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A randomized controlled trial of low-dose recombinant human interferons α-2b nasal spray to prevent acute viral respiratory infections in military recruits

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ABSTRACT

The military population has a high disease burden of acute viral respiratory infections in China. To assess the efficacy and safety of a low-dose recombinant human interferon α-2b (rIFNα-2b) nasal spray in preventing acute viral respiratory infections in military population, we performed this randomized controlled trial. The results showed that application of the rIFNα-2b nasal spray had the benefits in prevention of infections caused by influenza A virus, influenza B virus parainfluenza viruses 1–3 and adenovirus species B. However, no benefit was seen in preventing respiratory syncytial virus. No severe adverse events were reported. Therefore, the rIFNα-2b nasal spray was effective and well tolerated for preventing common viral respiratory infections in the military recruits.

1. Introduction

Acute respiratory tract infections are flourishing in closed and crowded environments. Military recruits are prone to outbreaks of acute respiratory tract infections because of their crowded living conditions in barracks, stressful work environments, frequent travels and exposure to novel strains of respiratory pathogens [1–3]. Over 90% of acute respiratory tract infections are caused by viruses, such as influenza virus, parainfluenza virus, respiratory syncytial virus, rhinovirus, adenovirus and coronavirus. Though most of viral respiratory infections usually represent mild, self-limited clinical manifestations, they are leading causes of morbidity in certain groups or populations (e.g., children, military population) and remain a heavy burden of disease [4,5]. In history, the famous "Spanish influenza" initiated from army recruits in 1916 [6]. In 1976, the novel A/New Jersey/76 (Hsw1N1) influenza virus caused severe respiratory illness in soldiers at Fort Dix [1]. Adenoviruses have been the most important cause of febrile acute respiratory disease in US military recruit populations [7]. In China, a comprehensive surveillance systems for influenza in military population showed that the average incidence of influenza was 1.4 episode per person-year from 1995 to 2000 [8]. Viral respiratory infections have become one of the most actual health problems in military population. Therefore, it is necessary to seek reasonable and effective measures to prevent outbreaks and epidemics of acute viral respiratory infections among military population.

Interferons (IFNs) are a family of cytokine mediators that are critically involved in alerting the cellular immune system to viral infections of host cells [9]. The previous studies have suggested that high dosage of intranasal interferons can prevent respiratory infections caused by viruses, such as influenza virus, rhinovirus, coronavirus and respiratory syncytial virus [10–14]. However, the major problem is higher frequency of local side effects such as mucosal irritation, dry mucous membranes, blood-tinged mucus and nasal mucosal erosion [15–17]. Recently, YUANCE Medicine Company (Beijing, China) has developed a low-dose recombinant human interferon α-2b (rIFNα-2b) nasal spray in order to reduce adverse reactions. To evaluate the efficacy and safety of this new nasal spray in preventing acute respiratory infections in military population, we performed this randomized, placebo-controlled, double-blind trial.

2. Materials and methods

2.1. Subjects

From November 2005 to December 2005, we did a randomized, placebo-controlled, double-blind, multi-center trial in the military trainees from 12 recruit training units in three geographically distinct cities in China (Guangzhou city and Foshan city in Guangdong

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province, Liuzhou city in Guangxi province). Candidate subjects were male recruits who aged 16–23 years, and finished army physical fitness examination. They were not admitted to the study if any of the following criteria were present: (1) on regular medical treatment or took other medications within two weeks, (2) history of serious allergies (e.g., asthma, urticaria, and eczema), (3) history of autoimmune disorders, (4) psychiatric disorders, and (5) acute or chronic illnesses. All candidates were observed two weeks before enrolling into the study, in order to screen eligible subjects. A total of 1500 trainees from three areas enrolled the trial initially. After the screening had been completed, 1449 eligible recruits remained in the subject pool. All participants understood the implications of the study, and provided informed consents.

The study was conducted during the basic training period of new military recruits at 12 recruit training units located in different districts. There were 120–150 new recruits in each independent training unit. All the trainees lived in barracks during a three-month training. Eight to 10 recruits shared a quarters room about 30 m^2.

2.2. Intervention

Participants in the experimental group received the rIFNα-2b nasal sprays, the metered spray device delivered 0.1 ml (3×10^5 IU of rIFNα-2b) per spray into each nostril and throat, that was a total of 9×10^5 IU of rIFNα-2b for each administration. The spray delivered twice daily after breakfast and supper respectively for five consecutive days. The control group was given placebo in the manner identical to the experimental group. The placebo contained components which were similar to the drug except rIFNα-2b. Participants were given instructions on when and how to use the sprays. Each nostril and pars laryngea pharyngis should be sprayed with breathing deeply. The rIFNα-2b nasal spray and placebo were stored at 4–8 °C at health clinics of training units. All subjects were followed up and observed for clinical signs and manifestations of respiratory infections for 10 days. Local symptoms (e.g., sore throat, dry pharynx, cough, nose running, sneezing, nose congestion), systemic symptoms (e.g., malaise, myalgia, headache, nausea, abdominal pain, diarrhea) and axillary temperature were recorded daily during the observed period. Severe adverse reactions (such as allergy, epistaxis, and nasal mucosa erosion) or complications should be reported immediately. Once the adverse reactions had occurred, subjects would stop the experiment and be given suitable treatments.

2.3. Sample size

Assuming a 18% viral respiratory infections attack rate in the group that received placebo and a 8% attack rate in the group that received rIFNα-2b nasal spray (based on the data obtained from our preliminary studies) and assuming that sufficient data would be collected for 90% of the cases to be included in the according-to-protocol population, we calculated that a sample of 508 recruits per group would provide more than 90% power to demonstrate the superiority of the rIFNα-2b nasal spray at a significant difference level of 0.01 (two tailed).

2.4. Randomization and blinding

A list of random numbers allocating to the each spray canister was determined via computer-generated randomization. The generation of randomized numbers and labeling of the spray canisters were performed by the third party (National Institute for Viral Disease Control and Prevention, China Center for Disease Control and Prevention) of this study. Participants were sequentially allocated to the treatments in the order in which they were recruited, i.e., the first person who was eligible for inclusion was given spray number 1, the second one spray number 2, and so on. When allocated, each participant's name was added to the label details on the spray container. The sequence was concealed until the data were analyzed. Both participants and researchers were blind to group assignment. Once accuracy of the data were confirmed, the database would be forwarded to the statistician who, only at this time, was supplied with the randomized list.

2.5. Follow-up and evaluation

Case report forms (CRF) consisted of subject demographics data, medical history, respiratory infection symptoms, adverse reactions including local reactions and systemic reactions and concomitant medications from the time that the spray was administered until 10 days later. Participants were given a diary CRF to record the spray administration, clinical signs and manifestations of respiratory diseases, adverse effects they might have experienced, and other medications they might have taken. Observers visited the participants daily and recorded severe adverse events, including high fever (axillary temperature, ≥39.0 °C), allergy, epistaxis, nasal mucosa erosion and hemafecia.

Serum samples for assessment of viral respiratory infections were collected on the days 0 and day 15 after the administration. ELISA (enzyme-linked immunosorbent assay) kits (Shenzhen Sciarray Biotech Co. LTD) were used to test IgM antibodies against adenovirus species B (ADV), respiratory syncytial virus (RSV), influenza A virus (Flu-A), influenza B virus (Flu-B), and parainfluenza viruses 1–3 (PIV 1–3). Coating antigens used in the kits were prepared from adenovirus (Strain adenoid 6), respiratory syncytial virus (RSV Long Strain), influenza A virus (H3N2, Strain A/Texas 1/77), influenza B virus (Strain HongKong 5/72), parainfluenza virus type 1(Strain VP1), parainfluenza virus type 2 (Strain Greer) and parainfluenza virus type 3 (Strain C243). The operating procedures of ELISA were according to the manufacturer's instructions. Briefly, 100 μl of 1:40 diluted serum specimens was applied to each well of the microtiter plate, the positive and negative standards (provided by the manufacturer) and blank control (dilution solution) were running with each plate to ensure accuracy, then the plate was incubated for 30 min at 37 °C. The specimen was removed and the plate was washed four times by washing solution. Anti-human IgM (μ-chain specific) conjugated to horse radish peroxidase was added and incubated for 30 min at 37 °C. The plate was washed four times again. TMB (3,3′,5,5′-tetramethylbenzidine) solution was added to each well and incubated for 15 min. The reaction was stopped by adding 100 μl stop solution (2M H_2SO_4) to each well and the optical density (OD) value at 450 nm was determined with an ELISA reader. Serum specific-IgM antibodies were defined as positive if OD 450 values were greater than two fold negative standard.

We compared the positive rates of the viral IgM antibodies between two groups. The subject whose serum IgM against any of five viruses was positive on the initial administration (day 0) was excluded for serological analysis. The recruit whose antibody was negative on day 0, positive on day 15 after the administration, was considered as having a recent infection with the corresponding virus. Serum specimens were collected from 1449 participants and detected for antibodies against three viruses (Flu-A, Flu-B and PIV1-3), among which 548 of specimens were detected for antibodies against five viruses (ADV, RSV, Flu-A, Flu-B and PIV1-3).

2.6. Statistical analysis

All study data were checked for range and consistency, and were double entered into databases. Data of antibodies were entered into Microsoft Excel 2003, while CRF and adverse events data were entered into Epidata3.1.

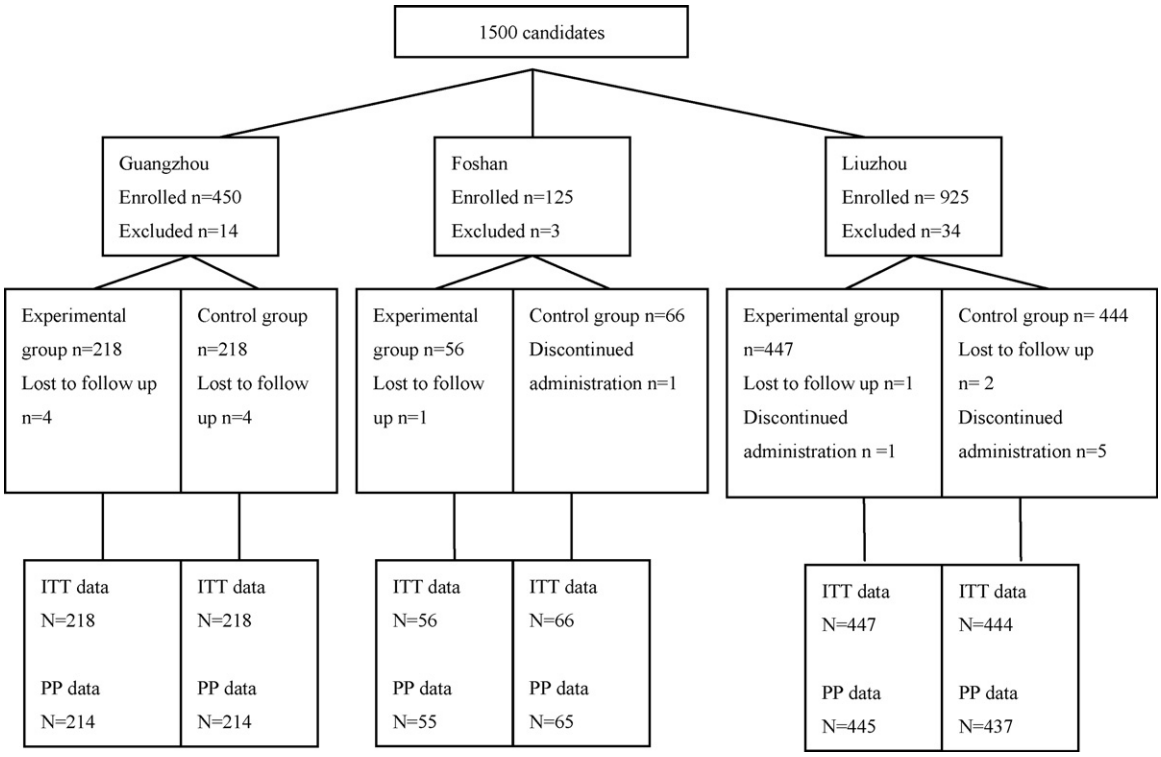

Fig. 1. The flow chart of the randomized controlled trial of the recombinant of human interferon α-2b nasal spray to prevent acute viral respiratory infections in military recruits.

All analyses were performed by using the SPSS 13.0 statistical software package. A descriptive statistical analysis was carried out to compare the baseline characters between two groups. Infection rates and adverse events were analyzed by χ^2 test. For the statistical analysis of body temperature parameters, a repeated measures ANOVA model was used. The level of statistical significance was established as $p < 0.05$. Meanwhile, intention-to-treat (ITT) and per protocol (PP) analysis were performed as the assessment. Parameters for assessment of benefits or harms of the intervention included: the rate at which events occur in the control group (the control event rate, CER), the rate at which events occur in the experimental group (the experimental event rate, EER), relative risk (RR), absolute risk reduction (ARR), relative risk reduction (RRR), and number needed to treat (NNT). Measures of RR, RRR, ARR and NNT were determined as these following formulae: RR = EER/CER, ARR = CER–EER, RRR = ARR/CER, NNT = 1/ARR [18].

2.7. Ethical approval

The research protocol was followed to the tenets of the Declaration of Helsinki and the Guidelines for Good Clinical Practice. The clinical trial (protocol number 2003L01500) was officially approved by State Food and Drug Administration, PR China in April 2003, and also approved by Ethical Committee of Southern Medical University.

3. Results

3.1. Recruitment and participant flow

Of 1500 recruits screened, 1449 recruits were eligible for the trial criteria. They were randomized to receive either treatment sprays ($n = 721$) or placebo control sprays ($n = 728$) twice daily for 5 days. During the trial, 12 recruits were lost to follow-up, among which, 6 in the experiment group and 6 in the control group. Another 7 subjects dropped off the study due to they were afraid of adverse events psychologically and quit the trial by themselves. Subjects who completed the study are shown in Fig. 1.

3.2. Baseline data

All subjects were male, recruited from different provinces, such as Guangdong, Guangxi, Hubei, Liaoning, Shandong. The mean age of subjects was 18.07 ± 1.05 years (range 18–23 years). The differences of age, educational level and other demographic characteristics were not statistical significance between two groups (Table 1). Compliance rates between two groups were not statistically significant different, which the experimental group was 99.03% and the control group was 98.35% ($p = 0.257$).

3.3. Effect assessment

The positive rates of IgM antibodies against the viruses in recruits whose were negative before the intervention are summarized in Table 2 (pp analysis) and Table 3 (ITT analysis). IgM positive rates of anti-ADV, anti-Flu-A, anti-Flu-B, and anti-PIV in the control group (CER) were significantly higher than that in the experimental group (EER) ($p < 0.05$) during the observational period. Although positive rate of anti-RSV IgM in the experimental group was higher than that in the control group, no significant difference was found. Values of RR were less one and their 95% confidence intervals (95% CI) did not cross one except for RSV that the 95% CI included one (Table 4). The results indicate that the risk of developing viral respiratory infections is less in the treatment group than that in the control group.

The PP analysis exhibited that RRRs (i.e., protection rates) of the rIFNα-2b against ADV, RSV, Flu-A, Flu-B and PIV1-3 were 59.4% (95% CI: 0.1–83.5%), 72.1% (95% CI: −35.6% to 94.3%), 76.4% (95% CI: 63.7–84.7%), 76.2% (95% CI: 61.2–85.4%), and 77.4% (95% CI: 63.2–86.1%), respectively (Table 4). Except for RSV, application of

Table 1
Baseline characteristics of participants.

	Experimental group (n = 721)	Control group (n = 728)	Test statistics	p
Age, Mean ± SD (years)	18.08 ± 1.06	18.07 ± 1.05	t = 0.188	0.851
Race				
Minority	55	58	χ^2 = 0.058	0.81
Han people	666	670		
Educational level				
Middle school	215	237	χ^2 = 0.1686	0.43
High school	471	462		
College	35	29		
Native place				
Southern	238	245	χ^2 = 0.068	0.799
Northern	483	483		
Household registered				
Countryside	396	406	χ^2 = 0.105	0.746
City	325	322		
Incompliant rate, N (%)	7 (0.97%)	12 (1.65%)	χ^2 = 1.258	0.257

Table 2
The detection of specific serum IgM antibodies to five viruses in the per protocol analysis.

Viruses	Experimental group		Control group		χ^2	p
	Total, N	Positive of serum IgM, N (%)	Total, N	Positive of serum IgM, N (%)		
ADV	229	7 (3.1)	236	17 (7.2)	4.083	0.043
RSV	251	2 (0.8)	250	7 (2.8)	1.827	0.177
Flu-A	466	30 (6.4)	452	102 (22.6)	48.480	0.000
Flu-B	514	22 (4.3)	512	81 (15.8)	37.824	0.000
PIV1-3	537	22 (4.1)	535	85 (15.9)	41.469	0.000

Table 3
The detection of specific serum IgM antibodies to five viruses in the intention-to-treat analysis.

Viruses	Experimental group		Control group		χ^2	p
	Total, N	Positive of serum IgM, N (%)	Total, N	Positive of serum IgM, N (%)		
ADV	234	7 (3.0)	240	17 (7.1)	4.127	0.042
RSV	256	2 (0.8)	255	7 (2.8)	1.826	0.177
Flu-A	472	30 (6.4)	460	104 (22.6)	49.988	0.000
Flu-B	519	22 (4.2)	520	84 (16.2)	40.251	0.000
PIV1-3	544	22 (4.0)	545	86 (15.8)	41.971	0.000

the rIFNα-2b decreased significantly the infection rates for ADV, Flu-A, Flu-B and PIV1-3 in the subjects. The NNTs of preventing ADV, RSV, Flu-A, Flu-B and PIV1-3 were 24(95% CI: 12.1–446.2), 50 (95% CI: −313.6 to 23.1), 7 (95% CI: 4.9–8.6), 9 (95% CI: 6.6–12.6) and 9 (95% CI: 6.5–12.1), which meant that 24 (12.1–446.2), 50 (−313.6 to 23.1), 7 (4.9–8.6), 9 (6.6–12.6) and 9 (6.5–12.1) subjects should be administrated with the rIFNα-2b nasal spray in order to prevent one case of the viral respiratory infection, respectively. The ITT analysis exhibited that RRRs (i.e., protection rates) of the rIFNα-2b against ADV, RSV, Flu-A, Flu-B and PIV1-3 were 59.5% (95% CI: 0.6–83.5%), 72.1% (95% CI: −35.6% to 94.3%), 76.8% (95% CI: 64.3–84.9%), 77.0% (95% CI: 62.6–85.9%), and 77.5% (95% CI: 63.5–86.1%), respectively, NNTs were 25 (95% CI: 12.5–553.9), 51 (95% CI: −319.3 to 23.6), 7 (95% CI: 4.8–8.4), 8 (6.1–11.3) and 9 (95% CI: 6.6–12.1), respectively (Table 5). The results showed that the PP analysis was consistent with the ITT analysis.

To sum it up, protective efficacy of the rIFNα-2b against four viruses arranged in descending order was Flu-A, PIV1-3, Flu-B, ADV. However, there was a 95% certainty that the rIFNα-2b had no effect for RSV because the 95% confidence intervals for the RR, RRR and NNT extended from a negative number (treatment may harm) to a positive number (treatment may benefit) (Tables 4 and 5).

3.4. Safety assessment

No participants withdrew from the trial due to intolerance of the spray. None of the participants were found to have allergy, high fever, nasal mucosa erosion or hemafecia during the follow-up

Table 4
Protective effects of the rIFNα-2b nasal spray against viral respiratory infections (per protocol analysis).

Viruses	EER (%)	CER (%)	RR (95% CI)	ARR (%) (95% CI)	RRR (95% CI)	NNT (95% CI)
ADV	3.1	7.2	0.406 (0.165–0.999)	4.1 (0.2–8.6)	0.594 (0.001–0.835)	24 (12.1–446.2)
RSV	0.8	2.8	0.279 (0.057–1.356)	2.0 (−0.3 to 4.3)	0.721 (−0.356 to 0.943)	50 (−313.6 to 23.1)
Flu-A	6.4	22.6	0.236 (0.153–0.363)	16.1 (11.7–20.6)	0.764 (0.637–0.847)	7 (4.9–8.6)
Flu-B	4.3	15.8	0.238 (0.164–0.388)	11.5 (7.9–15.2)	0.762 (0.612–0.854)	9 (6.6–12.6)
PIV1-3	4.1	15.9	0.226 (0.139–0.368)	11.79 (8.3–15.3)	0.774 (0.632–0.861)	9 (6.5–12.1)

EER: experimental event rate; CER: control event rate; RR: relative risk; ARR: absolute risk reduction; RRR: relative risk reduction (equal to protective rate); NNT: number needed to treat.

Table 5
Protective effects of the rIFNα-2b nasal spray against viral respiratory infections (intention-to-treat analysis).

Viruses	EER (%)	CER (%)	RR (95% CI)	ARR (%) (95% CI)	RRR (95% CI)	NNT (95% CI)
ADV	3.0	7.1	0.405 (0.165–0.994)	4.1 (0.2–8.0)	0.595 (0.006–0.835)	25 (12.5–553.9)
RSV	0.8	2.7	0.279 (0.057–1.356)	2.0 (−0.3 to 4.2)	0.721 (−0.356 to 0.943)	51 (−319.3 to 23.6)
Flu-A	6.4	22.6	0.232 (0.151–0.357)	16.3 (11.8–20.7)	0.768 (0.643–0.849)	7 (4.8–8.4)
Flu-B	4.2	16.2	0.230 (0.141–0.374)	12.6 (8.9–16.3)	0.770 (0.626–0.859)	8 (6.1–11.3)
PIV1-3	4.0	15.8	0.225 (0.139–0.365)	11.8 (8.3–15.2)	0.775 (0.635–0.861)	9 (6.6–12.1)

EER: experimental event rate; CER: control event rate; RR: relative risk; ARR: absolute risk reduction; RRR: relative risk reduction (equal to protective rate); NNT: number needed to treat.

observational period after administration. We found some flu-like symptoms including cough, sneeze, nose congestion and nose running were slightly higher in the drug group than those in the control group in the period of administrating the nasal sprays, particularly during the second to fourth days of the experiment, however, the differences were not significant ($p > 0.05$). The incidence rates of epistaxis in the treatment group were low, with from 1.2% (9/721) to 6.2% (45/721) during the period of administration, although the occurrences were significant higher in the treatment group than that in the control group on the third day (5.9%, 43/721, versus 2.1%, 15/728, $p < 0.001$) and the fourth day (6.2%, 45/721, versus 2.9%, 21/728, $p = 0.003$). The occurrence of dry pharynx was significantly higher in the treatment group than that in the control group during the whole drug administration period (21.3–31.9% in the treatment group, 12.1–20.7% in the control group). Average incidences of dry pharynx and epistaxis were higher in the experimental group (dry pharynx 27.94%, epistaxis 4.6%) than those in the control group (dry pharynx 16.16%, epistaxis 2.58%) ($p < 0.05$) during the follow-up period (Table 6). The peak of these symptoms was found during the first 5 days, thereafter declined. Both of the experimental group and the control group had high rates of myalgia (37.80%, 39.50%), arthralgia (21.50%, 21.44%). These symptoms happened after high intensity training and without significant difference between the two groups.

4. Discussion

Viral respiratory infections are caused by a variety of viruses, among which there are approximately 200 known ones including a vast number of serotypes, and undergo frequent changes in antigenicity [19]. Although several vaccines against respiratory viruses to prevent the infections have been proved useful in military populations [7,20–22], progress has been extremely slow. Furthermore, not all kinds of viral etiological respiratory infections have been available for specific prevention and still have unknown pathogens. In the recent decade, several novel respiratory viruses which caused serious illness have been identified, such as human metapneumovirus (Hmpv), new SARS-coronavirus (SARS-CoV) that associated with severe acute respiratory syndrome (SARS), H5N1 flu virus and novel H1N1 virus, which increase the difficulty of the immunological prevention for viral respiratory infections. In the past years, researchers found that IFN-α showed an inhibitory effect for SARS-CoV in vitro and in vivo [23–25]. Therefore, it suggests the potential benefits of IFN-α in preventing and controlling viral respiratory infections in specific population groups and in epidemic period.

Table 6
Comparison of the incidences of clinical features between the experimental group and the control group.

Symptoms	ARC (%)	ART (%)	RR	RR 95% CI	χ^2	p
Cough	10.94	11.62	1.06	0.79–1.418	0.158	0.691
Productive cough	10.44	11.52	1.10	0.82–1.479	0.426	0.514
Sneezing	8.46	8.94	1.06	0.76–1.481	0.059	0.808
Congested nose	16.3	17.46	1.07	0.85–1.346	0.329	0.566
Running nose	24.54	27.94	1.14	0.96–1.356	2.027	0.155
Dry pharynx	16.16	27.46	1.70	1.39–2.077	26.901	0.000
Sore throat	7.82	8.88	1.15	0.82–1.627	0.519	0.471
Epistaxis	2.58	4.60	1.85	1.06–3.226	4.051	0.044
Headache	3.32	2.88	0.84	0.47–1.509	0.178	0.674
Malaise	11.08	10.40	0.93	0.69–1.259	0.198	0.657
Abdominal pain	2.66	2.64	1.01	0.54–1.892	0.001	0.976
Diarrhea	4.34	4.20	0.98	0.60–1.60	0.049	0.825
Myalgia	39.50	37.82	0.96	0.84–1.09	0.439	0.507
Arthralgia	21.44	21.50	1.00	0.82–1.22	0.001	0.974
Rash	1.28	1.08	0.79	0.29–2.09	0.050	0.823

ARC: adverse event rate of control group; ART: adverse event rate of experimental group; RR: ART/ARC.

The common viral respiratory infections possess similar clinical signs and symptoms without special clinical manifestations, explicit diagnosis only depends on pathogen examination. Although isolation of virus from patients is the strongest evidence for confirming viral respiratory infections, the diagnostic procedures are complex and time-consuming because of the wide range of viruses, especially in a large sample epidemiological study. In addition, respiratory viruses are frequently detected in respiratory tract secretions samples from healthy people [26,27]. The determinations antibodies against respiratory viruses by comparing acute and convalescent serum specimens in infective people may sometimes be helpful to confirm a specific causative infection. Serum specific IgM against virus is a sensitive indicator of a recent onset of viral infection, thus, we used specific serum IgM as surrogate outcome variables to evaluate the preventive effects of the rIFN-α 2b for acute viral respiratory infections in this study.

The RR, RRR and ARR are common parameters used in reporting randomized clinical trials and epidemiological field trails. The RR is a ratio of the probability of the event rate occurring in the exposed (or experimental) group (EER) versus a non-exposed (or control) group (CER). In interventional trials, if the treatment arm is effective in preventing disease then the RR will be less than one, and vice versa. The RRR is the percent reduction in events in the EER compared with the CER. In other word, the RRR presents the percentage of the risk that has been reduced by the intervention in the control group. The ARR is the arithmetic difference in the event rate between treatment group and control group. In interventional trials, if the treatment arm is effective in preventing disease then ARR will be positive quantity, on the contrary, if the treatment arm is harm, ARR will be negative. RR and RRR can be used to quantify the relative magnitude of the protective (treatment) effects. The ARR can be used to measure the absolute difference in event rates between two populations. Therefore, ARR is considered as a more intuitive measure than RR and RRR. These years, the number needed to treat (NNT) has become a widely used index for interpreting the magnitude of treatment benefits or harms. The NNT is the inverse of the ARR. It represents the expected number of persons who must be treated with an intervention in order to prevent one additional adverse outcome event (or, depending on the context, to expect one additional beneficial outcome), compared to the expected event rates under the control. That is to say, the smaller the NNT, the more effective the treatment. Therefore, besides RR, RRR and ARR, we used the NNT to express the size of efficacy of the rIFNα-2b in the present analysis.

In our study, serological detection exhibited that serum positive rates of IgM antibodies against the five viruses were higher in the control group than those in the experimental group on the 15th day of the administration. In ITT analysis, highly antiviral effects for Flu-A, Flu-B and PIV1-3 were seen, all with the RR of <1 and the RRR (i.e., protective rates) of >70%. A protective effect also was seen for ADV, with the RR of <1 and the RRR of 59.5%. However, there were wide range of 95% confidence intervals of the RR (0.165–0.999) and RRR (0.6–83.5%), which indicated the low reliability. No certain effect was found for RSV. Although the RR was less than 1 and RRR = 72.1%, the 95% CI ranges of RR (0.057–1.356) and RRR (−35.6% to 94.3%) were very wide and included null values (RR 95% CI included 1, RRR 95% CI included negative). Because of small size samples for detecting serum ADV antibodies and RSV antibodies and less people of anti-ADV and anti-RSV IgM positive in both groups and a very wide confidence interval of protection rates in ADV and RSV, we considered that the sample of this study was not enough for assessing preventive effects of the rIFN-α2b for these two viruses.

In the ITT analysis of the study, NNT values were 7 (95% CI: 4.8–8.4), 8 (95% CI: 6.1–11.3) and 9 (95% CI: 6.6–12.1) for Flu-A, Flu-B and PIV1-3 infections respectively, which meant 7, 8 and 9 people need to administrate the rIFN-α2b to prevent one infection with relevant virus. The NNT for ADV was 25 (95% CI: 12.5–553.9), which indicated that the benefits of preventing ADV infection with the rIFN-α2b may be less than above infections with any three viruses above. The NNT for RSV was 50 (95% CI: −313.6 to 23.1), which indicated no effect in preventing RSV infection among recruits with the rIFN-α2b nasal spray.

ITT analysis is a method of analysis for randomized trials in which all subjects randomly assigned to one of the treatments are analyzed together, regardless of whether they completed or received that treatment or not. On the other hand, PP analysis is a method based only on those patients who complete the entire treatment protocol. In this study, similar results were observed for subjects in the ITT and PP populations, which indicated no significant missingness and protocol deviation.

On the whole, the intranasal rIFNα-2b with relative low dose and short term administration (1.8×10^6 IU daily for five days) was well tolerated. Most of the clinical features reported were comparable between the control and the experimental groups. Except epistaxis, no other known severe adverse events (such as allergy, high fever, nasal mucosa erosion and hemafecia) of the intranasal interferon were reported during the trial. The RRs of dry pharynx and epistaxis were 1.70 (95% CI: 1.39–2.08) and 1.85 (95% CI: 1.06–3.23), respectively, which indicated that dry pharynx and epistaxis might be linked to the interferon. However, the incidence of epistaxis was low and the clinical signs were mild and transitory in present study. The results are the same as our previous study [28] and the risk is much lower than that appraised and summarized in evidence-based medicine (OR = 4.52, 95% CI = 3.78–5.41) by Jefferson and Tyrrell [29].

In summary, this randomized controlled trial suggested that the recombinant human interferon α-2b nasal spray can be used to prevent common acute viral respiratory infections caused by Flu-A, Flu-B, PIV1-3 and ADV and was generally well tolerated among military recruits. However, the limitations of this trial may be found on sampling and sample size. All subjects enrolled were healthy and young male recruits, so it may be difficult to extrapolate the conclusion to other populations. The sample was not large enough for evaluating the effects for ADV and RSV infections which had relative low incidence in the army recruits. The efficacy of preventing viral respiratory infections by the rIFNα-2b nasal spray should be evaluated further in different population groups, such as children and the elderly, and more samples should be involved in the further study.

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References

[1] Gaydos JC, Top Jr FH, Hodder RA, Russell PK. Swine influenza A outbreak, Fort Dix, New Jersey, 1976. Emerg Infect Dis 2006;12(1):23–8.
[2] McNeill KM, Vaughn BL, Brundage MB, Li Y, Poropatich RK, Gaydos JC. Clinical presentations for influenza and influenza-like illness in young, immunized soldiers. Mil Med 2005;170(1):94–7.
[3] Sencer DJ, Millar JD. Reflections on the 1976 swine flu vaccination program. Emerg Infect Dis 2006;12(1):29–33.
[4] Ison MG, Mills J, Openshaw P, Zambon M, Osterhaus A, Hayden F. Current research on respiratory viral infections: fourth international symposium. Antiviral Res 2002;55(2):227–8.
[5] Kolavic-Gray SA, Binn LN, Sanchez JL, Cersovsky SB, Polyak CS, Mitchell-Raymundo F, et al. Large epidemic of adenovirus type 4 infection among military trainees: epidemiological, clinical, and laboratory studies. Clin Infect Dis 2002;35(7):808–18.
[6] Löwy I. The social history of medicine: beyond the local. Soc Hist Med 2007;20(3):465–81.
[7] Lyons A, Longfield J, Kuschnera R, Straight T, Binn L, Seriwatana J, et al. A double-blind, placebo-controlled study of the safety and immunogenicity of live, oral type 4 and type 7 adenovirus vaccines in adults. Vaccine 2008;26(23):2890–8.
[8] Peiruo Duan, Chungang Ma, Lili Zhang, Hongyan Han, Ronghui Zhang, Zhao Hua, et al. Influenza surveillance on the army population. J Prev Med Chin PLA 2002;20(1):23–6.
[9] Katerina MA, Emmanouil A, Demosthenes B. Interferon and their application in the diseases of the lung. Chest 2003;123(1):209–16.
[10] Higgins PG, Barrow GI, Tyrrell DA, Isaacs D, Gauci CL. The efficacy of intranasal interferon alpha-2a in respiratory syncytial virus infection in volunteers. Antiviral Res 1990;14(1):3–10.
[11] Turner RB, Felton A, Kosak K, Kelsey DK, Meschievitz CK. Prevention of experimental coronavirus colds with intranasal alpha-2b interferon. J Infect Dis 1986;154(3):443–7.
[12] Sung RY, Yin J, Oppenheimer SJ, Tam JS, Lau J. Treatment of respiratory syncytial virus infection with recombinant interferon alfa-2a. Arch Dis Child 1993;69(4):440–2.
[13] Douglas RM, Moore B, Miles HB, Pinnock CB. Could preventive intranasal interferon lower the morbidity in children prone to respiratory illness? Med J Aust 1990;152(10):527–8, 524.
[14] Monto AS, Shope TC, Schwartz SA, Albrecht JK. Intranasal interferon-alpha 2b for seasonal prophylaxis of respiratory infection. J Infect Dis 1986;154(1):128–33.
[15] Scott GM, Onwubalili JK, Robinson JA, Doré C, Secher DS, Cantell K. Tolerance of one-month intranasal interferon. J Med Virol 1985;17(2):99–106.
[16] Samo TC, Greenberg SB, Palmer JM, Couch RB, Harmon MW, Johnson PE. Intranasal applied recombinant leukocyte A interferon in normal volunteers. II. Determination of minimal effective and tolerable dose. J Infect Dis 1984;150(2):181–8.
[17] Hayden FG, Mills SE, Johns ME. Human tolerance and histopathologic effects of long-term administration of intranasal interferon-alpha 2. J Infect Dis 1983;148(5):914–21.
[18] Forrest GL. Glossary of evidence-based terms. J Evid Base Dent Pract 2008;8:55–61.
[19] Abed Y, Boivin G. Treatment of respiratory virus infections. Antiviral Res 2006;70(2):1–16.
[20] Kitchen LW, Vaughn DW. Role of U.S. military research programs in the development of U.S.-licensed vaccines for naturally occurring infectious diseases. Vaccine 2007;26(41):7017–30.
[21] Russell KL, Ryan MAK, Hawksworth A, Freed E, Irvine M, Daum LT, et al. Effectiveness of the 2003–2004 influenza vaccine among U. S. military basic trainees: a year of suboptimal match between vaccine and circulating strain. Vaccine 2005;23(16):1981–5.
[22] Amelio R, Biselli R, Calì G, Peragallo MS. Vaccination policies in the military: an insight on influenza. Vaccine 2002;20(Suppl. 5):B36–9.
[23] Ströher U, DiCaro A, Li Y. Severe acute respiratory syndrome-related severe coronavirus is inhibited by interferon-alpha. J Infect Dis 2004;189(7):1164–7.
[24] Duan ZJ, Zhang LL, Xie ZP, Yu Z, Zhang LP, Zhang B, et al. Anti-SARS virus activities of different recombinant human interferons in cell culture system. Chinese J Exp Clin Virol 2003;17(13):205–8.
[25] Gao H, Zhang LL, Wwi Q, Duan ZJ, Tu XM, Yu Z, et al. Preventive and therapeutic effects of recombinant IFN-b nasal spray on SARS-CoV infection in *Macaca mulata*. Chinese J Exp Clin Virol 2005;19(3):207–10.

[26] Graat JM, Schouten EG, Heijnen ML, Kok FJ, Pallast EGM, de Greeff SC, et al. A prospective, community-based study on virologic assessment among elderly people with and without Symptoms of acute respiratory infection. J Clin Epiemiol 2003;56(12):1218–23.
[27] Alper CM, Doyle WJ, Winther B, Hendley JO. Upper respiratory virus detection without parent-reported illness in children is virus-specific. J Clin Virol 2008;43(1):120–2.
[28] Chen Q, Zhang LL, Yu DX, Yu Z, Liu Y, Zhang LP, et al. A field trial for evaluating the safety of recombinant human interferon α-2b for nasal spray. Chinese J Exp Clin Virol 2005;19(3):211–5.
[29] Jefferson TO, Tyrrell D. Vaccines for the common cold (protocol for a Cochrane review). In: The Cochrane Library, Issue 3. Chichester, UK: John Wiley & Sons, Ltd.; 2004.