Video Article

Effects of Exposure of Formaldehyde to a Rat Model of Atopic Dermatitis Induced by Neonatal Capsaicin Treatment

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URL: https://www.jove.com/video/55987
DOI: doi:10.3791/55987

Keywords: Immunology, Issue 127, Formaldehyde, exposure, air pollutant, atopic dermatitis, capsaicin, animal model

Date Published: 9/27/2017

Citation: Han, R.T., Back, S.K., Lee, J., Kim, H.y., Kim, H.J., Na, H.S. Effects of Exposure of Formaldehyde to a Rat Model of Atopic Dermatitis Induced by Neonatal Capsaicin Treatment. *J. Vis. Exp.* (127), e55987, doi:10.3791/55987 (2017).

Abstract

Atopic dermatitis is chronically relapsing pruritic eczema and prevails around the world especially in developed countries. Complex interactions between genetic and environmental factors are known to play an important role in the pathophysiology of atopic dermatitis. However, we still lack a detailed picture of the pathogenesis of this disease. Thus, it is of importance to develop appropriate animal models for elucidating the progression of atopic dermatitis. Moreover, investigating the effect of environmental factors such as air pollutants on atopic dermatitis expands understanding of the disease. Here, we describe a method for inducing atopic dermatitis in rats with neonatal capsaicin treatment and a protocol for exposure of a constant concentration of formaldehyde to rats to reveal effects on the development of atopic dermatitis in infantile and adolescent periods. These protocols have been successfully applied to several experiments and can be used for other substances.

Video Link

The video component of this article can be found at https://www.jove.com/video/55987/

Introduction

To interrogate the effects of formaldehyde (FA) inhalation on atopic dermatitis (AD) progression, we established a protocol for AD induction and developed a conventional circulation chamber for chronic exposure of FA.

AD is a chronic inflammatory skin disease attributed to the complex interactions between genetic predisposition and environmental triggers[1,2]. A proper animal model is required to study AD in the preclinical state. This is most important when elucidating the pathogenesis of AD under the influence of environmental factors. Moreover, to better investigate the effects of the environmental triggers on the AD progression, it is essential to separate the individual environmental factors and quantify the degree of exposure.

We have demonstrated a novel rat model of AD induced by neonatal capsaicin treatment. This AD model[3] has some merits over other AD models such as the AD models induced by epicutaneous sensitization, the genetically-engineered AD models, and the spontaneous mouse models of AD[4]. First, this model develops AD-like symptoms at the age of 3 weeks, which corresponds to infant AD and is earlier than other spontaneous mouse models of AD such as the NC/Nga mouse. In some cases, unlike the genetically-engineered AD models, the symptoms of the AD models subside and relapse spontaneously over a 20-week period. Moreover, the induction process of AD-like symptoms in the model is easier than that of the epicutaneous sensitization model, which is more similar to the allergic contact dermatitis in the perspective of the induction process.

We have introduced the novel protocol for FA exposure to an animal model by using an acrylic glass chamber and a conventional medical oxygen regulator[5] to study the effects of FA inhalation on the development of AD in infantile and adolescent periods. The regulator has allowed us to expose the animals to a constant concentration of gas by adjusting the flow rates and the solution concentration. Previous studies have reported several ways of exposure of FA to animal models. Xu *et al.* described the exposure method in which FA solution was directly applied to the skin[6]. Another recent study addressed the protocol of FA gas exposure using a specialized volatile solvents generator, model 4912[7]. Compared to methods of previous studies, the new method is more easily equipped and can be applied for other solutions in addition to FA.

Protocol

All methods described here have been approved by the International Animal Care and Use Committee (IACUC) of Korea University College of Medicine. 6 weeks after birth, experimenters can induce enthansasia and collect various samples such as the skin and serum of rats.

1. Induction of AD by Neonatal Capsaicin Treatment

1. To prepare 5 mg/mL capsaicin solution, add 50 mg of capsaicin to 1 mL of Tween 80 and 1 mL of 100% ethanol in a 15 mL conical tube.
2. Add 8 mL of normal saline to the conical tube. Shake the tube vigorously until no separated layers are visible
3. Inject the capsaicin solution (10 µL/g) into the subcutaneous tissue, on the midline and backside of the neck of a neonatal Sprague-Dawley rat.
    1. Deliver the capsaicin between 12 h to 48 h after birth (CRITICAL).
    NOTE: If the injection is delayed by more than a few days, dermatitis will not develop or it will persist only for few weeks. If the injection is too early, respiratory distress can occur due to severe pain.
    2. To prevent neonatal rats from respiratory arrest due to the capsaicin injection, push the chest of neonates to encourage breathing or keep the neonates in an oxygen chamber until the respiration becomes normal. Since capsaicin-induced pain can discourage breathing, adding general anesthesia could possibly worsen breathing difficulty. Thus, experimenters should not use anesthesics to increase survival of rats.

2. Evaluation of Pruritus and Dermatitis in a Rat Model of AD

1. **Measurement of pruritus by counting spontaneous scratching behavior**
    1. Prepare plastic chambers (20 cm x 30 cm x 20 cm) equipped with a mirror to allow for full coverage for viewing and holes to allow the rats to breathe.
    2. Set up and adjust a digital video camera properly to record the mirror views and front views of the rats simultaneously.
    3. Place rats in the plastic chamber and cover the ceiling of the chamber with a heavy object to prevent from opening.
    4. Record the spontaneous behaviors of rats for 1 h, play back the video clips, and count the number of scratching behaviors.

2. **Assessment of skin lesion using a scoring system**
    1. Place the rat in the induction chamber.
    2. Turn the oxygen flowmeter to 2 L/min flow rate and adjust the isoflurane vaporizer to 3% for anesthesia.
    3. Identify the extent and severity of dermatitis in the ears and other parts of a rat.
    4. Define the unit size for the extent of skin lesions as 0.25 cm^2 and the severity of skin lesions by following **Table 1**: severity index; degree of dermatitis score by multiplying the extent and severity (see dermatitis score in **Figure 1B**).

3. Exposure of Gaseous FA to Animals

1. **Preparation of the inhalation chamber**
    1. Prepare an acrylic glass box (55 cm x 40 cm x 35 cm) that has two outlets on opposite sides.
    2. CAUTION. For circulation of FA gas, place the outlet higher than the inlet and make the size of the outlet larger than that of the inlet.
    3. CAUTION. For the measurement of internal gas concentration, on another side of the outlets, drill three small passages, which can be closed.
    4. Connect tubes to both outlets. Place an outlet tube to circulation hood or outside of the building for emission of the gas, and connect the other outlet tube to a medical oxygen regulator.
    5. Place a conventional rat cage inside the acrylic glass box.
    6. Put rats in the cage from one week after birth for 5 weeks (2 h/day and 5 days/week).
    7. Seal the acrylic glass box as tightly as possible to prevent gas leakage.
    NOTE: For tight sealing, the chamber lid should contain rubber. Apply cling film to wrap the chamber for tight sealing.

2. **Generation of gaseous FA by using a medical oxygen regulator**
    1. Dissolve 5 g of FA into 1 L of water.
    2. Put the 0.5% FA solution in the humidifier bottle of the medical oxygen regulator.
    3. After connecting the inlet tube to the regulator, turn on the regulator and set the flow rate to 5 L/min.
    4. Adjust the solution concentration and the flow rate to determine the concentration of gas in the chamber.

Representative Results

The AD-like skin inflammation of the capsaicin-treated AD rat model at 7 weeks of age is presented in **Figure 1A**. Robust scratching behaviors of the capsaicin-treated AD rat model is shown in **Video 1**. Neonatal capsaicin treatment elicited dermatitis and aggravated scratching behaviors in rats 3 weeks of age, and AD-like symptoms persisted for several weeks (**Figure 1B, C**). Though the degree was less severe, some rats suffered from relapsing dermatitis at 16 weeks of age after resolution of early AD-like symptoms (**Figure 1D**). Furthermore, serum IgE level, which is a biological marker for AD severity[8], was elevated in the capsaicin-treated AD rat model (**Figure 1E**). The mRNA expressions of IL-4 and IL-13 also increased in the capsaicin-treated AD rat model (**Figure 1F, G**).

Representative photographs showed that FA induced the aggravation of dermatitis in the capsaicin-treated AD rat model at 5 weeks of age (**Figure 2A**). Exposure of 1.2 ppm FA aggravated the scratching behaviors and dermatitis in capsaicin-treated AD rat model for more than 2 weeks. However, 0.8 ppm FA exposure didn't exacerbate scratching behaviors and dermatitis in the capsaicin-treated AD rat model (**Figure 2B, C**). Moreover, 1.2 ppm FA exposure elevated the serum IgE level in the capsaicin-treated AD rat model (**Figure 2D**).

For exposure of gaseous FA, the ventilation chamber was set up as described in the **Protocol** (**Figure 3A**). Before the exposure, the concentration of FA in the chamber was repeatedly measured for assurance of consistent concentration. When changing the concentration of FA solution, the concentration of gaseous FA in the chamber was measured. As the concentration of the solution increased, the concentration of the gas in the chamber slowly increased and concentration of the gas in the chamber from the same solution did not vary significantly in repeated measurements (**Figure 3B**).

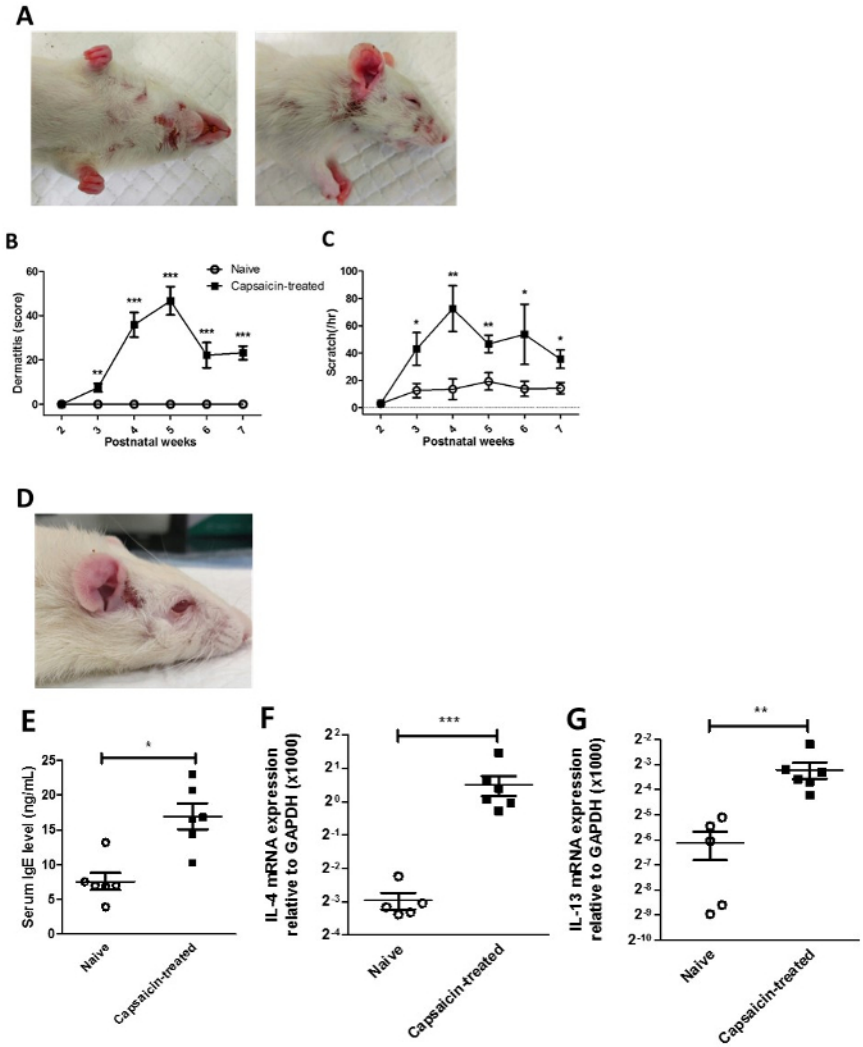


Figure 1: Neonatal Capsaicin Treatment Induced Relapsing AD-like Skin Inflammation and Pruritus in Rats. Representative photographs of skin inflammation in the capsaicin-treated rat (**A**). Chronological features of dermatitis (**B**) and scratching behaviors (**C**) of the capsaicin-treated rats. Representative image of relapsing skin lesions of 16 week-old capsaicin-treated rats (**D**). (**E**) Significant elevation of serum IgE levels in capsaicin-treated animals. (**F**) Significant elevation of IL-4 mRNA expression in capsaicin-treated animals. (**G**) Significant elevation of IL-3 mRNA expression in capsaicin-treated animals. All results were expressed as means ± SEM and analyzed using the Student's t-test. *p < 0.05, **p < 0.005, ***p < 0.001. Please click here to view a larger version of this figure.

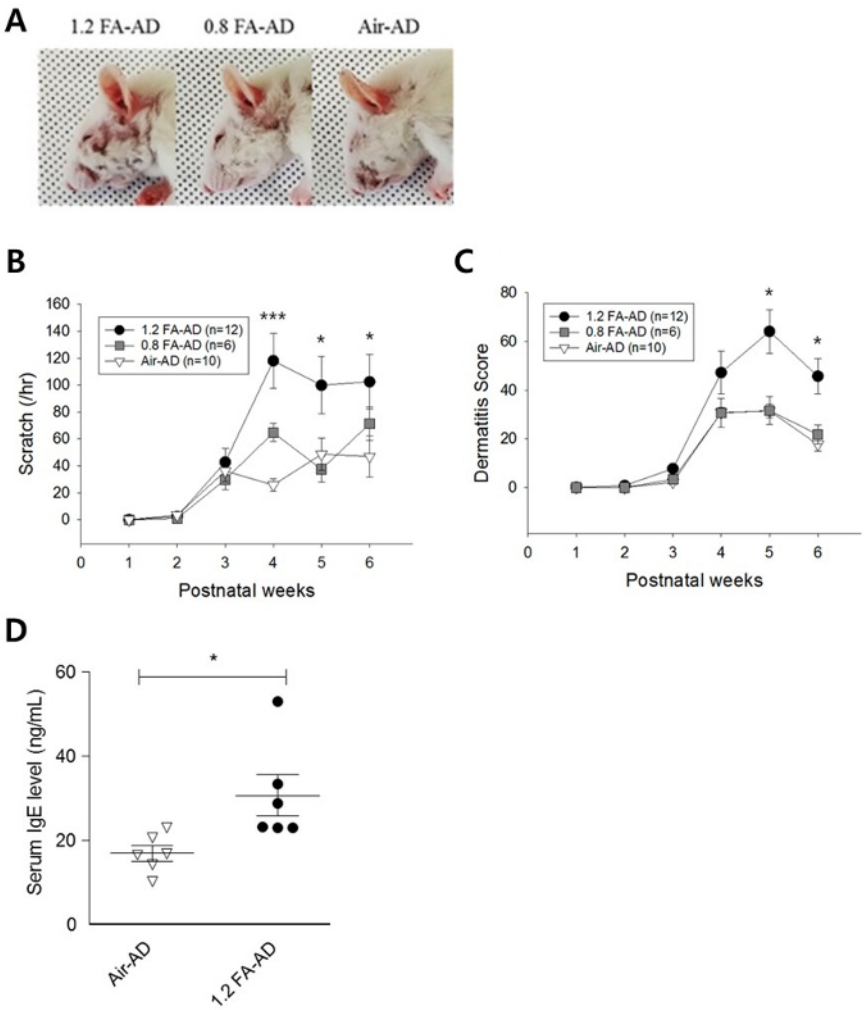


Figure 2: FA Exposure Aggravated Pruritus and Dermatitis in the Capsaicin-treated AD Rat Model. Representative photographs of the 5th week of treatment using 1.2 ppm and 0.8 ppm FA (**A**). Exposure of 1.2 ppm FA, but not 0.8 ppm FA exposure, significantly exacerbated pruritus (**B**) and dermatitis (**C**) in the capsaicin-treated AD model. Serum IgE level increased in the capsaicin-treated AD rat model treated with 1.2 ppm FA exposure (**D**). Results were expressed as means ± SEM and analyzed using a one-way ANOVA followed by Tukey's test or the Student's t-test. *$p < 0.05$, ***$p < 0.001$ (vs rats exposed to fresh air). 1.2 FA-AD: Capsaicin-treated AD rat model exposed to 1.2 ppm FA; 0.8 FA-AD: Capsaicin-treated AD rat model exposed to 0.8 ppm FA; and Air-AD: capsaicin-treated AD rat model exposed to fresh air. Please click here to view a larger version of this figure.

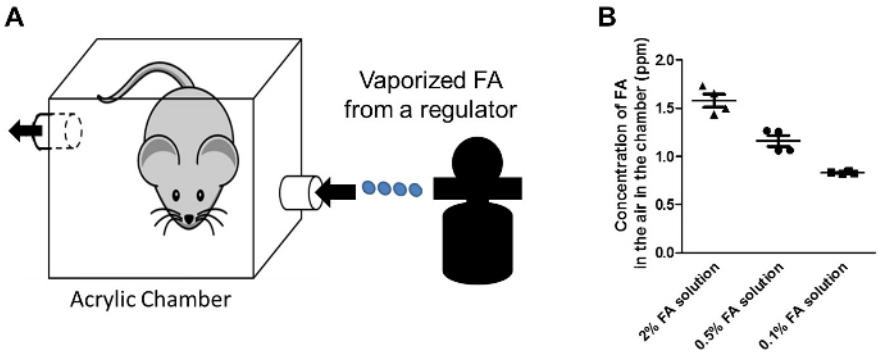
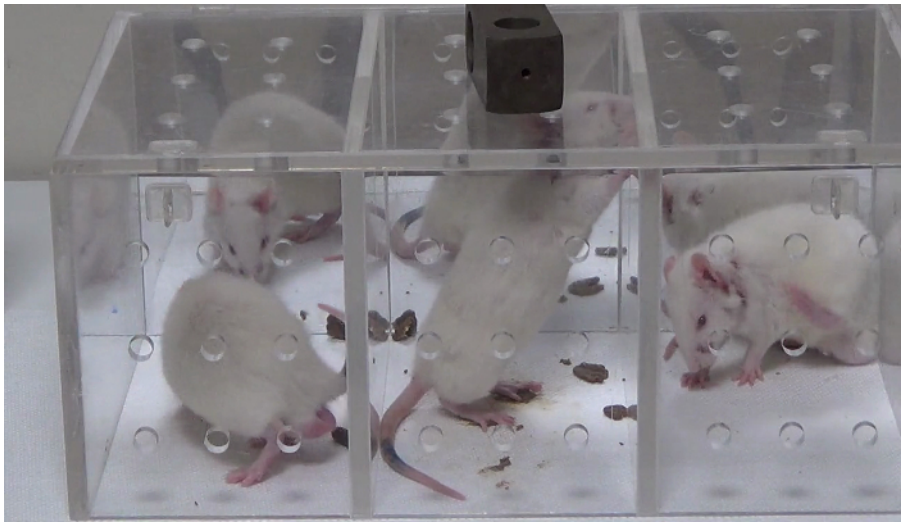


Figure 3: FA Treatment. Schematic illustration of the regulator and the gas chamber (**A**). The concentration of gaseous FA in the chamber was proportionate to the concentration of FA solution (**B**). Results were expressed as means ± SEM. Please click here to view a larger version of this figure.



Video 1: Bouts of Scratching Behaviors of Capsaicin-treated Rats were Recorded for Measurement of Pruritus. Please click here to view this video. (Right-click to download.)

Region	Score	Description
Except ears	0	Normal
	1	Wispy fur
	2	Alopecia or flare
	3	Bleeding or Scab
Ears	0	Normal
	1	Flare
	2	Bleeding or Scab
	3	Loss of the tissue

Table 1: Severity Index.

Discussion

The protocol described here for the induction of AD-like symptoms in rats can be adapted for studying AD pathophysiology and used as a screening tool for drug test. The instrument for the exposure of gaseous FA to capsaicin-treated rats can be applied to evaluate the effects of exposure to various gases on diverse animal models.

The neonatal capsaicin-treated AD model relies on a more simple procedure than other atopic models such as the DNCB treatment model[9] and epicutaneous sensitization model[4], and is more affordable than the NC/Nga mouse[4]. The manifestation of AD-like symptoms in the AD model is consistent. Moreover, in some cases, this protocol elicits spontaneous relapsing AD-like skin lesions in the affected rats without any additional treatment[3]. Overdose capsaicin injection evoked neuronal cell death and neurocutaneous inflammation[10] and caused severe pain in the rats. Some of them possibly suffered from respiratory arrest due to the pain. It is of importance to prevent them from respiratory arrest. Thus, after the capsaicin injection, the pups should be placed in an oxygen chamber and some pups may require chest compression. Neonatal capsaicin treatment does not induce AD-like symptoms in mice, and the cause for this is still unknown. Thus, genetic intervention is hardly applicable to this AD model using a knock-out strain, but other genetic approaches such as siRNA plausibly compensate for this shortcoming. Furthermore, the consistent manifestation of AD-like symptoms of the rat model is beneficial for studying AD disease pathogenesis, as well as applying it to a drug screening tool.

The establishment of the circulatory chamber for FA exposure only requires conventional laboratory equipment including an acrylic box and a medical oxygen regulator. Furthermore, by changing the solution in the humidifier bottle of the oxygen regulator, diverse types of gases can be applied in the experiment. Concentration of the gas in the chamber can be easily maintained by adjusting the flow rate of the regulator and the concentration of the solution. It is critical to ensure a tight sealing in the installation of circulatory system for FA exposure to the capsaicin-treated AD rat model using an acrylic chamber and a medical oxygen regulator. Not only because the concentration of gas in the chamber could differ each time due to the leakage, but also the leaking gas can harm the experimenters. Vaporization of some volatile organic compounds (VOC) often blocks the tube by crystallization. Thus, it is relevant to avoid clogging through continuous tube management for long-term use by regularly replacing the tubes. This vaporization system reflects more precisely the disease progression in patients than the topical application methods[11]. This method revealed distinct features of the effects of FA inhalation on a rat model of AD compared to the previous studies which exposed FA to naïve animals[7,12].

Disclosures

The authors have nothing to disclose.

Acknowledgements

This research was supported by the Public Welfare & Safety research program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2014M3C8A5030612), and supported by The Chung Yang, Cha Young Sun, M.D. and Jang Hi Joo Yeu Sa Memorial Fund.

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