A hypothesis of the Effect of a New Nasal Spray Made from Natural Medicines on Allergic Rhinitis in Animals

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Abstract. To verify the effect of a new nasal spray made from natural medicines on allergic rhinitis in animals. Methods: The main natural medicines contained in Acusine nasal spray plus essential traditional Chinese medicine contained in drugs for allergic rhinitis in Chinese market were used. By preparation process of extraction of traditional Chinese medicine such as steam distillation, ethanol extraction, a new nasal spray made from natural medicines was prepared. In the meantime, 24 BALB/c mice and New Zealand white rabbits were used. Then, mice were randomly divided into four group; control group, beclomethasone dipropionate group, Acusine group and new spray group, 6 mice in each group. Moreover, the effect of the new nasal spray made on passive cutaneous anaphylaxis was conducted by detecting absorptions of Evan’s blue (620nm) in the four groups. Allergic rhinitis models in 40 New Zealand white rabbits were established. Consequently, 40 allergic rhinitis models in rabbits were randomly divided into control group, Acusine group and new spray group, 10 rabbits in each group. The four groups were sprayed nasally with saline, Acusine spray and new spray respectively, three times/d, for 30 days. The nasal resistances in the four groups were measured with a rhinorresistometer. Moreover, their nasal mucosa was taken for HE staining. Consequently, their pathological manifestations were observed. The results: Absorption of Evan’s blue (620nm) of new spray group will be found significantly lower than Acusine group ($P<0.05$) and will have no significantly difference compared with beclomethasone dipropionate group($P>0.05$). On the other hand, absorption of Evan’s blue (620nm) of beclomethasone dipropionate group will be significantly lower than Acusine group ($P<0.05$). Moreover, The nasal resistances of new spray group will be significantly lower than Acusine group ($P<0.05$) and will have no significantly difference compared with beclomethasone dipropionate group ($P>0.05$). Moreover, the nasal resistances of beclomethasone dipropionate group will be significantly lower than Acusine group ($P<0.05$). While, the nasal mucosa of control group will be found typical as nasal mucosa of allergic rhinitis. However, the nasal mucosa of beclomethasone dipropionate group, Acusine group and new spray group will be better than control group. Of them, new spray group will be the best. The nasal mucosa of beclomethasone dipropionate group will show the manifestation of drug-induced rhinitis. The conclusion: The invented new spray will not only have a better treatment effect on allergic rhinitis, similar with beclomethasone dipropionate, than Acusine Nasal Spray but also will not leave side effect on nasal mucosa, compared with beclomethasone dipropionate. Thus, the new spray made from natural medicines can be further studied as a better prescription drug, rather than an OTC like Acusine Nasal Spray, to replace beclomethasone dipropionate nasal spray. However, further study that would be required to produce a more meaningful answer.

1. Introduction
Allergic rhinitis is a common but recurrent refractory disease. In the meantime, Beclomethasone...
asal ointment, a compound, mezereum, administered orally, unobstructed breathing, nasal spray.

However, allergic rhinitis is equal to "bigiu" in Chinese medicine, including the symptoms of lung Qi deficiency, spleen deficiency and kidney Yang deficiency.

The marketed drugs for allergic rhinitis in China are listed as follows: 1. nasal drops: 1) Tongda nasal drops (Zhunzi B20020318, Pharmaceutical Factory of Guangxi Medicinal Botanical Garden.) 2) Cang Yi nasal drops (Zhunzi B20020682, Guangxi Nanning Dezhi Pharmaceutical Company Limited.)

3) Nasal smooth drops (Zhunzi Z20030105, Shenyang Shuangding Pharmaceutical Company Limited.) 4. Thirteen-ingredient Flos Magnoliae nasal drops (Zhunzi B20020131, Shanxi Yellow River Traditional Chinese Medicine Company Limited.) 5.) Ditong drops for rhinitis (Zhunzi Z33020423, Zhejiang Kangenbei Pharmaceutical Company Limited.) 2. Nasal ointment: Compound Hibiscus mutabilis nasal ointment (compound Hibiscus mutabilis ointment) (Zhunzi Z20025809, Guizhou Liangji Pharmaceutical Co., Ltd). 3. nasal spray: 1) Ditong biyanshui spray (Zhunzi Z20050412, Guangxi Boke Pharmaceutical Co Ltd); 2) Nasal Ning spray (Zhunzi Z20025757, Baihua Pharmaceutical Group Co. Limited); 3) Dafoeshui nasal spray (Hong Kong Meijiakang Healthcare Pharmaceutical Co.) and so on.

The above nasal drugs made from traditional Chinese medicine are still not ideal for allergic rhinitis.

Acusine Nasal Spray (Nestmann Pharma, Zapendorf/Bamberg), a marketed OTC for allergic rhinitis, is produced from natural medicines, but has been included in the Homeopathic Pharmacopoeia of the United States (HPUS), but has not been registered as a foreign drug in China Food and Drug Administration. The spray is used to relieve nasal congestion, nasal dryness, unobstructed breathing, runny nose, nasal inflammation and nasal bleeding due to allergic rhinitis or colds. Its main ingredient is hydrastis canadensis and its auxiliary ingredients are ephedra vulgaris, lycopodium clavatum, mezereum, petroleum, pulsatilla, thuja occidentalis. The efficacy of the spray is satisfactory.

Therefore, it’s reasonable to speculate that a new nasal spray combining the ingredients in Acusine Nasal Spray with the essential traditional Chinese medicines contained in the marketed drugs for allergic rhinitis in China will be more effective to treat allergic rhinitis than Acusine Nasal Spray alone.

So, an animal experiment was designed to verify the above hypothesis in terms of the effectiveness and safety of the new nasal spray made from natural medicines.

2. Materials and Methods

2.1. Preparation of a new nasal spray made from natural medicines.

The main natural medicines contained in Acusine nasal spray: hydrastis Canadensis, ephedra vulgaris, lycopodium clavatum, mezereum, pulsatilla, thuja occidentalis, plus essential traditional Chinese medicine contained in drugs for allergic rhinitis in Chinese market: cocklebur fruit, flos magnoliae, mint, herba centipedae, scutellaria baicalensis, hibiscus mutabilis, angelica dahurica, cordate houttuynia, herba asari, astragalus mongholicus, atractylodes macrocephala, radix saposhnikoviae were used. Weight of each of all the natural medicines except petroleum was 80g. Moderate petroleum was used.

Moreover, preparation process of extraction of traditional Chinese medicine such as steam distillation, ethanol extraction, a new nasal spray made from natural medicines was prepared.

2.2. Effect of the new nasal spray made on passive cutaneous anaphylaxis in BALB/c mice.

However, Acusine liquid and new spray liquid were taken out from Acusine nasal spray and the new nasal spray. 24 BALB/c mice were randomly divided into control group, beclomethasone dipropionate group, Acusine group and new spray group, 6 mice of each group. Then, control group was injected intradermally with 30μL of saline and the other three groups were sensitized intradermally with anti-DNP IgE. On day 2, 3 and 4, control group, beclomethasone dipropionate group, Acusine group and new spray group were administered orally 50mg/kgbw of saline, beclomethasone dipropionate, Acusine liquid and new spray liquid respectively. On day 5, all the four groups were injected
intravenously with DNP-HSA containing 0.5% Evan’s blue dye. 30 minutes after dye injection, mice were sacrificed and tissue sections around the intradermal injection site were excised and weighed. Evan’s blue dye was then extracted from the tissue by incubation of biopsies in 0.5ml formamide at 55°C for 24h and quantitated by absorbance at 620nm[18].

3. Effect on allergic rhinitis in rabbits

3.1 Establishment of Allergic Rhinitis Models

3.1.1 Animals. 40 New Zealand white rabbits, half male and half female, weighing 1.5-2kg.

3.1.2 Drugs. Toluene-2,4-diisocyanate (TDI) was added into olive oil to prepare into a 10% oil solution as an allergen.

3.1.3 Methods. Allergization period: 20μl TDI oil solution was dripped into the bilateral anterior nostrils of the rabbits with a pipette, 10μl per side, once a day for seven days. Provocation period: From day nine dripped once every other day for eight days, total four provocations. The nasal symptoms were observed for 30 minutes after every provocation and were observed for 30 minutes again after 2 hours, then were scored (Table 1). According to the standard superposition method, the total score of each rabbit was calculated. The rabbit with more than five points was an established model[19].

Table 1: Scoring criteria for allergic rhinitis models in rabbits

| degree of nasal itching | sneezing         | runny nose                        |
|-------------------------|------------------|----------------------------------|
| 1 point                 | scratching several times | 1-3, flowing to anterior nostrils |
| 2 points                | scratching faces constantly | 4-10, beyond anterior nostrils    |
| 3 points                | rubbing          | ≥11, faces full of tears         |

3.2 Groups and dripping noses
40 allergic rhinitis models in rabbits were randomly divided into control group, Acusine group, new spray group, 10 rabbits each group. The four groups were sprayed nasally with saline, Acusine spray and new spray respectively, three times/d, for 30 days.

3.3 Nasal resistance measurement
The nasal resistances in the four groups were measured with a rhinoresistometer.

3.4 Pathological examination
The four groups were sacrificed. Their nasal mucosa was taken for HE staining. Their pathological manifestations were observed under light microscopy[20-22].

If the new type of natural medicine spray against acute and chronic inflammation, nasal resistance of new natural medicine group rabbits was significantly lower than that of Acusine group, beclomethasone group and blank control group (P < 0.05), and nasal mucosa do pathological examination showed that the pathological model of natural medicine group to eliminate the rabbit allergic rhinitis change is better than that of Acusine group and beclomethasone group and control group, and no drug rhinitis, new natural medicine spray proof developed has good curative effect and adverse reaction of drug rhinitis on allergic rhinitis.

4. The Results

4.1. Effect of new nasal spray made from natural medicines on passive cutaneous anaphylaxis in BALB/c mice:
Absorption of Evan’s blue (620nm) of beclomethasone dipropionate group, Acusine group and new
spray group will be significantly lower than control group ($P<0.05$). Absorption of Evans’s blue (620nm) of new spray group will be significantly lower than Acusine group ($P<0.05$) and will have no significantly difference compared with beclomethasone dipropionate group ($P>0.05$). Absorption of Evans’s blue (620nm) of beclomethasone dipropionate group will be significantly lower than Acusine group ($P<0.05$).

4.2. Effect on allergic rhinitis in rabbits:

4.2.1 Nasal resistance measurement. The nasal resistances of beclomethasone dipropionate group, Acusine group and new spray group will be significantly lower than control group ($P<0.05$). The nasal resistances of new spray group will be significantly lower than Acusine group ($P<0.05$) and will have no significantly difference compared with beclomethasone dipropionate group ($P>0.05$). The nasal resistances of beclomethasone dipropionate group will be significantly lower than Acusine group ($P<0.05$).

4.2.2 Pathological examination. The nasal mucosa of control group will be typical nasal mucosa of allergic rhinitis, such as separated epithelium, loose submucosa, edema in interstitial space, dilatated blood vessels, eosinophil-dominated inflammation cells filtrated in lamina propria. The nasal mucosa of beclomethasone dipropionate group, Acusine group and new spray group will be better than control group, i.e. more complete and evenly aligned epithelium, less edema in interstitial space, less dilatated blood vessels. Of them, new spray group will be the best. The nasal mucosa of beclomethasone dipropionate group will show the manifestation of drug-induced rhinitis.

5. Discussion

Acusine Nasal Spray is a popular OTC for allergic rhinitis in Europe and the U.S. [9], but no literatures on it were retrieved in Pubmed. Fortunately, its detailed information including its ingredients, effect, indication, etc can be openly accessed. Its ingredients consist of hydrastis Canadensis, ephedra vulgaris, lycopodium clavatum, mezereum, petroleum, pulsatilla, thuja occidentalis. These natural medicines from Europe have antianaphylaxis and anti-inflammation effects. Similarly, the essential traditional Chinese medicines contained in the marketed drugs for allergic rhinitis in China such as cocklebur fruit, flos magnoliae, mint, herba centipedae, scutellaria baicalensis, hibiscus mutabilis, angelica dahurica, cordate houttuynia, herba asari, astragalus mongholicus, atracyloides macrocephala, radix saposhnikoviae also have such effects. Moreover, some of them such as scutellaria baicalensis, cordate houttuynia still have antibacterial effect [23-24]. Astragalus mongholicus, atracyloides macrocephala and radix saposhnikoviae together, called yupingfeng powder, exert immune regulation and enhance immunity of immunosuppressive mice through adjusting nonspecific and cellular immunity, inhibit the activity of mast cells in allergic rhinitis and exert antioxidant effect [25-27]. On the other hand, according the theory of traditional Chinese medicine, these Chinese medicines can treat "biqiu" by relieving stuffy nose and supplementing spleen Qi and lung Qi [10]. So, integration of the two kinds of natural medicines from Europe and Chinese will have a synergistic treatment effect on allergic rhinitis.

The designed study will investigate the effect of new nasal spray on allergic rhinitis in vitro and in vivo by an experiment in mice and an experiment in rabbits respectively. If absorption of Evans’s blue (620nm) of new spray group is significantly lower than Acusine group ($P<0.05$) and have no significantly difference compared with beclomethasone dipropionate group ($P>0.05$) and absorption of Evans’s blue (620nm) of beclomethasone dipropionate group is significantly lower than Acusine group ($P<0.05$), then the new spray will have a higher anti-type I allergic effect, similar with beclomethasone dipropionate, than Acusine Nasal Spray in vitro. In vivo, if the nasal resistances of new spray group is significantly lower than Acusine group ($P<0.05$) and have no significantly difference compared with beclomethasone dipropionate group ($P>0.05$) and the nasal resistances of beclomethasone dipropionate group is significantly lower than Acusine group ($P<0.05$), then the new spray will have a better treatment effect on allergic rhinitis, similar with beclomethasone dipropionate,
than Acusine Nasal Spray in vivo. Furthermore, if among the four groups, pathological observation of the nasal mucosa of new spray group is the best without the manifestation of drug-induced rhinitis shown in beclomethasone dipropionate group, then a conclusion can be drawn that the invented new spray, or improved Acusine Nasal Spray, will not only have a better treatment effect on allergic rhinitis, similar with beclomethasone dipropionate, than Acusine Nasal Spray but also will not leave side effect on nasal mucosa, compared with beclomethasone dipropionate. Then the new spray made from natural medicines can be further studied as a better prescription drug, rather than an OTC like Acusine Nasal Spray, to replace beclomethasone dipropionate nasal spray.

To make the above ideas become reality, the study and its further studies need the funds from foundations home or aboard, Nestmann Pharma or other visionary pharmaceutical companies, to be supported for its excellent academic and commercial interests.

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