BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Jul; 16 (4): 308—310

Toxicity of human recombinant interferon-γ in rats and dogs

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AIM: To study the acute and chronic toxicities of human recombinant interferon-gama (Hu-rIFN-γ) in mice, rats, and dogs. **METHOD:** Twenty mice were administrated Hu-rIFN-γ (im or iv) 4.4×10^9 IU m^{-2} to observe the acute toxicity. In chronic studies, 1×10^7, 5×10^7, 1×10^8 IU m^{-2} d^{-1} were given to 80 rats and 5×10^5, 5×10^7 IU m^{-2} d^{-1} were injected to 14 dogs im for 3 months, treatment-related changes were measured in the hematologic, chemical, urinalysis values, ECG and pathologic profile of organs and tissues. **RESULTS:** The maximal tolerance dose (MTD) im or iv in mice was 4.4×10^9 IU m^{-2}, 4400 times the recommended clinical dosage (1×10^6 IU m^{-2}). No adverse effects were found in chronic toxicity studies. **CONCLUSION:** Human recombinant interferon-γ did not produce toxic reaction in rats and dogs.

KEY WORDS recombinant interferon-gamma; toxicology; pathology

Human recombinant interferon-γ (Hu-rIFN-γ), an active glycoproteinpróduced from *Escherichia coli*, has a variety of bioactivities, including immune regulation, antitumour, and antiviral effects. Human interferon (HuIFN) was recognized in early days as a kind of endogenous protein. Although having been used in clinical practice, there were few reports on its toxicity in animals. In recent years, HuIFN has caused several kinds of side effects to human[1,2]. However, there is no noticeable toxicity in the HuIFN treatment in animals[3-5]. The reason that HuIFN produced toxicity in human beings while not in animals was suggested to be the difference of inherent species specificity between animals and human beings. HuIFN elevated ALT and lowered of WBC in chimpanzee[6], which may be the suitable animal in evaluating its toxicity. This experiment, the acute and chronic toxicities of Hu-rIFN-γ were studied in mice, rats, and dogs.

MATERIALS AND METHODS

Hu-rIFN-γ Provided by Institute of Medical Biotechnology and Genetics of Second Military Medical University. It was dissolved in normal saline. The biological activity was 2×10^{11} IU L^{-1}, purity 99.1 %, pH 7.0, concentration of protein 146.4 mg L^{-1}. It was stored at -20 ℃.

Acute toxicity in mice Twenty Kunming mice of either sex weighing $20\pm s$ 2 g were injected im with Hu-rIFN-γ 4.4×10^9 IU m^{-2}. (4 400 times the recommended clinical dose) and observed for 7 d.

Chronic toxicities in rats and dogs Forty ♂ and forty ♀ Wistar rats (age 7 wk) were divided randomly into 4 groups, 10 ♂ and 10 ♀ in each group, 7 male and 7 female beagle dogs (age 6—12 months) obtained from Shanghai Medical University were maintained on a standard diet in separate cages. The dogs were divided into 3 groups with equal sex distribution. Hu-rIFN-γ was injected im 1×10^7, 5×10^7, and 1×10^8 IU m^{-2} d^{-1} in rats and 5×10^6, 5×10^7 IU m^{-2} d^{-1} in dogs for 3 months. We observed hematologic, chemical, and urinalysis values, electrocardiogram was recorded in dogs. The skin temperature of rats was measured with a VMT-01 digital thermometer in the armpit.

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Received 1992-03-31 Accepted 1994-11-19

Half of the animals in each group were killed at the end of 3-month study, and the other were killed after another 1-month recovery period for pathological examination. The specimens were embeded in paraffin, sectioned, stained with H&E, and examined with light microscope.

RESULTS

Acute toxicity All mice survived after 7 d. Their activity, hair, stool and food intake remained unchanged. There were no toxic responses at im or iv dosage as high as 4.4×10^9 IU m^{-2}(MTD).

Chronic toxicity There were no abnormal changes in animals' moter activity, food intake, body weight, stool, urine, hair, heart rate, respiratory rate, pupils, lacrimation, nausea, vomiting, salivation, amyostasia, and the injection site, all items were recorded before and after the medication. The skin temperature of rats and the rectal temperature of dogs were also in normal.

Blood samples were collected for the determination of RBC, Hb, Hct, MCV, MCH, MCHC, WBC and DC, Plt and coagulation time with CELL-DYN610 hemocytometer. UNIFAST-2 analyzer was used to determine the serum ALT, AST, ALP, LDH, BUN, Glu, Tch, TBill, Cr, TP and Alb. No treatment-related changes were found.

Urinalysis was done in dogs, including urinary nitrite, pH, protein, glucose, ketone body, bilirubin, urobilinogen, occult blood, RBC, WBC, and cast in concentrated urine. and no abnormal changes were found in urinalysis values.

There were no differences in ECG of dogs recorded before and after treatment.

The major pathologic findings were found in dogs (Tab 1) . No difference was seen between controls and treated animals in heart, lung, liver, spleen, stomach, kidney, adrenal gland, brain, thymus, mensenteric nodes, testis, and epididymides (or uterus and ovary), skin, skelatal muscle (in the site of injection), in beagle dogs, further 21 organs or tissues were sampled, including thyroid, prostate, duodenum, jejunum, colon, rectum, cecum, hypophysis, spinal cord, sciatic nerve, pancreas, gall bladder, skin and muscle, and blood vessel at the site of injection.

Tab 1. Pathologic changes after chronic toxicity test of Hu-rIFN-γ in beagle dogs.

Organ	Pathologic changes	Saline ($n=4$)	Hu-rIFN-γ 5×10^5 ($n=4$)	(IU m^{-2} d^{-1}) 5×10^7 ($n=6$)
Liver	Focal inflammation	2	0	4
Kidney	Interstitial inflammation	1	1	1
	Cortical miliary abscess	0	1	0
Duodenum	Mucosal epithelial cell occasional normal division phase	2	1	1
Jejunum	Mucosal epithelial cell occasional normal division phase	0	1	1
Colon	Mucosal epithelial cell occasional normal division phase	2	0	0

DISCUSSION

The results showed that there was no toxic reaction of Hu-rIFN-γ in acute toxicity test of mice and chronic toxicity test of rats and beagle dogs. We may say that: Hu-rIFN-γ given continuously for 3 months, a dosage 100 times in rats and 50 times in dogs higher than the recommended clinical dosage, did not show any toxic reaction. Now, HuIFN is widely used, and several kinds of side effects were known, such as fever, WBC decrease, central nerve system toxicity and so

on[1,2]. But when studying the toxic reactions of HuIFN in animals, people could not found any toxicity[3-5]. This study showed similar results. Why did HuIFN produce toxicity in human while not in lab animals? We also thought that the difference was resulted from the species specificity between human beings and animals. So routine method and animal were improper for assessing the toxicity of HuIFN. The chimpanzee should be recommended in assessing the toxicity of HuIFN[6]. But according to the evolutionary point of view that monkey is most kin to human and it is obtainable, we suggested that monkey be used in assessing the toxicity of HuIFN so as to get the better results. The proper high dosage is 100 times as the clinical dosage, or the dosage that can produce antibody, raising the dosage infinitely high is not wise.

ACKNOWLEDGEMENTS Thanks to GUO Feng-Chuan, DAI Zu-Rui for their help in sampled blood, to YANG Jun-Hua and CHEN Wei in pathological examination.

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人重组γ干扰素对大鼠和狗的毒性

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目的：研究人重组γ干扰素(Hu-rIFN-γ)对小鼠的急性毒性及对大鼠、狗的长期毒性。**方法**：小鼠肌注或静注 Hu-rIFN-γ 4.4×10^9 IU m^{-2}观察一周。长期毒性研究中，肌注给予临床推荐剂量1×10^6 IU m^{-2}的10，50及100倍(大鼠)及5和50倍(狗)，观察血液学、血液生化、尿分析、心电图和组织器官的病理变化。**结果**：Hu-rIFN-γ 对小鼠 im 或 iv 的最大耐受剂量为4.4×10^9 IU m^{-2}。长期毒性试验中未发现药物相关的毒性。**结论**：人重组γ干扰素对大鼠及狗不产生毒性反应。

关键词 人重组γ干扰素；毒理学；病理学