-oxidative effect is to protect lens from oxidative injury mainly by resisting lipid peroxidative reaction. Further research shows that Tau may react with hypochlorous acid or hypochlorous acid metal compound to eliminate the redundant hydroxyl free radical, and inhibit cell edema by adjusting osmotic pressure at the same time[10]. NAC and Tau have different effects on lens damages induced by hyperoxia. Therefore, the GSH content and all enzymes activities were distinguishable.

Our present study has demonstrated that oxidative injury induced by hyperoxia could lead to the opacification of lens. All measured parameters showed the effect of the NAC was superior to Tau. And these results are the first report on a possible role for NAC in the prevention of the hyperoxia-induced damages in rabbit lens. It appears that the protective effects of NAC against oxidative lenticular damage are evidently, through its favorable effect on GSH, catalase and Na, K-ATPase activities. In conclusion, these results of the present investigation suggest that NAC is superior than Tau to significantly retard experimental hyperoxia-induced lens damages and provides futher evidence for the research of NAC.

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## NAC 和牛磺酸对高氧诱导的离体兔晶状体混浊的抑制作用

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**摘要**

**目的**:研究高氧环境下N-乙酰半胱氨酸(NAC)和牛磺酸(Tau)对离体兔晶状体的作用。

**方法**:新鲜兔晶状体24只随机分为对照组、高氧组、20mmol/L NAC处理组和80mmol/L Tau处理组。除对照组正常培养外,其余各组培养在高氧环境(氧浓度>80%)下4h/d×7。每天观察其透明度,培养7d后检测各项生化指标。

**结果**:培养7d后高氧组晶状体出现重度皮质混浊,正常组和80mmol/L牛磺酸处理组中度皮质混浊,20mmol/L NAC处理组仅轻度皮质混浊;高氧组与对照组相比谷胱甘肽含量及Na, K-ATPase活性分别下降了37.8%($P<0.05$)和53.5%($P<0.05$),两药物处理组均有不同程度升高,20mmol/L NAC处理组较80mmol/L Tau处理组升高更明显;过氧化氢酶、谷胱甘肽还原酶活性及水溶性蛋白含量的变化在各组间没有统计学意义。

**结论**:NAC对高氧环境下兔晶状体皮质混浊的抑制作用优于Tau。

**关键词**:N-乙酰半胱氨酸;牛磺酸;高氧;晶状体;兔