·论著(译文)·

Cerebral Apoptosis Gene Expression on Rats with Passive Smoking from Intrauterine to Weaning

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Abstract: Objective To study the effect of various concentrations of tobacco smoke exposure in the environment on cerebral apoptosis gene expression in pup rats in utero and postnatally. **Methods** Pregnant SD rats were exposed to air or to different concentrations of smoke for 5 hours daily, 7 days a week from day 2 of pregnancy until delivery. Then their pups were exposed to the same environment of passive smoking till weaning. Rapid competitive reverse transcriptase-PCR was used to analyze the relative expression of Bax mRNA and Bcl-2 mRNA semi-quantitatively of the cerebral hemisphere in the pups. **Results** The relative expressions of Bax mRNA of the cerebral hemisphere was 0.31 in the control group, 0.47 in the low concentration group, 0.55 in the moderate concentration group, and 0.60 in the high concentration group. It was significantly higher in the moderate and high concentration groups than that in the control group ($P < 0.05$). The relative expressions of Bcl-2 mRNA were of no significant difference among the passive smoking groups and the control group. **Conclusions** The increase of the relative expression of BaxmRNA in the pups' brain shows that cell apoptosis may play a role in brain damage of rats with passive smoking from intrauterine life to weaning.

Key words: Passive smoking; Intrauterine growth retardation; Cerebra; Apoptosis; Pup rats

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Adverse effects of passive smoking on fetal and infant development have been well established in both clinical and animal studies[1]. We report the results of a study of both prenatal and postnatal exposure to passive smoking on the expression of cerebral apoptosis genes.

1 Materials and Methods

1.1 Animals

Pregnant SD rats and pup rats were used in the study.

1.2 Preparation of animal models and samples

Twelve pregnant SD rats were randomly assigned to the exposure to air or different concentrations of smoke (the concentration of carbon monoxide in the smoke was 5～10 ppm in the low concentration group, 50 ppm in the moderate concentration group, and 200 ppm in the high concentration group) for 5 hours daily, 7 days weekly from d 2 of gestation until delivery. After birth, the female rats were randomly preserved to litters of 10 pups/dam which were then exposed to the same exposure condition as before. At day 21 of life, the pups were weaned and weighed, and then sacrificed. The brain was removed and weighed, and then frozen at -80 ℃ for later analyses.

1.3 Assay

1.3.1 RNA extraction Total RNA was extracted by using the Totally RNA Extraction Kit as per the manufacturer's instructions (Huashun, Shanghai, China).

1.3.2 Designing and synthesis of primers The primers were designed by using Oligo 5.0 software, and were synthesized by Gybersyn Genetic Engineering Company, USA.

1.3.3 Preparation of the internal standard (IS) preparation of IS refer to the document[2].

1.3.4 Recovering of the glue The glue was recollected by using the Glue Recollecting Kit (Huashun, Shanghai, China).

1.3.5 Competitive RT-PCR For each sample 2 μg of the total RNA was routinelyf used for cDNA

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synthesis. Then PCR was conducted with a GeneAmp PCR System 2400 (Gene, USA) in a total volume of 25 μl. The PCR products were separated by 2 % agarose gels (containing 0.5 μg/ml ethidium bromide) electrophoresis. Band intensities of photographed ethidiumed bromide - stained gels were determined with an imaging instrument. The relative amount of sample mRNA was calculated by the densitometric analysis of both samples and IS bands.

1.3.6 Statistic analysis Results are presented as means ±SD. Data were analyzed with Statistical Software 5.0 for Windows. Analysis of variance was used for all analyses. Results were considered to be significant at the $P < 0.05$ level.

2 Results

2.1 Body and brain weights of the pup rats

In every passive smoking group, both the body weight and the brain weight of the pup rats decreased significantly. The higher the smoke concentration was, the lighter the body weight and brain weight (Table 1).

Table 1 Effects of in utero and postnatal passive exposure on the body weight and brain weight ($\bar{x} \pm s$, g, n = 30)

Group	Body Weight	Brain Weight
Control	31.81 ±5.05	1.28 ±0.07
Low Concentration	24.61 ±3.97[a]	1.22 ±0.05[a]
Moderate Concentration	23.44 ±3.38[a]	1.19 ±0.07[a]
High Concentration	18.66 ±3.11[a,b,c]	1.08 ±0.08[a,b,c]

a vs control group, $P < 0.01$; b vs low concentration group, $P < 0.01$; c vs moderate concentration group, $P < 0.01$

2.2 Preparation of the internal standard (IS)

We generated DNA fragments that were used as IS. In its bands, both the target and IS were clear. The amount of Bax IS from the right band to the left band was 320, 160, 80, 40, 20, 10, 5, 2.5, and 1.25 fg. The amount of target cDNA was 0.3 μg. Bax PCR products was 436 bp and IS was 224 bp. The amount of IS mRNA corresponding to the 8th band (2.5 fg) was the same as the amount of competitive IS (Figure 1). The amount of Bcl-2 IS from the right band to the left band was 160, 80, 40, 20, 10, 5, 2.5, 1.25, and 0.625 fg, and the amount of target cDNA was 0.4 μg. Bcl-2 PCR products was 431 bp, and IS was 223 bp. The amount of IS mRNA corresponding to the 7th band (2.5 fg) was the same as the amount of competitive IS (Figure 2).

Figure 1 Determination of the internal standard of Bax

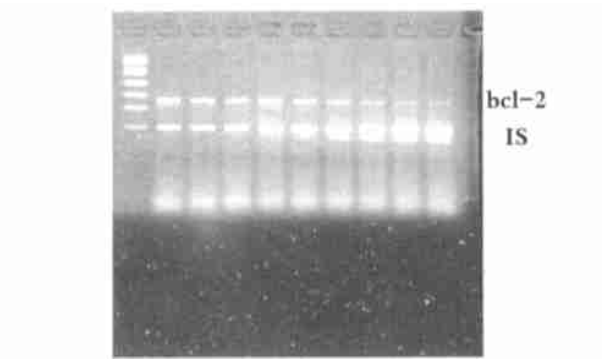

Figure 2 Determination of the internal standard of Bcl-2

2.3 Relative expressions of Bax-mRNA and BCL-2 mRNA in the brain of the pup rats (Table 2, Figure 3 and Figure 4)

Table 2 Relative expressions of Bax mRNA and Bcl - 2 mRNA in the brain of the pup rats ($\bar{x} \pm s$, n = 10)

Group	Bax mRNA	Bcl-2 mRNA
Control	0.31 ±0.18	0.37 ±0.23
Low Concentration	0.47 ±0.27	0.38 ±0.21
Moderate Concentration	0.55 ±0.22[a]	0.44 ±0.33
High Concentration	0.60 ±0.21[a]	0.46 ±0.40

a vs control group, $P < 0.05$

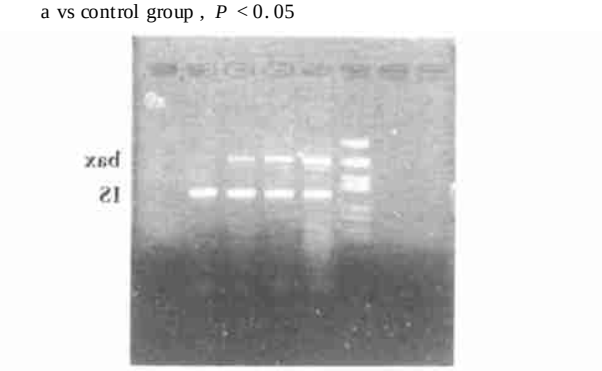

Figure 3 PCR electrophoresis photograph of Bcl-2 mRNA at the cerebral hemisphere of the pup rats

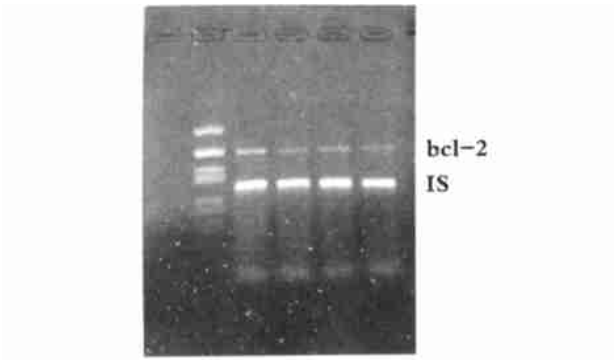

Figure 4 PCR electrophoresis of Bcl-2 mRNA at the cerebral hemisphere of the pup rats

3 Discussion

This study demonstrates that perinatal exposure of pregnant mice to tobacco smoke affects both the body and brain weight of pup rats. Although the relative expression of bcl-2 mRNA in the brain of pup rats was not affected, it did increase the relative expression of Bax mRNA in the brain of the pup rats exposed to smoke from intrauterine life to the time of weaning. Therefore, the ratio of Bax mRNA/Bcl-2 mRNA was increased in those rats exposed to smoke passively. Apoptosis, also known as programmed cell death (PCD), is regulated by genes that include two categories, one of them triggering PCD, such as Bax, and the other, such as Bcl-2, preventing it. When cells are damaged, dead proteins would be formed following the up-regulation of genes inducing PCD, and trigger off endogenous endonuclease activity, leading to nuclear chromatin condensation and break. Permanent cell damage ensues. Bcl-2 enables cells to survive by preventing apoptosis in the cells induced by a number of challenges[3]. The ration of Bax/bcl - 2 plays a key role in the regulation of apoptosis or survival in cells which have been challenged. Overexpression of Bax leads to apoptosis. Instead, overexpression of bcl - 2 allows the survival of cells[4]. The decrease of the brain weight in rats exposed to smoke from intrauterine to the weaning period may be associated with the increase of the ration of Bax/Bcl-2. The method used was semi - quantitatived Rapid Competitive Reverse Transcriptase-PCR. mRNA can also be examined by Northen blot.

In this study, the pup rats were exposed to smoke in utero. Nicotine and carbon monoxide in tobacco lead to the dysfunction of the placenta, affecting intrauterine growth and nutrition[1]. After birth, the injurious matter in tobacco produces harmful effects directly. Carbon monoxide has adverse effects both on the combination of hemoglobin with oxygen and on oxygen saturation of the blood, so that the risk of hypoxia is increased. Hydride in tobacco both reduces the level of Vitamin B_{12} and Vitamin B_1 in the blood, and inhibits the activity of cytochrome reductase, so as to satisfy the hypoxic state. Ferrer[5] reported that hypoxia caused overexpression of Bax in the brain of rats. In our study, the increase of the relative expression of Bax mRNA in the brain of the pup rats may be associated with chronic hypoxia.

4 Conclusion

The increase of the relative expression of Bax mRNA in the pups' brain shows that apoptosis may play a role in the brain damage of rats with exposure to smoke from intrauterine to weaning.

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