ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Aug; 20 (8): 709 – 714
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical

Antiviral effects of rhIFN-alpha 1 against seven influenza viruses

LI Fang[1], WANG Qiu-Juan, ZHU Bao-Li[2], WANG Min[3]
(*Department of Physiology, [3]Research Division of Biochemistry, China Pharmaceutical University, Nanjing 210009; [2]Jiangsu Occupational Disease Prevention and Therapy Institute, Nanjing 210028, China*)

KEY WORDS recombinant interferon alfa; antiviral agents; influenza A virus; influenza B virus; influenzavirus C; viral pneumonia; cultured cells; ribavirin

ABSTRACT

AIM: To study the antiviral effects of rhIFN-α_1 (Chinese silkworm gene recombinant interferon α_1) on 7 influenza viruses in MDCK cells and in mouse pneumonia caused by PR_8 virus. **METHODS**: 100$TCID_{50}$ virus (H_1N_1, H_2N_2, H_3N_3, type B, type C, clinical A_1, and clinical B) were inoculated into MDCK cells. PR_8 viruses were dropped nasally in mice, the antiviral effects of rhIFN-α_1 were observed. **RESULTS**: The minimal effective concentrations of rhIFN-α_1 against these 7 influenza viruses were 12.5, 25, 50, 25, 12.5, 25, and 12.5 $kU \cdot L^{-1}$, respectively. The infectious therapeutic indices of rhIFN-α_1 to these viruses in MDCK cells were 8×10^3, 4×10^3, 2×10^3, 4×10^3, 8×10^3, 4×10^3, and 8×10^3, respectively. The inhibitory indices of rhIFN-α_1 to the 7 influenza viruses in MDCK cells were 3.6, 4.7, 3.5, 3.3, 3.9, 4.6, and 3.5, respectively. The rhIFN-α_1 inhibited the intracellular replication of influenza viruses effectively, but did not kill viruses directly. The rhIFN-α_1 prolonged the life span of mice infected with pneumonia by influenza virus A strain PR_8 to 94.2 % – 132.7 %. It inhibited the inflammation and hyperplasia of interstitial fibers, and decreased the virus titer. The inhibitory rates of rhIFN-α_1 to pulmonary-indice were 14.8 % – 37.4 %. **CONCLUSION**: rhIFN-α_1 inhibited the proliferation of influenza virus and improved the symptom of mouse pneumonia caused by influenza virus.

[1] Correspondence to Dr LI Fang. Phn 86-25-330-5996, ext 324. Fax 86-25-330-1655. E-mail pharmcol@mails.cpu.edu.cn
Received 1998-01-13 Accepted 1998-12-30

INTRODUCTION

Clinically, vaccinia and amantadine are used for prevention and cure of influenza, but their effects are not definite. Intravenous injection of human interferon inhibits proliferation and development of influenza. Inactive or active influenza virus can induce interferon production, which inhibits the propagation of virus conversely[1,2].

BmN cell line obtained from silkworm *Bombyx mori* was cultured with microcarrier cytodex 3, then inoculated with BmNPV-IFNα for 48 – 60 h. After 5 d, rhIFN-α_1 was efficiently expressed[3].

The antiviral effects of rhIFN-α_1 on *Herpes simplex* virus type Ⅰ and Ⅱ[4,5] were studied. In this study, we observed the antiviral effects of rhIFN-α_1 on MDCK cells infected with influenza viruses as well as its protective effects on pneumonia mice infected by mouse PR_8 virus via nasal dripping.

MATERIALS

Virus Influenza virus type A – H_1N_1 (A/FM/1/47), H_2N_2 (A/Singapore/1/57), H_3N_3 (A/Hongkong/1/68), strain PR_8, influenza virus type B (B/England/5/66), and influenza virus type C (C/GL/1167/54) were provided by Center for Influenza in Institute of Viral Diseases, Chinese Academy of Preventive Medicine.

Influenza virus type A and B clinical lines (A_1/Beijing 86-1, B/Shanghai 91-3) were provided by Jiangsu Sanitary Epidemic Prevention Station.

Eggs 9 – 11-d-old, purchased from Nanjing Maigaoqiao Chicken Farm.

Cells MDCK cells were provided by Jiangsu Sanitary Epidemic Prevention Station.

Drugs Ribavirin injection was produced by the Third Pharmaceutical Factory of Nanjing. rhIFN-α_1 was provided by Molecular Genetics Laboratory,

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Aug; 20 (8)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Nanjing Military Medical Research Institute. Culture solution was DEM/F_{12} (1 : 1). Drugs were prepared with culture solution.

Mice Kunming mice (♂ and ♀) weighing 18 – 22 g were provided by the University (Grade Ⅱ, Certificate № 97007).

METHODS

Effects on influenza virus inoculated in MDCK cells

Toxicity test of influenza virus MDCK cells were grown monolayer on 96-well plate. Influenza virus type A (H_1N_1, H_2N_2, H_3N_3), B, and C were added into MDCK cell culture with concentrations of 10-fold serial dilution, each concentration was incubated at 37 ℃ in 5 % CO_2 for 3 d. Cytopathic effects (CPE) were observed and viral titers ($TCID_{50}$) were measured. The experiment was repeated twice.

Cytotoxicity of rhIFN-α_1 in MDCK cells rhIFN-α_1 diluted 10-fold serially were added to monolayer MDCK cells and cultured at 37 ℃ in 5 % CO_2 for 4 d. Each concentration was tested in 3 parallel tubes. The cells were dyed with trypan blue and counted.

Antiviral effects of rhIFN-α_1 on influenza virus in MDCK cells Monolayer MDCK cells together with 100$TCID_{50}$ viruses were co-incubated for 2 h. Viruses-solution were decanted from monolayer MDCK cells, then rhIFN-α_1 100 kU·L^{-1} and its 2-fold serial dilution or ribavirin 0.1 g·L^{-1} were added. Cells were incubated at 37 ℃ in 5 % CO_2 for 3 d to observe CPE and cytopathy progress. Each experiment was repeated twice.

Monolayer MDCK cells were inoculated with 7 influenza viruses which were diluted by 10-fold serial dilution, then cultured at 37 ℃ in 5 % CO_2 for 2 h. The viruses-solution was decanted. rhIFN-α_1 100 kU·L^{-1} or ribavirin 0.1 g·L^{-1} were added and incubated for 5 d to examine CPE everyday. The culture solution was changed every 2 d. $TCID_{50}$ was calculated. The inhibitory indices were defined as the difference of – lg $TCID_{50}$ of control and the treated.

100$TCID_{50}$ H_1N_1 was inoculated into monolayer MDCK cells at 37 ℃ in 5 % CO_2 for 1 h. After 1 h, rhINF-α_1 100 kU·L^{-1} was added, culture solution was collected at 6, 12, 24, 48, 72, and 96 h and preserved at – 60 ℃. Four parallel samples at each point. The infectious titers of H_1N_1 were tested in MDCK cells.

Effects on mouse pneumonia caused by influenza virus strain PR_8

Toxicity test of mouse influenza virus strain PR_8 Fifty mice were divided into 5 groups and inoculated with PR_8 virus (Chick allantoic fluids) by nasal dripping at 1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} diluted solutions. The toxic signs in mice were recorded daily for 14 d. LD_{50} was calculated according to Bliss methods.

Protective effects of rhINF-α_1 on mouse pneumonia caused by PR_8 virus Fifty mice were administered 10LD_{50} PR_8 virus (Chick allantoic fluids) by nasal dripping, then im rhINF-α_1 3.0×10^6, 2.0×10^6, and 1.0×10^6 IU·kg^{-1}, ribavirin 120 mg·kg^{-1}, and NS immediately. Drug therapy continued for 6 d and responses to treatment were observed for 14 d. The death number, life span, and the pathological change of lung were recorded. Other 50 mice were infected and administered as above. Mice were killed 6 d later. The lungs were taken, weighed, and homogenized to measure the titer with type O blood. Ten normol mice were killed as control.

RESULTS

Effects of rhINF-α_1 on influenza virus inoculated in MDCK cells

The toxicity test of influenza virus The $TCID_{50}$ of the 7 influenza viruses were 3.7×10^{-4}, 2.7×10^{-5}, 5.3×10^{-5}, 5.3×10^{-6}, 5.7×10^{-5}, 2.3×10^{-6}, and 1.2×10^{-6}, respectively. (Tab 1)

Tab 1. $TCID_{50}$ of 7 influenza viruses.

	1	2	3	$\bar{x}$
H_1N_1	1×10^{-4}	5×10^{-4}	5×10^{-4}	3.7×10^{-4}
H_2N_2	1×10^{-5}	2×10^{-5}	5×10^{-5}	2.7×10^{-5}
H_3N_3	1×10^{-4}	1×10^{-5}	5×10^{-5}	5.3×10^{-5}
B	1×10^{-5}	1×10^{-6}	5×10^{-6}	5.3×10^{-6}
C	1×10^{-4}	2×10^{-5}	5×10^{-5}	5.7×10^{-5}
Clinical A_1	1×10^{-6}	1×10^{-6}	5×10^{-6}	2.3×10^{-6}
Clinical B	1×10^{-6}	2×10^{-6}	5×10^{-7}	1.2×10^{-6}

Cytotoxicity of rhIFN-α_1 in MDCK cells rhIFN-α_1 at concentrations of $< 10^5$ kU·L^{-1} showed no significant cytotoxicity. We selected 100 kU·L^{-1} as test concentration. (Tab 2)

Tab 2. Cytotoxicity of rhIFN-α_1 in MDCK cells. $n = 6$ wells. $\bar{x} \pm s$.

rhIFN-α_1 concentration/kU·L^{-1}	Death rate/ %
10^5	5.0 ± 1.4
10^4	4.5 ± 1.1
10^3	3.9 ± 1.3
10^2	3.5 ± 1.3
10	3.0 ± 0.7
1	3.3 ± 1.1
0.1	3.1 ± 1.1
Control	3.4 ± 0.8

Antiviral effects of rhIFN-α_1 on influenza virus in MDCK cells The degree of CPE was divided into 5 levels according to pathological changes (%) in cells: >90 %, 75 %, 50 %, 25 %, <10 %. The highest diluted solution which appeared 50 % CPE as minimal effective concentrations of rhIFN-α_1 to virus H_1N_1, H_2N_2, H_3N_3, B, C, clinical A_1, and clinical B were 12.5, 25, 50, 25, 12.5, 25, and 12.5 kU·L^{-1}, respectively.

The infection therapeutic indices T_I (maximal nontoxicity concentration/minimal effective concentration) of rhIFN-α_1 to the 7 viruses in MDCK cells were 8×10^4, 4×10^3, 2×10^3, 4×10^3, 8×10^4, 4×10^4, and 8×10^3, respectively. Under this condition, ribavirin 0.1 g·L^{-1} inhibited the CPE of virus wholly (<10 %) (Tab 3).

Tab 3. Effect of rhIFN-α_1 on CPE caused by H_1N_1 virus.

No	rhIFN-α_1/100 kU·L^{-1}								Ribavirin
	1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	Control	0.1 g·L^{-1}
1	-	-	-	++	++±	+++	++++	++++	++++	-
	-	-	-	++	++±	+++	++++	++++	++++	-
	-	-	-	+	+++	+++	++++	++++	++++	-
	-	-	-	++	+++	+++	++++	++++	++±+	-
2	-	-	++	+++	++++	++++	++++	++++	++±+	-
	-	-	-	++	+++	+++	++±+	++++	++±+	-
	-	-	+	++	+++	+++	++±+	++++	++±+	-
	-	-	-	++	+++	+++	++++	++++	++±+	-
3	-	-	++	+++	±++	+±+±	++++	++++	++++	-
	-	-	-	++	±+±	+++	++++	++++	++±+	-
	-	-	-	+±	++±	+++	++++	++++	++±+	-
	-	-	+	+++	+++	+++	++++	++++	++±+	-

Percentage of pathological change in cells: -) <10 %, +) 25 %, ++) 50 %, +++) 75 %, ++++) >90 %.

Antiviral effects of shIFN-α_1 100 kU·L^{-1} was equivalent to ribavirin 0.1 g·L^{-1} on influenza viruses (Tab 4).

Tab 4. Inhibitory indices of rhIFN-α_1 100 kU·L^{-1} and ribavirin 0.1 g·L^{-1} on 7 influenza viruses in MDCK cell line. $n = 4$ wells, $\bar{x}$.

	Infectious titer (-lg $TCID_{50}$)			Inhibitory indices	
	Control	Ribavirin 0.1 g·L^{-1}	rhIFN-α_1 100 kU·L^{-1}	Ribavirin 0.1 g·L^{-1}	rhIFN-α_1 100 kU·L^{-1}
H_1N_1	4.5	0.9	1.3	3.6	3.2
H_2N_2	5.7	1.0	1.2	4.7	4.5
H_3N_3	5.3	1.8	1.8	3.5	3.5
B	5.2	1.9	1.3	3.3	3.9
C	5.6	1.7	1.2	3.9	4.4
Clinical A_1	5.9	1.3	0.9	4.6	5.0
Clinical B	4.3	0.8	0.8	3.5	3.5

rhIFN-α_1 100 kU·L^{-1} decreased the titer of H_1N_1 virus and effects did not appear until 6 and 12 h later, when the titers between control group and rhIFN-α_1 group showed no significant difference. It was suggested that rhIFN-α_1 inhibited the replication but could not kill the virus directly. (Tab 5)

Tab 5. Effects of rhIFN-α_1 100 kU·L^{-1} on replication of infectious H_1N_1. $n = 4$ wells. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Time/h	Control (Titer of virus)	rhIFN-α_1 (-lg $\bar{x} \pm s$)	Inhibitory indice
6	0.75 ± 0.33	0.48 ± 0.35	0.3
12	1.68 ± 0.29	1.25 ± 0.33	0.4
24	2.68 ± 0.29	1.08 ± 0.59^b	1.6
48	3.58 ± 0.34	1.25 ± 0.33^c	2.3
72	5.50 ± 0.23	1.75 ± 1.17^c	3.8
96	7.32 ± 0.29	1.40 ± 0.42^c	5.9

Effects of rhIFN-α_1 on mouse pneumonia caused by influenza virus strain PR_8

Toxicity test of mouse influenza virus strain PR_8 Mice died successively since 3 d after nasal dripping PR_8 virus until 9 d. The mice showed loss of body weight, asthenia, reduced food intake, loss of hair and luster, asthma, and hemorrhage spot or

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Aug; 20 (8)
E-mail aps@server.shcnc.ac.cn Phn Fax 86-21-6474-2629

necrosis in lungs which appeared to be live-colored. LD_{50} of virus to mice was 9.6×10^{-3} of dilution titer and 95 % confidence limits were 7.5×10^{-3} to 1.2×10^{-2} of dilution titer.

Protective effects of rhIFN-α_1 on mouse pneumonia caused by PR_8 virus rhIFN-α_1 1.0×10^6, 2.0×10^6, 3.0×10^6 IU·kg^{-1} decreased the death rate and prolonged the life span of PR_8 virus-infected mice. (Tab 6)

The effects of rhIFN-α_1 and ribavirin on the pathological changes in murine pneumonia caused by PR_8 virus were shown in Fig 1. The pulmonary-indice (lung weight/body weight) was decreased by rhIFN-α_1. CPE was also monitered according to the criterion (0: normal, 1: some inflammatory cells and inflammatory exudate, 2: inflammatory exudate increased and interstitial fibers partly proliferated, 3: most or whole of lung tissue consolidated). The CPE

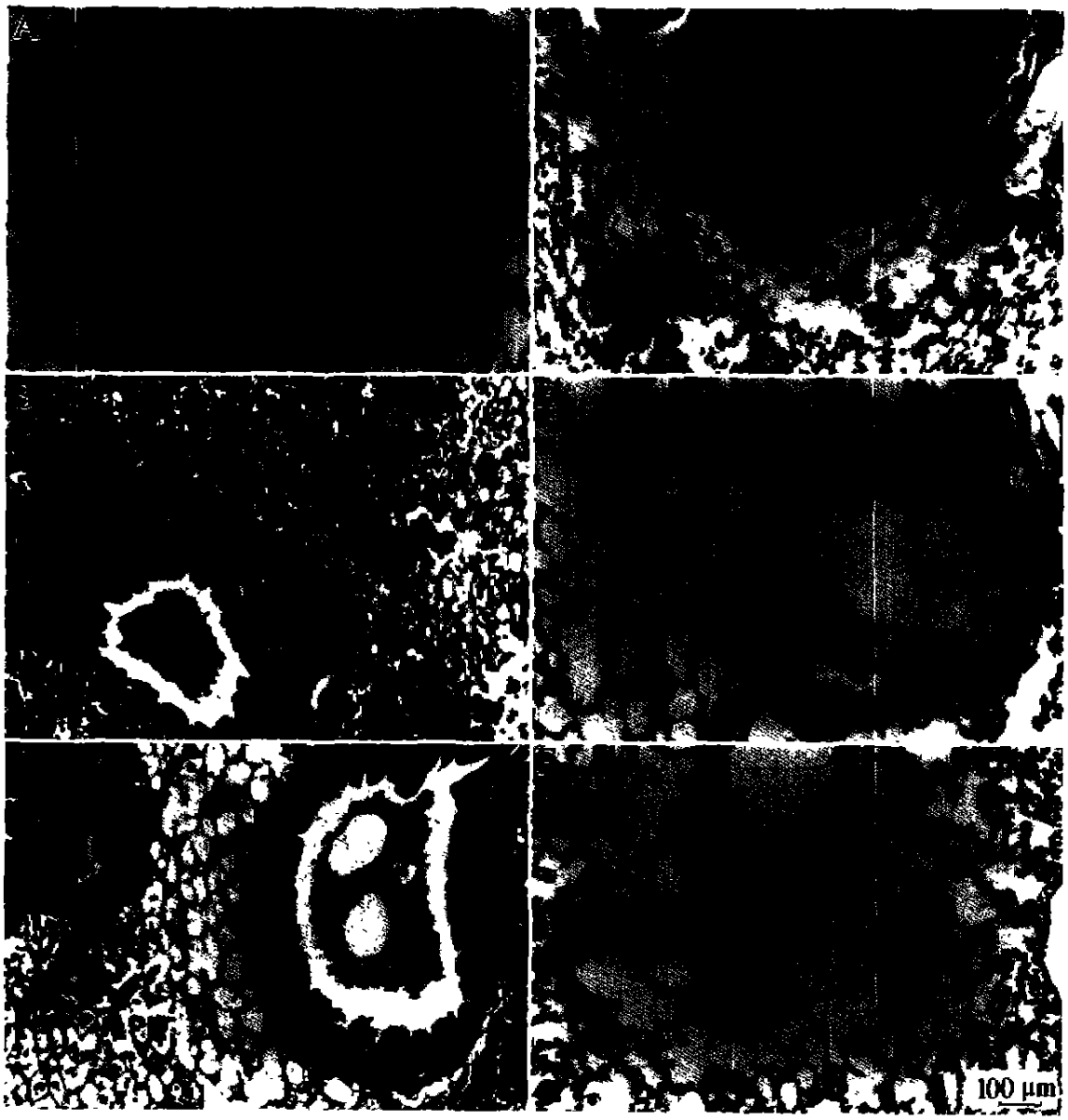

Fig 1. The effects of rhIFN-α_1 and ribavirin on the pathological changes in murine pneumonia caused by PR_8 virus nasally. (HE stain, ×100). A) Normal; B) NS (Model); C) Ribavirin 120 mg·kg^{-1}; D) rhIFN-α_1 1.0×10^6 IU·kg^{-1}; E) rhIFN-α_1 2.0×10^6 IU·kg^{-1}; E) rhIFN-α_1 3.0×10^6 IU·kg^{-1}.

Tab 6. The living time prolonged rate of rhIFN-α_1 on PR_8-infected mice. $n = 10$ mice, $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

Groups	Died	Lile span	Prolongation/%
rhIFN-α_1 1.0×10^6 IU·kg^{-1}	7	10.1 ± 2.9^c	94.2
rhIFN-α_1 2.0×10^6 IU·kg^{-1}	5	11.4 ± 2.8^c	119.2
rhIFN-α_1 3.0×10^6 IU·kg^{-1}	4	12.1 ± 2.5^c	132.7
Ribavirin 120 mg·kg^{-1}	5	10.9 ± 3.5^c	109.6
Control	10	5.2 ± 1.3	–

were inhibited by rhIFN-α_1, but not by ribavirin. rhIFN-α_1 also lowered the virus titer of lung homogenate. The rhIFN-α_1 and ribavirin both suppressed the infiltration of inflammatory cells, in contrast to edema, purulent inflammation, and necrosis in untreated mice (Fig 1, Tab 7, 8).

Tab 8. Effect of rhIFN-α_1 on reproduction of infectious PR_8 virus. $n = 10$ mice. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

Groups	$-\lg_2$ (titers of virus)	Inhibitory indice
rhIFN-α_1 1.0×10^6 IU·kg^{-1}	3.0 ± 0.8^c	1.3
rhIFN-α_1 2.0×10^6 IU·kg^{-1}	2.6 ± 0.8^c	1.7
rhIFN-α_1 3.0×10^6 IU·kg^{-1}	1.8 ± 0.8^c	2.5
Ribavirin 120 mg·kg^{-1}	3.4 ± 0.7	0.9
Control	4.3 ± 0.7	–
Normal	–	–

DISCUSSION

rhIFN-α_1 expressed in microcarrier culture of silkworm BmN cell had efficient inhibitory effects on influenza viruses type A, B, and C. It inhibited the CPE caused by the 7 influenza viruses at the minimal effective concentrations 12.5 – 50 kU·L^{-1} without cytotoxicity on MDCK cells. The inhibitory indices of rhIFN-α_1 100 kU·L^{-1} were 3.2 – 5.0. rhIFN-α_1 inhibited H_1N_1 replication effectively. In mice, rhIFN-α_1 can improve mouse pneumonia symptom caused by PR_8 infection significantly, prolong the life span more than 100 %, and reduce the mortality more than 40 %.

Data showed that the rhIFN-α_1 could be used to cure the viral pneumonia caused by influenza virus in clinical settings. Interferons induce a number of immuno-active proteins that mediate the antiproliferative, antiviral, and immunomodulatory functions. Mx in mouse and rat, and p78 in human may be the factor of resistance of interferon type A to influenza[9], or not involved in the antiviral mechanism[10]. The mechanism of rhIFN-α_1 on antiviral effects needs further studying.

In our study, it showed that rhIFN-α_1 could improve the mouse pneumonia caused by PR_8 virus. Others' findings suggest that type Ⅱ pneumocytes may be a major source of alveolar interferon for antiviral state and modulating alveolar cell function requisite for lung integrity[11]. We suggested that rhIFN-α_1 might have the same function as interferons induced from type Ⅱ pneumocytes and alveolar macrophages.

rhIFN-α_1 inhibited the proliferation of influenza virus B to some extent *in vitro*. But interferon α_2 as a spray did not prevent influenza B infection or modify the resulting illness[12].

REFERENCES

1 Kouichi T, Keith EJ, Joel W. Passive interferon pretection

Tab 7. Effect of rhIFN-α_1 on CPE of mouse pneumonia caused by PR_8 virus. $n = 10$ mice. $x \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Groups	Body weight/g	Lung weight/g	Pulmonary-indices	Inhibition/%	CPE marks
rhIFN-α_1 1.0×10^6 IU·kg^{-1}	22.3 ± 4.1	0.29 ± 0.06	1.32 ± 0.18^b	14.8	1.9 ± 0.5^b
rhIFN-α_1 2.0×10^6 IU·kg^{-1}	22.0 ± 4.2	0.27 ± 0.05^c	1.23 ± 0.17^c	20.6	1.8 ± 0.8^b
rhIFN-α_1 3.0×10^6 IU·kg^{-1}	23.7 ± 1.9	0.23 ± 0.04^c	0.97 ± 0.20^c	37.4	1.3 ± 0.8^c
Ribavirin 120 mg·kg^{-1}	22.0 ± 2.2	0.26 ± 0.03^c	1.22 ± 0.20^c	21.3	2.1 ± 0.7
Control	22.5 ± 3.4	0.33 ± 0.04	1.55 ± 0.20	–	2.8 ± 0.4
Normal	26.0 ± 3.0^c	0.19 ± 0.03^c	0.74 ± 0.08^c	–	–

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Aug; 20 (8)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

in mouse influenza. Proc Soc Exp Biol Med 1963; 114: 472 – 5.
2 Gershenfeld L. Interferon — an inhibitor of virus multiplication. Am J Pharm 1961; 133: 183 – 9.
3 Deng XZ, Zhang LY, Zhou YC, Chen YF, Li GF, Diao ZY. Experimental research of microcarrier culturation of silkworm BmN cell and high efficiency expression of HuIFN-a gene. Pharm Biotechnol 1994; 1: 1 – 4.
4 Wang QJ, Li YM, Wang M, Wu WT, Zhu BL. The study on antiviral effect of injection of HIFN-α against herpes simplex virus type Ⅱ. J Chin Pharm Univ 1996; 27: 557 – 61.
5 Wang QJ, Li YM, Wang M, Wu WT, Zhu BL. The study on antiviral effect of HIFN-α injection against herpes simplex virus type Ⅰ. Pharm Biotechnol 1996; 3: 224 – 8.
6 Melen K, Ronni T, Broni B, Krug RM, von-Bonsdorff CH, Julkunen I. Interferon-induced Mx proteins from oligomers and contain a putative leucine zipper. J Biol Chem 1992; 267: 25898 – 907.
7 Horisberger MA. Virus-specific effects of recombinant porcine interferon-gamma and the induction of Mx proteins in pig cells. J Interferon Res 1992; 12: 439 – 44.
8 Pavlovic J, Zürcher T, Haller O, Staeheli P. Resistance to influenza virus and vesicular stomatitis virus conferred by expression of human MxA protein. J Virol 1990; 64: 3370 – 5.
9 Goetschy JF, Zeller H, Content J, Horisberger MA. Regulation of the interferon-inducible IFI-78K gene, the human equivalent of the murine Mx gene, by interferons, double-stranded RNA, certain cytokines, and viruses. J Virol 1989; 63: 2616 – 22.
10 Horisberger MA, McMaster GK, Zeller H, Wathelet MG, Dellis J, Content J. Cloning and sequence analyses of cDNAs for interferon- and virus-induced human Mx proteins reveal that they contain putative guanine nucleotide-binding sites: functional study of the corresponding gene promoter. J Virol 1990; 64: 1171 – 81.
11 Hahon N, Castranova V. Interferon production in rat type Ⅱ pneumocytes and alveolar macrophages. Exp Lung Res 1989; 15: 429 – 45.
12 Foy HM, Cooney MK, Allan ID, Albrecht JK. Influenza B in households: virus shedding without symptoms or antibody response. Am J Epidemiol 1987; 126: 506 – 15.

细胞基因工程重组人 α_1 干扰素对 7 种流感病毒的抗病毒作用

李 放[1]，王秋娟，朱宝立[2]，王 旻[3]
（中国药科大学生理学教研室，[3]生物化学研究室，南京 210009；
[2]江苏省职业病防治研究所，南京 210028，中国）

关键词 重组干扰素 α；抗病毒剂；流感病毒 A；流感病毒 B；流感病毒 C；病毒性肺炎；培养的细胞；利巴韦林

目的：研究细胞基因工程人 α 干扰素（rhIFN-α_1）对体外 7 种流感病毒感染的 MDCK 细胞以及对甲型流感病毒鼠肺适应株 PR_8 引起的小鼠肺炎的对抗作用。**方法**：接种 7 种病毒液（H_1N_1，H_2N_2，H_3N_3，B 型，C 型，A_1，B 分离株）于 MDCK 细胞中，小鼠用 PR_8 病毒液滴鼻，观察 rhIFN-α_1 的抗病毒作用。**结果**：rhIFN-α_1 对 7 种病毒液的最小有效浓度分别为 12.5，25，50，25，12.5，25 和 12.5 $kU \cdot L^{-1}$；在 MDCK 细胞上对 7 种病毒液的感染治疗指数为 8×10^3，4×10^3，2×10^3，4×10^3，8×10^3，4×10^3 和 8×10^3；抑制指数为 3.6，4.7，3.5，3.3，3.9，4.6 和 3.5；rhIFN-α_1 能有效地抑制流感病毒的细胞内复制，却不能直接杀伤病毒；rhIFN-α_1 对小鼠病毒肺炎有抑制作用，使肺组织炎症，纤维间质增生明显改善，降低病毒滴度，延长生命率为 94.2 % – 132.7 %，肺指数抑制率为 14.8 % – 37.4 %。**结论**：rhIFN-α_1 对流感病毒的增殖有抑制作用，并且能够改善流感病毒引起的小鼠肺炎症状。

（责任编辑 朱倩蓉）