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Long-term toxicity of modified recombinant human tumor necrosis factor in *Macaca mulatta*

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KEY WORDS *tumor necrosis factor*; *Macaca mulatta*; toxicity tests

AIM: To study the long-term toxicity of modified recombinant human tumor necrosis factor (rhTNF-NC) in *Macaca mulatta* compared with recombinant human tumor necrosis factor (rhTNF). **METHODS**: rhTNF-NC 93, 9.3 GU/m^2, and rhTNF 62 GU/m^2 were injected iv daily to 16 *Macaca mulatta* for 1 month and 10 d, respectively. Hematologic, chemical, urinalysis values, ECG, specific antibody, bone marrow, and pathologic profile of organs were measured. **RESULTS**: No more adverse effects of rhTNF-NC were found in spite of anorexia in 4 monkeys and palpebral edema in 2 monkeys of 93 GU/m^2 group. Besides, in rhTNF group, the injury of liver and kidneys, the decrease of erythron, the phlebitis, and thrombosis at injection site occurred. Both drugs caused the production of specific antibody. **CONCLUSION**: No serious adverse effects of rhTNF-NC were found in *Macaca mulatta*. The toxicity of rhTNF-NC was much lower than that of rhTNF.

Tumor necrosis factor-alpha (TNF) is cytostatic and/or cytotoxic against tumors *in vitro* and *in vivo*. The recombinant human TNF (rhTNF) has similar activities to those of natural human TNF[1]. However, the adverse effects were shown in clinical and animal experiment, so its clinical application was limited. The structure of amino terminal of TNF was closely related to its antitumor activity, and that of carbozyl terminal was related to its toxicity[2]. The modified recombinant human tumor necrosis factor (rhTNF-NC) is a new active protein based on rhTNF. At its carbozyl terminal 157-Leu was mutated to Gln, while at the amino terminal 7 amino acids were deleted, and Pro Ser Asp at 8, 9, 10 position were mutated to Arg Lys Arg respectively to modify TNF on purpose of increasing its antitumor activity and decreasing its toxicity. Experimental pharmacodynamics demonstrated that rhTNF-NC was superior to rhTNF[3]. In this paper, the long-term toxicity of rhTNF-NC in *Macaca mulatta* was compared with that of rhTNF, so as to provide experimental evidence of safety for its clinical trial.

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MATERIALS AND METHODS

Drugs *rhTNF-NC with biological activity of 100* GU·L^{-1} and specific activity of 800 GU/g protein, rhTNF 10 GU·L^{-1} and 30 GU/g were manufactured by Department of Neurobiology of Second Military Medical University. Both of the two liquids were colorless and transparent. They were stored at -14 ℃, and were prepared with saline before use.

Monkeys *Macaca mulatta* from Anhui Province of either sex, age 2 - 4 a, weighing 2 - 6 kg, were trained for 4 wk before experiment.

Long-term toxicity in monkeys[4, 5] *Macaca mulatta* (8 ♂ and 8 ♀) maintained in separate cages were divided into 4 groups, 4 monkeys/group (2 ♂, 2 ♀). 1) normal saline control (NS); 2) rhTNF-NC 9.3 GU/m^2 iv daily (15 times of the recommended clinical dose) for 1 month; 3) rhTNF-NC 93 GU/m^2 iv daily (150 times of the recommended clinical dose) for 1 month; 4) rhTNF 62 GU/m^2 iv daily (100 times of the recommended clinical dose) for 10 d. General symptoms, *hematologic*, *chemical*, *urinalysis values*, *specific antibody*, *and* ECG were observed. Half of the monkeys in each group were killed at the end of medication, and the other half were killed after another 15-d recovery period for pathological and bone marrow examination.

Statistic analysis Data were expressed as $x \pm s$ and analyzed by t test.

RESULTS

General symptoms In NS group, there were no abnormal changes in motor activity, food-intake, body weight, stool, urine, fur, lacrimation, nausea, vomiting, salivation, amyostasia and the injection site. The food-intake of low dose of rhTNF-NC group

slightly decreased, and that of high dose of rhTNF-NC greatly decreased, while that of rhTNF group extreamely decreased (Tab 1).

In high dose group of rhTNF-NC and rhTNF group, especially the latter, the monkeys showed lassitude, the activity obviously diminished, and the fur was loose and mat. After the first medication, palpebral edema occurred in these 2 groups, and the edema in half of monkeys in high dose rhTNF-NC group was moderate or mild, in rhTNF group, 2 mild, 1 moderate, and 1 severe. There was no obvious palpebral edema in the other 2 groups. The palpebral edema lasted 1 – 3 d and disappeared spontaneously. On d 8 and d 10, there was separately a death in rhTNF group, so the group received no longer rhTNF after d 10. The body weight of rhTNF group was decreased because of the reduction of food-intake. There was no significant difference between groups in rectal temperature, heart rate, respiratory rate, pupil size, Ⅱ lead P wave, R wave, P – R interval, QRS complex, Q – T interval, and T wave of ECG.

Hematologic examinations RBC, Hb, and Hct of rhTNF group were notably lower than those of NS group (Tab 1). MCV and MCH of high dose of rhTNF-NC were obviously higher than those of NS group, but still in normal ranges. There were no abnormal values in the remaining values of RBC, Hb, Hct, MCV, MCH, MCHC, WBC, and DC, coagulation time, and platelet count.

Serum chemical analysis No significant differences were found between groups in serum ALT, AST, ALP, TP, Alb, BUN, Crea, Tch, TBill, and Glu. But in 2 monkeys of rhTNF group, the preagonal AST(♂ 5 d_0 258 and d_w 982, ♀ 11 d_0 154 and d_w 1050 nmol·s^{-1}·L^{-1}), BUN (♂ 5 d_0 4.35 and d_w 18.4, ♀ 11 d_0 3.82 and d_w 12.4 mmol·L^{-1}) and Crea (♂ 5 d_0 66.6 and d_w 126.0, ♀ 11 d_0 63.4 and d_w 101.0 μmol·L^{-1}) obviously increased, while in NS group the $\bar{x} \pm s$ were AST 312 ± 56 nmol·s^{-1}·L^{-1}, BUN 4.9 ± 1.0 mmol·L^{-1}, Crea 73 ± 11 μmol·L^{-1}, which showed the damages of liver and kidneys.

Urinalysis No abnormal changes were found in 8 urinalysis values: urinary nitrite, pH, protein, glucose, ketone body, bilirubin, urobilinogen, and occult blood.

Pathological examination The major pathologic changes in rhTNF group were found. Vacuolar degeneration and hemorrhagic necrosis of hepatocyte, and degeneration even necrosis of renal tubular epithelial cells occurred in 2 monkeys. One of them had phlebitis and thrombosis at injection site, which were toxic pathologic changes. After recovery period, the phlebitis of 2 monkeys recovered, and some hyperplastic fibrous tissues remained around the blood vessel (Fig 1).

Bone marrow examination No abnormal changes were found but all the bone marrow cells of one of the rhTNF group were lower than NS group while the shapes were normal.

Antiserum titer examination Only 1 in 4 monkeys of low dose of rhTNF-NC group produced

Tab 1. Changes of food-intake (F-I), RBC, Hb, and Hct in long-term toxicity of rhTNF-NC in *Macaca mulatta*. $n = 4$ **monkeys.** $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* (1). $^eP < 0.05$, $^fP < 0.01$ *vs* (2). $^iP < 0.01$ *vs* (3). d_0 (day before administration). d_w (day of withdraw).

	Date	(1) NS	(2) rhTNF-NC (9.3 GU/m^2)	(3) rhTNF-NC (93 GU/m^2)	(4) rhTNF (62 GU/m^2)
F-I (kg/m^2)	d_0	2.2 ± 0.5	2.4 ± 0.4	2.4 ± 0.6	2.4 ± 0.3
	d_w	2.2 ± 0.6	2.2 ± 0.4	1.4 ± 0.4^e	0.65 ± 0.09^{efi}
RBC (T/L)	d_0	5.58 ± 0.19	5.2 ± 0.4	5.6 ± 0.5	5.3 ± 0.4
	d_w	5.6 ± 0.9	5.8 ± 0.4	4.7 ± 1.0	4.2 ± 0.5^{bf}
Hb (g/L)	d_0	124 ± 7	118 ± 4	114 ± 13	119 ± 9
	d_w	120 ± 8	128 ± 11	109 ± 5^e	107 ± 6^{bf}
Hct	d_0	0.351 ± 0.021	0.336 ± 0.021	0.333 ± 0.022	0.27 ± 0.05
	d_w	0.33 ± 0.04	0.349 ± 0.026	0.27 ± 0.05^e	0.24 ± 0.04^{bf}

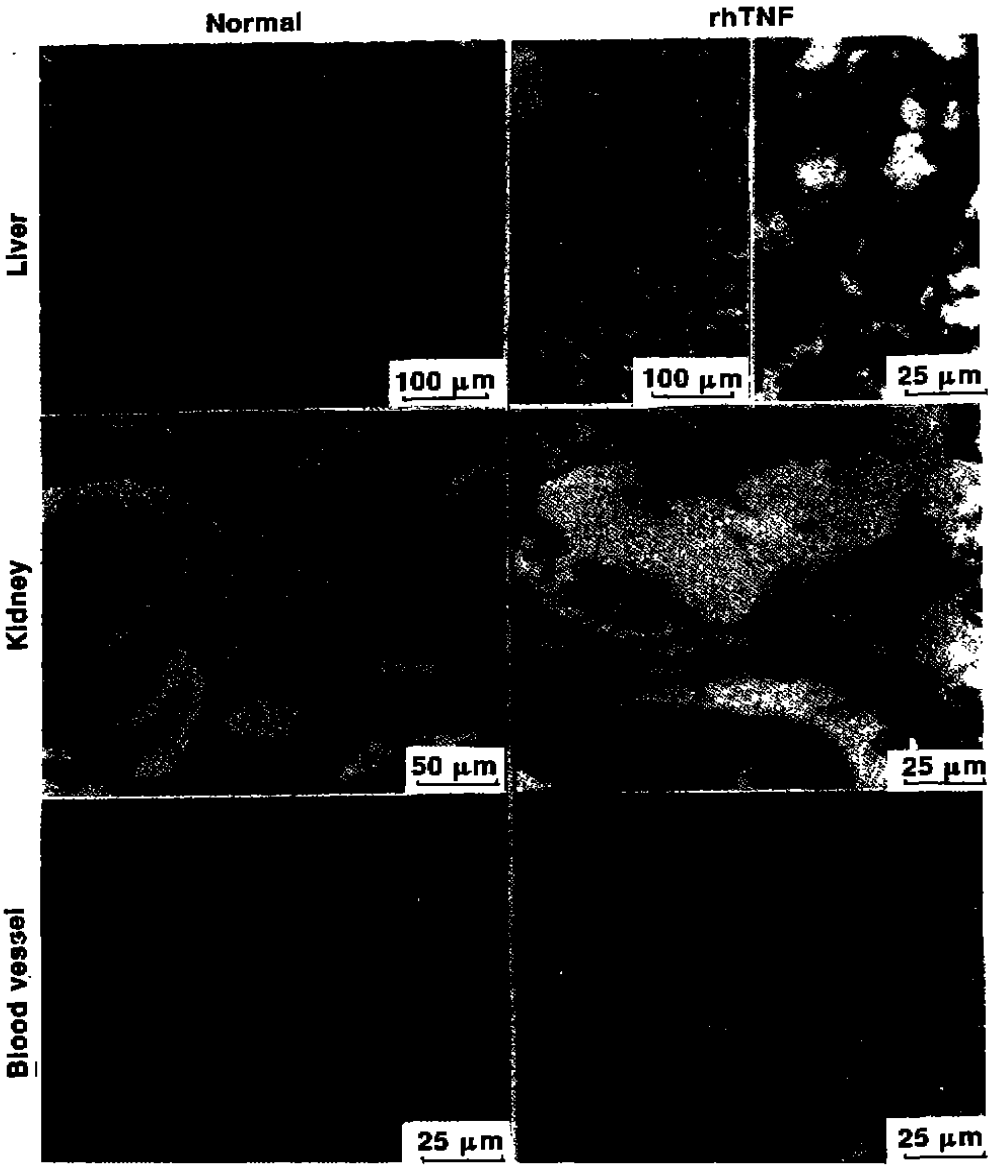

Fig 1. Pathologic changes after long-term toxicity test of rhTNF in *Macaca multta* (stained with HE).

special antibody. The ratio of high dose of rhTNF-NC increased to 2/4, and the titer was rather high. In one of 2 survival monkeys of rhTNF group, antibody was found. The titer of antibody reached maximum level in 20 – 30 d after iv both drugs.

DISCUSSION

Administration of rhTNF-NC and rhTNF in high dose caused tiredness and dispiriting. Moreover, weight loss and anemia occurred in rhTNF group, which was in conformity with clinical reports[6,7], but no fever, shiver, hypotension, and diarrhea were found. The transient palpebral edema appeared in 1 – 3 d after iv rhTNF-NC or rhTNF.

The decrease of erythron suggested that high dose of rhTNF could cause anemia in monkeys.

rhTNF caused damage of liver and kidneys in *Macaca mulatta*. Hepatic transferase and renal function

were abnormal, which conformed to reference [8]. Pathologic examination also showed the damages of liver and kidneys in rhTNF group.

Besides, we found the phlebitis and thrombosis at injection site. Biopsy of the injection site in a limited number of patients revealed a mixed cellular infiltration. The inflammatory reaction consisted of induration and erythema peaking at 1 – 3 d and resolving within 5 – 7 d[7].

The antibodies appeared dose-relatively. It was suggested that the production of drug resistance should be paid attention to in the later clinical trial.

All toxicities recovered in 15 d after the last medication. rhTNF has been found to have a plasma half-life of approximately 10.5 min and less than 30 min in murine models and human respectively[9]. So this was probably the reason that rhTNF did not easily cause the irreversible toxicity.

rhTNF-NC 93 GU/m^2 daily injected iv *Macaca mulatta*, no severe drug-induced toxicity was found. By contrast, rhTNF 62 GU/m^2 caused obvious toxicities. It was demonstrated that the toxicity of rhTNF-NC was much lower than that of rhTNF when same active dose was used, and rhTNF-NC may be safer in clinical trial.

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新型重组人肿瘤坏死因子对恒河猴的长期毒性

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关键词 肿瘤坏死因子; 恒河猴; 毒性试验

目的: 研究新型重组人肿瘤坏死因子(rhTNF-NC) iv 对恒河猴的长期毒性, 并与重组人肿瘤坏死因子(rhTNF)作比较. **方法**: 16 只恒河猴分 4 组分别每日 iv rhTNF-NC 93 和 9.3 GU/m^2 1 个月和 rhTNF 63 GU/m^2 10 d, 检测其一般症状, 血液学, 血液化学和尿液生化指标, 心电图, 特异性抗体, 骨髓, 对组织器官作病理检查等. **结果**: iv rhTNF-NC 后除大剂量组出现纳差和部分动物眼睑水肿之外, 未见明显的药物性毒性反应, 而 rhTNF 组除上述反应外, 还伴有肝肾受损, 红系降低, 注射局部有静脉炎及血栓形成等病理改变. 此外两种 TNF 均能使猴产生特异性抗体. **结论**: rhTNF-NC 对恒河猴的毒性比 rhTNF 要小得多.