

The formation of rats' choroidal neovascularization induced by acrolein

Guan-Feng Wang, Xiu-Lan Zou, Dong-Hao Li, Chen Wang, Wen-Li Li, Rong-Biao Pi

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Department of Ophthalmology, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou 510150, Guangdong Province, China

Correspondence to: Xiu - Lan Zou. Department of Ophthalmology, The Third Affiliated Hospital of Guangzhou Medical University, Lihua Road No. 111, General Hospital of PLA Guangzhou Military Area, Guangzhou 510010, Guangdong Province, China. xlzou2003@yahoo.com.cn

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丙烯醛诱导大鼠脉络膜新生血管的形成

王观峰, 邹秀兰, 李东豪, 王琛, 李文立, 皮荣标

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(作者单位: 510150 中国广东省广州市, 广州医科大学附属第三医院眼科)

作者简介: 王观峰, 毕业于暨南大学, 硕士研究生, 住院医师, 研究方向: 眼底病。

通讯作者: 邹秀兰, 毕业于德国柏林 Charité 医学院, 博士研究生, 硕士研究生导师, 研究方向: 眼底病. 149642427@qq.com

摘要

目的: 探讨丙烯醛 (AC) 诱导大鼠脉络膜新生血管 (CNV) 的形成。

方法: 选取远交群大鼠 (SD) 12 只, 随机将大鼠分为三组, 即空白组, AC 诱导 4wk 组和 AC 诱导 8wk 组, 其中空白对照组每日 200 μ L 新鲜自来水灌胃, 实验组为 200 μ L AC 溶液 (2.5 mg/kg/d) 每日灌胃, 分为诱导 4wk 组和 8wk 组, 取材后视网膜组织石蜡包埋, 切片及 HE 染色。

结果: 空白对照组及 AC 诱导 4wk 组均见 RPE-Bruch 膜连续性完整, 未见明显异常; AC 诱导 8wk 组发现 RPE-Bruch 膜连续性缺失, 脉络膜新生血管长入视网膜神经上皮层内。

结论: 长期使用 AC 可以诱导大鼠脉络膜新生血管形成。

关键词: 丙烯醛; 脉络膜新生血管; 大鼠

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Abstract

• **AIM:** To investigate the formation of rats' choroidal neovascularization (CNV) induced by acrolein.

• **METHODS:** Twelve Sprague-Dawley rats were randomly divided into three groups. Acrolein 200 μ L (2.5 mg/kg/d) was poured into the rats' stomach for 4wk as acrolein 4wk and for 8wk as acrolein 8wk group. The same volume of fresh water was also done to the rats as the control group. Remove all eye balls and embed into paraffin with HE staining.

• **RESULTS:** The RPE-Bruch membrane was intact with no obvious abnormality in the control group and acrolein 4wk group. Lost in the continuity of RPE and the movement of choroidal neovascularization were found in the acrolein 8wk.

• **CONCLUSION:** The long time use of acrolein can induce the formation of choroidal neovascularization in rats.

• **KEYWORDS:** acrolein; choroidal neovascularization; rat DOI:10.3980/j.issn.1672-5123.2016.4.01

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INTRODUCTION

Choroidal neovascularization (CNV) which lead to the average of millions patients with low vision and hundreds of thousands blind people in progress has become the main cause of blindness in our country now. Patients often have the performance of both eyes at the same time or successively irreversible, progressive vision decline^[1-2]. But the pathogenesis of the disease lack of efficacy and side effects of the treatment in clinic is not clear. The disease is also easy to relapse. Thus, to study the occurrence and development of the disease have great clinical and social significance in exploration of prevention and treatments.

The cause of CNV is not clear at present, but smoking has been recognized as the most important risk factors by the domestic and foreign scholars. The relative risk (RR) of CNV in smokers is 2.4 times than that of non-smokers, and the RR of complement 402H CC genome is 3.4 times proved by statistical data^[3-4]. Aldehydes, nitrogen oxides and other toxic substances which were founded in the cigarettes can not only significantly reduce the levels of ascorbic acid and hydrogenated sulfur protein in retinal pigment epithelium (RPE), but also cause the oxidation of fat and protein^[5-8]. However, the toxicity of acrolein (AC) is 10 times to that of formaldehyde and 100 times to the acetaldehyde. The AC, meanwhile, can be directly reacted with the hydrogen sulfide

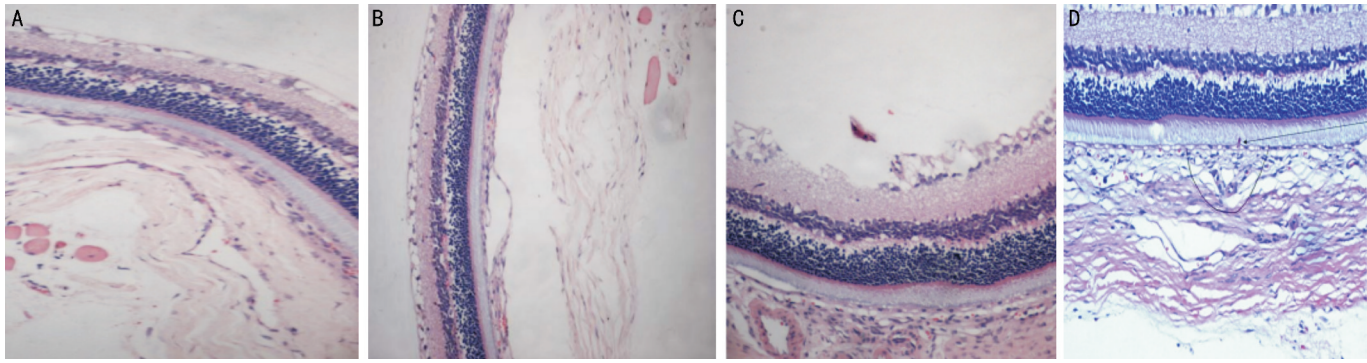


Figure 1 HE staining (10×20) A: Control group; B: Acrolein 4wk group; C: Acrolein 8wk group; D: Acrolein 8wk group.

protein to have a higher risk index than other five kinds of toxic aldehydes in cigarettes. In earlier years, we succeeded in replicating the damage process in RPE cell research by AC. Thus, we will explore the induction of CNV rats to provide more experimental data for studying the formation and development of CNV disease.

SUBJECTS AND METHODS

Experimental Animals and Feed Sources Randomly selected 12 male, 8 month old Sprague Dawley (SD) rats weighted about 270 to 300 g. These animals were fed at the animal house of Sun Yat-Sen University with the temperature of (25±1) centigrade. The relative humidity of (50±5)%, 12h light cycle on a day and related feed were purchased from the the experimental animal center of Sun Yat - Sen University.

Grouping and the Route of Administration Accorded to the the random number table, all SD rats were randomly divided into three groups as the blank group, 4wk for acrolein and 8wk for acrolein. The amount of 2.5 mg/kg acrolein dissolved into 200μL fresh water which was daily gavaged in SD rats within 2h as the treatment group. At the same time, the control group was given the same volume of fresh water.

Hematoxylin-Eosin Staining for Paraffin Section Eyes, collected from these experimental rats, were removed into the fixed radium of 4% paraformaldehyde for the whole night. Then these organizations were dehydration by gradient alcohol, embedded in the paraffin and sliced with HE staining. Observed and photoed them by optical microscope.

Statistical Methods Using the SPSS 13.0 statistical software to make a statistical analysis. Experiment data were expressed as mean ± standard deviation by single factor analysis of variance in the complete random block design. The data was statistically significant when $P < 0.05$.

RESULTS

HE staining of optic nerve and choroida control group: the structure of the retina was regular with the complete RPE-Bruch film continuity by optical microscope (Figure 1A).

Acrolein 4wk group: the structure of the retina was regular with the complete RPE-Bruch film continuity by optical microscope (Figure 1B).

Acrolein 8wk group: the RPE-Bruch film continuity

interrupted with the invasion of choroidal neovascularization into the neuroepithelial layer as a typical uplift (Figure 1C, 1D).

DISCUSSION

More than 300 kinds of substances, as formaldehyde, acetaldehyde, acrolein, crotonaldehyde and so on, have been found in the daily food or raw materials like bread, fish, alcohol and etc. In addition, the contact of oil, sugar and fatty substance can make the production of aldehydes by peroxidation during the cooking. But these aldehydes which contained of aldehyde (CHO) and hydroxyl (-OH) or hydrogen (H) atoms have a strong reduction and oxidation. The maximum amount of average adult daily contact is about 7mg/kg in the epidemiological studyings, included of 5mg/kg^[7-11] acrolein, 2 mg/kg formaldehyde and acetaldehyde exposure. Otherwise, foreign scholars constructed the acute myocardial toxicity model with the concentration of 5 mg/kg/day acrolein in the treatment for 30min, the chronic vascular tissue and nerve tissue toxicity model with the concentration of 2.5 mg/kg/day acrolein in the treatment for 8wk to make a great contribution in the animal toxicity model. Therefore, we take 2.5 mg/kg/day acrolein as a 50% total unsaturated aldehydes to investigate the chronic injury of rat retina and choroid.

We found the interruption of RPE-Bruch membrane and invasion of CNV from the choroidal tissue which might be possible to form as follows^[12-15]. The production of vascular endothelial growth factor (VEGF) not only promotes the formation of new blood vessels, but also can stimulate endothelial cells division, increasing of microvascular permeability and the formation of some proteases. Nicotine in cigarettes can increase the ratio of VEGF and pigment epithelium-derived factor (PEDF) which could cause the formation of CNV in RPE cells. The levels of VEGF and TGFβ2 were double than that of the control group on the acute toxicity of RPE cells model^[16]. Another, the level of VEGF was 1.417 times to that of the TGF-β/SMAD3. We believe the induction of TGF-β which was an important factor in CNV activity and fibrosis could cause the formation of VEGF and CNV. On the other hand, acrolein can cause the production of cyclooxygenase-2 (COX-2), nitric oxide (NO), and the

oxidative damage of nitric, proteins and other biological molecules. It was found the deficiency in COX-2 which made a decline on the expression of VEGF and formation of CNV^[17]. NO can also promote the release of proteases to stimulate the proliferation, migration of endothelial cells and increasing permeability of capillaries as an important signal molecule transduction. Therefore, AC *in vitro* and *vivo* can cause the chronic effects on the formation of CNV which expand the development of CNV disease in rats.

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