The Differential Expression of Androgen Receptor between with and without Medication Administration Guilong Kechuanning for the Male Rats under the Exposure of Environmental Tobacco Smoking (ETS)

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Abstract: This article demonstrates the expression in difference of androgen receptor with and without Guilong Kechuanning medication administration for heavy environmental tobacco smoke (ETS) treated rats. The discrepancy expression of androgen receptor reveals the efficiency of the Guilong Kechuanning medication administration in pneumotherapy. [The Journal of American Science. 2007;3(1):84-87].

Introduction

Guilong Kechuanning is a traditional Chinese material drug in pneumotherapy. The basic effect of medication administration has been studied in plenty [1-3], including the influence of cinnamaldehyde, TNF and IL-1β in Serum of mouse with chronic bronchitis. However, little investigation into the effect of Androgen Receptor (AR) is conducted in Guilong Kechuanning (GLKCN) medication administration. AR consists of eight exons with coding nucleoprotein being composed of 918 amino acids [4]. Androgen can diffuse into both target and non-target organs. But, it only functions in target organs. Similar like steroid hormone, AR is also a transcriptional factor. AR, if excited by Androgen, can recognize the target factor in a specific segment in DNA and combine with it to adjust the gene transcription expressing a new protein as well as changing the function of the cell [4]. It is well known that tobacco smoking can affect the shape and function of testis [5-6]. However, no evidence has shown any correlation between AR and tobacco smoking. In this article, we use RT-PCR and immunostaining LAB-SA to exam the mRNA expressing, in addition, the concentrations of the AR in tracheal sac, testis and lung of the rat with and without medication administration of Guilong Kechuanning (GLKCN).

Material and methods

36 Wistar healthy male mice with body weight 180-220g provided by the Henan Province experimental animal center were randomly divided into three groups, group A was for ETS exposure, group B for being as control group, and group C as natural ETS exposure group. Each group has two cages. Each cage was raised six mice. An ETS room, 1740mm×1100mm×1500mm was constructed by acrylic plate with a 2mm×3 mm air hole on top for exposing tobacco smoke and air. Group A was in ETS exposed 60 minutes, twice a day for the first 38 days and changed to being in exposure for once a day 60 minutes for another 38 days. Rats (including control, ETS exposure and GLKCN groups) should be taken off the eyeball to draw blood, chopped rapidly for taking the trachea, lung and testicle after ETS exposure. Then, the chopped organs can be saved in freezer tubes separately with entering liquid nitrogen for freezing immediately in -70℃. (All instruments pass through deactivating procedure of RNA enzyme).Taking 50mg organ sample from - 70℃ freezer and putting it into 800 μl (10^{-6} liter) reagent A (guanidine thiocyanate - phenol solution) for vibrating 30 seconds, sample was laid aside for 30 minutes in room temperature and then, added 200 μl reagent B (the chloroform: Isoamyl Alcohol in 24: 1) for being in 14000rpm centrifugal for 5minutes. Supernatant should be carefully drawn 400 μl to mix with 400 μl isoamyl alcohols. Taking 50μl mixed solution for14000rpm centrifugal 10min, we draw off supernatant and added 500ul 75% ethyl alcohol to sediment shaking uniformly and put it for 14000rpm centrifugal 5min. Then, we draw off supernatant again to get the sediment in dry being ready for use. In the mean time, reaction of reverse transcriptase can be performed by using 2mM $MgCl_2$ and 200μm dNTP to add 1.5μM random primer 5-CTACTGCGCT-3 1.5μm primer 5-TTACAGCAGAGGCAGGAGACT-3 and 1.3μm primer 5-AGGCAGCTGCTCAGGGTGGC-3 mixed with buffer as a reactive solution, and then, 20μl solution can be taken to mix with MLV with keeping in 37℃ incubator for an hour. PCR reaction was performed to compare the observed expression.

Results

The expression of AR mRNA is depicted in Table I and Figure 1 for the organs of trachea, Table II and Figure 2 for the organs of lung and Table III and Figure 3 for the organs of testis of the rats.

The immunostaining also can be depicted to have the similar results (figures not shown). Moreover, the expression of AR levels is depicted in Table IV and Figure 4 for the organs of trachea and Table V and Figure 5 for the organs of lung of the rats.

Table I. The expression level of AR mRNA in the trachea of the rats

Groups	n	1 n	-1 n	-2 n	-3 n
Normal control group	12	4	4	2	0
ETS exposure group	12	1	2	3	4
GLKCN group	12	6	3	1	0

Table II. The expression level of AR mRNA in the lung of the rats

Groups	n	1 n	-1 n	-2 n
Normal control group	12	5	4	1
ERS exposure group	12	3	5	2
GLKCN group	12	6	3	1

Table III. The expression level of AR mRNA in the testis of rats

Groups	n	0 n	1 n	-1 n	-2 n
Normal control group	12	0	5	3	2
ERS exposure group	12	7	3	0	0
GLKCN group	12	1	8	1	0

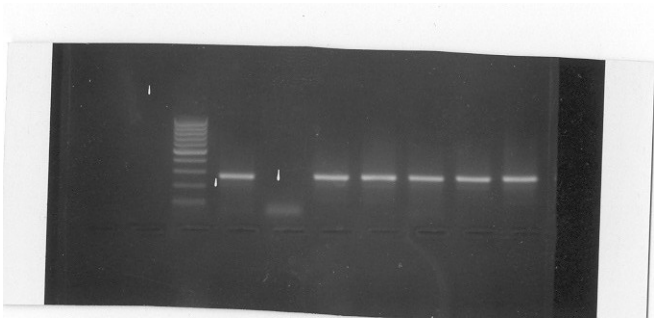

Figure 1. The expression level of AR mRNA in the trachea of rats

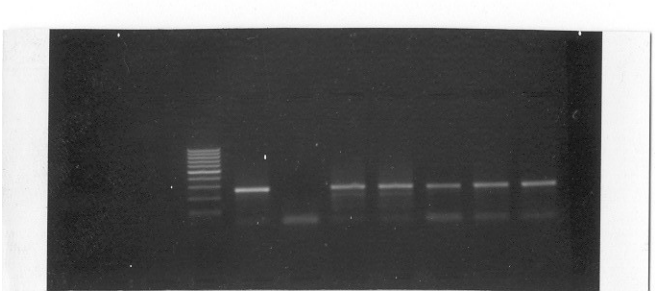

Figure 2. The expression level of AR mRNA in the lung of rats

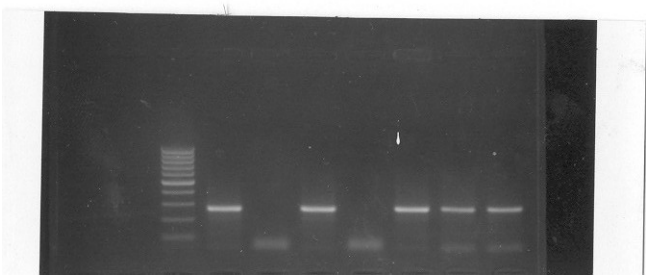

Figure 3. The expression level of AR mRNA in the testis of mice

Table IV. The expression level of AR in the trachea of rats (x±s)

Groups	n	mean ash density	mean optical density
Normal control group	12	$99.9\pm7.75^{**}$	$0.293\pm0.05^{**}$
ETS exposure group	12	$87.0\pm9.60^{\Delta\Delta}$	$0.358\pm0.06^{\Delta\Delta}$
GLKCN group	12	$106.7\pm11.29^{***}$	$0.265\pm0.04^{***}$

Compared with that of chronic bronchitis model group rats, ** P<0.01, *** P<0.001, compared with normal control group Δ P<0.05, ΔΔ P<0.01

Table V. The expression level of AR in the lung of rats (x±s)

Groups	n	mean ash density	mean optical density
Normal control group	12	$140.5\pm6.04^{*}$	$0.114\pm0.015^{**}$
ETS exposure group	12	$134.4\pm5.92^{\Delta}$	$0.143\pm0.023^{\Delta\Delta}$
GLKCN group	12	$149.9\pm15.21^{**}$	$0.092\pm0.034^{***}$

Compared with that of chronic bronchitis model group rats, * P<0.05, ** P<0.01, *** P<0.001, compared with normal control group rats, Δ P<0.05 and ΔΔ P<0.01

Conclusion

The study revealed that ETS exposure influences AR levels of the organ of trachea, lung as well as testis of the rats. ETS also lowers the AR mRNA expression in the organ of testis of rats. However, GLKCN can have significant improvement to lower the damage of ETS to the organs of the rats. Since the Sertoli cells and solenocytes [7] are the major cells to be able to express the AR, ETS can damage the germinal epithelium and Sertoli cells as well as primary spermatocytes. The ETS can be direct or indirectly to influence the growth of spermatoblast [8] and the damage of organs of testis causing spermid abnormal [9-12] of the rats. Our research confirms that ETS can cause the pathological alternation in testes sperm duck being resulted lower the AR mRNA significantly. However, the mechanism is still unknown. The RT-PCR experiment result has shown that ETS can affect AR mRNA expression in the organ of trachea and lung being increased. The immunostaining findings also showed that ETS affects AR expression strongly in comparison with normal control group. We found mucous membrane around the trachea and the bronchial tube, if the phlogocyte were found more, the AR expression was stronger. Basically, androgen gets the negative control of AR mRNA expression. But this kind of function needs the normal function of AR [13-15] in organs. Smoking may cause the blood serum androgen standard to reduce, therefore, the low standard androgen causes AR the high expression. Nevertheless, because AR mRNA expression depresses in testicle under ETS resulting blood serum androgen reduced is not only in trachea but also in the lung, the AR high expression can be the primary reason for the ETS damage. On the other hand, due to ETS may cause high AR expression in the trachea and lung organization, we extrapolate ETS may also affect organ of liver [22]. Therefore, whether or not in trachea and lung, phenomenon of high AR expression should be revealed the possibility that lung cancer being caused by ETS. The most interesting results of our research are that GLKCN administration can cause the lower of AR mRND expression and protect the increasing of AR levels in organs of trachea and lung of rats. The drug mechanism is still unclear. We may not conclude that GLKCN can recover the damage of the ETS, at least, we can say GLKCN helps to the lower the damage of the ETS.

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