International Journal of Medical Physics, Clinical Engineering and Radiation Oncology, 2016, 5, 33-41
Published Online February 2016 in SciRes. http://www.scirp.org/journal/ijmpcero
http://dx.doi.org/10.4236/ijmpcero.2016.51004

The Hematopoietic and Immunomodulatory Effect of rhIL-12 for Liver Cancer

Xiaojing Gong[1*], Na Guo[2], Lingqin Wan[1], Xifeng Jia[2], Yishan Wang[2#]

[1]Binzhou Medical University, Yantai, China
[2]Center for Tumor Diagnosis and Treatment of the Whole Army, 107th Hospital of the People's Liberation Army, Yantai, China
Email: [#]wangyishan288@163.com

Received 11 November 2015; accepted 13 February 2016; published 16 February 2016

Abstract

Purpose: To explore the effect of rhIL-12 on the number of the blood cells and CD4/8+ T, CD45+ leukocytes, and CD56+ NK cells in liver cancer patients following radiation therapy. Methods: We selected forty liver cancer patients who carried out by cyber knife (the patients were given 5 Gy every time for 5 times continuously) to observe the size of the tumor. After thirty hours, rhIL-12 was injected into the liver cancer patients via subcutaneous at the concentration of 50 ng/kg, 100 ng/kg, 200 ng/kg and 300 ng/kg in different patients, respectively. And there were ten patients in the four groups, respectively. The twenty patients who were selected from the hospital without rhIL-12 treatment were used as controls. All the blood cells were collected from different groups on day 0, hour 12, day 7, day 14, day 21 and day 28 after rhIL-12 treatment, respectively. The full number of blood cells in every group was analyzed by ELISA. The number of CD4/8+ T, CD45+ leukocytes, and CD56+ NK cells were detected by Flow Cytometry. After one month with rhIL-12 treatment, ECOG and WHO were used to evaluate the prognosis of liver cancer. Results: In present study, we found that the number of blood cells was significantly decreased on day 0 - day 3, while recovered from day 7 - day 14 and down-regulated on day 21 after rhIL-12 treatment. The number of CD4/8+ T, CD45+ leukocytes, and CD56+ NK cells was elevated with any concentration of rhIL-12. Furthermore, results showed that number of white blood cells was obviously higher than in patients without rhIL-12 treatment ($P < 0.05$). However, there was no significant difference of erythrocyte and platelet, between groups treated with rhIL-12 and control groups. In addition, the immune cells including CD4/8+ T, CD45+ leukocytes, and CD56+ NK cells were reduced on day 0 - day 3, recovered from day 7, and then decreased from day 21 in rhIL-12 treatment groups related to control groups ($P < 0.05$). Furthermore, studies showed that five patients developed symptoms of fever, bilirubin increased and liver dysfunction with the dose of 300 ng/kg. So we found that the safe and well-tolerated human dose of 200 ng/kg is within this efficacious range based on expo-

[*]The first author.
[#]Corresponding author.

How to cite this paper: Gong, X.J., Guo, N., Wan, L.Q., Jia, X.F. and Wang, Y.S. (2016) The Hematopoietic and Immunomodulatory Effect of rhIL-12 for Liver Cancer. *International Journal of Medical Physics, Clinical Engineering and Radiation Oncology*, **5**, 33-41. http://dx.doi.org/10.4236/ijmpcero.2016.51004

sure parameters through the research. Higher ECOG and WHO scores were observed in rhIL-12 treatment groups compared to control groups (P = 0.025, P = 0.044, respectively). Conclusion: Our results suggested that rhIL-12 could recover the liver cancer induced aberrant blood cell number and CD4/8+ T, CD45+ leukocytes, and CD56+ NK cells , which may be an effective method to alleviate the progress of liver cancer and played an important role in treating liver cancer.

Keywords

rhIL-12, Liver Cancer, Blood Cells, CD4/8+ T, CD45+ Leukocytes, CD56+ NK Cells

1. Introduction

Liver cancer is one of the most common malignancies of human, which accounts for more than one million around the world and seriously impacts human body health. At present, surgical removal, as the most effective method, extensively was used in clinical treatment for liver cancer [1] [2]. However, almost patients suffered from liver cancer were detected at the middle-late progress of liver cancer, leading to missing the opportunity to be treated with surgical removal. In addition, chemotherapy and radiotherapy treatment were also used in treatment liver cancer, but didn't play a significant role in liver cancer treatment. Therefore, it is necessary to explore an effective target to treat the liver cancer recently.

Interleukin-12 (IL-12), previously called cytokine lymphocyte maturation factor (CLMF) or NK cell stimulatory factor (NKSF), is considered as key regulation factor in the process of cellular immune responses. Some results reported that rhIL-12 involved in many immune regulation including activating NK/LAK cells, promoting T cell proliferation and differentiation, inducing IFN-gamma production. Some other results showed that rhIL-12 also could regulate the expression of surface molecules of lymphocyte, participating in the synthesis of cytokines and inhibit the formation of tumor blood vessels [3]-[5]. In addition, some studies showed that the serum rhIL-12 was significantly lower in cancer patients. Meanwhile, some results tell that the level serum of rhIL-12 also decreases in gastric cancer and colon cancer [6]. Interestingly, some have found that supplement of rhIL-12 was benefit for improving the symptom of liver cancer in mice model [7] [8]. However, if injection of IL-12 has an effect on the patients undergoing liver cancer is still needed to be further explored in clinical.

In this study, we injected rhIL-12 into the patients suffered from liver cancer after cyberknife treatment to investigate the role of rhIL-12 on the number of the blood cells, immune cells and the clinical imaging change in liver cancer patients. It implies a potential method for treatment the liver cancer.

2. Materials and Methods

2.1. Clinical Data

The research selected thirty patients who came to the Center for Tumor Treatment, The People's Liberation Army 107th Hospital, for palliative treatment from May 2015 to November 2015. A total of 20 cases of male and 10 cases of female who aged 32 - 68, and with an average age of 50. Meanwhile, twenty patients were selected with cyber knife radiotherapy of liver malignant cancer as the controls, with 10 cases of female and 10 cases of male who aged 45 - 70, and with an average age of 57.5. All the patients accept the conventional inspecting after admission, as well as they also accept the inspect which including the clotting time and cardiac function, renal function, liver function, imaging examination such as (CT, MRI） and so on. Above all, the patients all signed the informed consent. The study was approved by the Ethics Committee of the People's Liberation Army 107th Hospital affiliated to Binzhou Medical College (Yantai, China).

2.2. Methods

The liver cancer patients were carried out by cyber knife to observe the size of the tumor. After thirty hours, rhIL-12 was injected into the liver cancer patients via subcutaneous at the concentration of 50 ng/kg, 100 ng/kg, 200 ng/kg and 300 ng/kg in different patients, respectively. And there were ten patients in the four groups, respectively. The twenty patients who were selected from the hospital without rhIL-12 treatment were used as con-

trols. All the blood cells were collected from different groups on day 0, hour 12, day 7, day 14, day 21 and day 28 after rhIL-12 treatment, respectively. In the last, we selected the whole blood cells of the patients, and observe the changes of RBC, WBC and PLT as well analysis the change of immune factor CD4/8+ T, CD45+ leukocytes, and CD56+ NK cells.

2.3. Efficacy Evaluation Criteria

1) Test evaluation: Some patients will suffered from the decreasing in blood cells in a short time through a short course radiation therapy, and also decreasing of immune function. At the same time, the patients were observed the ability of recovery of the hematopoietic function, and immune function with rhIL-12 treatment.

2) Objective curative effect evaluation (WHO): CR: It means that (complete relief) all the signs and symptoms disappear entirely in four weeks; PR: It means that (partial relief) tumor size is estimated to reduce by fifty percent or higher at least four weeks; NC: It means that (no change) lesions have no obvious change at least four weeks, tumor size is estimated to increase by fifty percent, reduced by less than fifty percent; PD: It means that(new progress)new lesions were observed or the original lesions are estimated to increase by fifty percent or higher.

3) Zubrod ECOG-WHO score: Zero score is that the patients have normal activity; One score is that the patients have mild symptoms, but almost entirely could activity freely; Two score is that the patients sometimes stayed in bed, but the number is no more than fifty percent during the day time; Three score is that the patients need stay in bed, and the number is more than fifty percent during the day; Four score is that the patients need stay in bed all the day; Five score is that the patients have died. The total effective rate was calculated with the following equation: (CR + PR)/total cases × 100%.

2.4. Statistical Methods

SPSS 17.0 statistical software was used for data analysis. Chi-square test was used for the comparison of the rate between the two samples. Differences were considered statistically significant with a $P < 0.05$.

3. Results

3.1. General Condition

The studies showed that the number of blood cells and the immune factors had recovery mostly, except there is a transient decreased on hour 12, with a peak around day 3, and again returned to baseline on day 28. Meanwhile, such changes were not seen without rhIL-12 treatment. Furthermore, studies showed that have five patients developed symptoms of fever, bilirubin increased and liver dysfunction with the dose of 300 ng/kg. So we found that the safe and well-tolerated human dose of 200 ng/kg is within this efficacious range based on exposure parameters through the research.

3.2. Blood Picture Test Results

We found that there was no significantly difference of erythrocyte and platelet, between groups treated with rhIL-12 and control groups. (**Table 1**, **Figure 1**) However, the number of blood cells was significantly decreased from day 0-day 3, while recovered from day 7-day 14 and down-regulated on day 21 after rhIL-12 treatment. Furthermore, results showed that number of white blood cells was obviously higher than in patients without rhIL-12 treatment (**Table 2**, **Figure 2**) ($P < 0.05$).

3.3. Immune Index Test Results

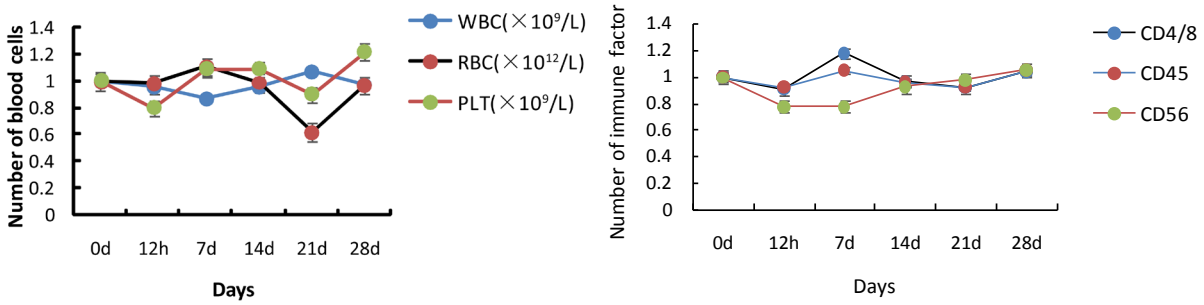
The results showed that the number of CD4/8+ T, CD45+ leukocytes, and CD56+ NK cells was elevated and improved with concentration of rhIL-12. In addition, they were reduced by day 0-hour 12, recovered from day 7, and then decreased from day 21 in treatment groups than control groups (**Table 3**, **Figure 3**) ($P < 0.05$).

3.4. The Results of the ECOG and WHO Score

We found that the ECOG score and WHO score were higher than the controls after one month. It's great to be

Table 1. The control group routine blood indicators ($\bar{x} \pm s$, n = 6).

Indicators	0 d	12 h	7 d	14 d	21 d	28 d
WBC ($\times 10^9$/L)	7.21 ± 0.27	6.92 ± 0.04	6.01 ± 0.12	5.74 ± 0.38	6.21 ± 0.01	6.41 ± 0.41
RBC ($\times 10^{12}$/L）	3.71 ± 2.72	3.62 ± 0.21	4.01 ± 0.12	3.98 ± 2.01	3.85 ± 0.21	3.76 ± 2.01
PLT ($\times 10^9$/L）	374 ± 0.41	301 ± 0.21	327 ± 12.1	357 ± 0.41	321 ± 0.17	392 ± 0.27
CD4/8	17.2 ± 1.2	15.6 ± 2.4	18.4 ± 1.7	17.9 ± 2.1	16.5 ± 4.2	17.2 ± 3.1
CD45	76.3 ± 2.4	69.2 ± 4.2	72.7 ± 3.7	69.7 ± 4.2	64.2 ± 3.8	67.2 ± 2.1
CD56	13.7 ± 1.2	12.8 ± 3.0	14.2 ± 2.1	13.2 ± 4.1	12.9 ± 2.1	13.7 ± 2.1



(a) (b)

Figure 1. The control group ((a) WBC; RBC and PLT change; (b) immune factor change).

Table 2. Each experimental group routine blood indicators ($\bar{x} \pm s$, n = 6).

group	indicators	0 d	12 h	7 d	14 d	21 d	28 d
50 ng/kg	WBC ($\times 10^9$/L)	7.66 ± 0.82	5.5 ± 0.67	4.3 ± 0.48	4.21 ± 0.62	4.69 ± 0.38	4.89 ± 0.63
	RBC ($\times 10^{12}$/L)	3.92 ± 0.31	4.26 ± 0.43	3.93 ± 0.22	4.38 ± 0.36	3.86 ± 0.34	3.89 ± 0.28
	PLT ($\times 10^9$/L)	358 ± 0.3	339 ± 31.4	298 ± 19.2	325 ± 0.31	356 ± 0.29	371 ± 0.26
100 ng/kg	WBC ($\times 10^9$/L)	7.4 ± 10.25	5.7 ± 17.89	5.6 ± 0.98	6.1 ± 0.64	5.73 ± 0.47	2.7 ± 0.21
	RBC ($\times 10^{12}$/L)	6.3 ± 0.72	3.37 ± 0.43	4.2 ± 0.37	4.78 ± 0.46	4.5 ± 0.42	4.6 ± 0.34
	PLT ($\times 10^9$/L)	231 ± 272	185 ± 10.7	195 ± 12.8	166 ± 10.9	182 ± 12.5	205 ± 15.3
200 ng/kg	WBC ($\times 10^9$/L)	7 ± 0.8	5.2 ± 0.3	3.5 ± 0.32	3.6 ± 0.27	4.3 ± 0.31	3.4 ± 0.43
	RBC ($\times 10^{12}$/L)	4.6 ± 0.51	3.2 ± 0.41	4.5 ± 0.3	4.4 ± 0.2	3.72 ± 0.34	3.5 ± 0.42
	PLT ($\times 10^9$/L)	278 ± 36	183 ± 19	258 ± 22	299 ± 24	307 ± 25	201 ± 21
300 ng/kg	WBC ($\times 10^9$/L)	3.6 ± 0.3	3 ± 0.37	4 ± 0.4	4.2 ± 0.3	4.9 ± 0.4	5 ± 0.4
	RBC ($\times 10^{12}$/L)	3.6 ± 0.3	3.3 ± 0.2	3.5 ± 0.3	3.4 ± 0.3	3.7 ± 0.2	4.1 ± 0.3
	PLT ($\times 10^9$/L)	364 ± 35	235 ± 21	241 ± 22	243 ± 19	242 ± 21	212 ± 20

that the patients have recovered. The important is that the quantity of life is improving (**Table 4**, **Table 5**).

3.5. Imaging Evaluation Result

We selected the imaging pictures from one patients that was carried out by cyber knife (the patients were given 5 Gy every time for 5 times continuously) and with 200 ng/kg of rhIL-12 treatment. And we observe the day 0, day 7, day 28 respectively. The results showed that the lesions size became increasing on day 0, day 7, day 28

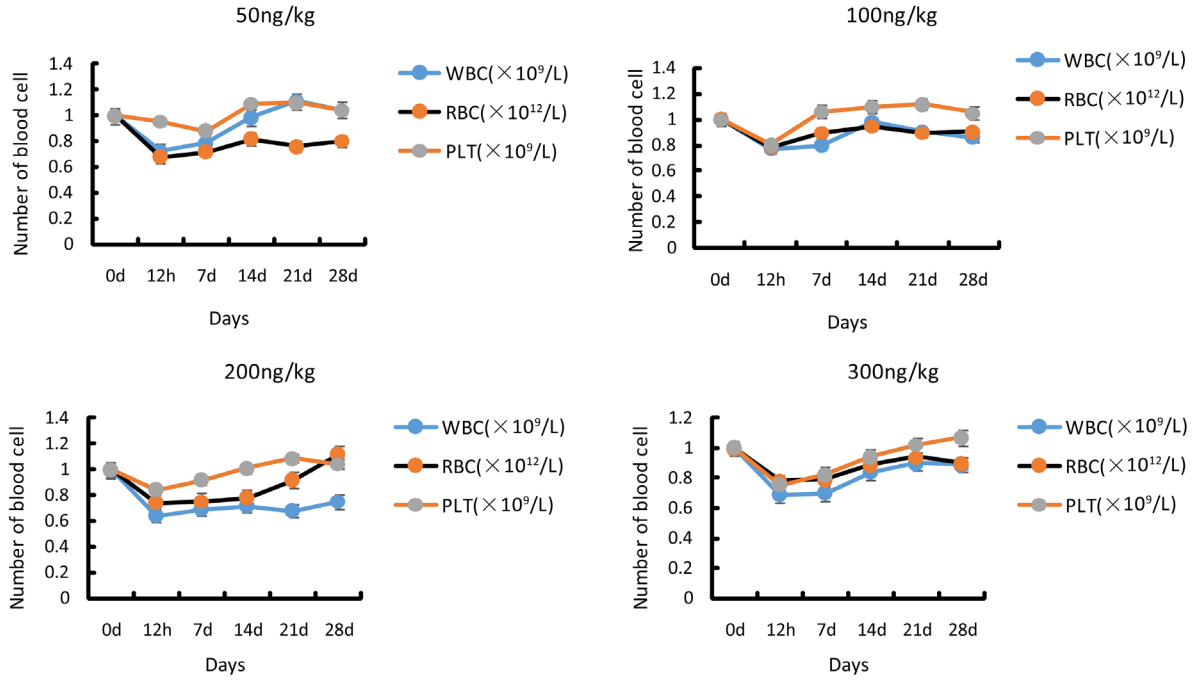


Figure 2. Different experimental blood cells change.

Table 3. Each group immune index results ($\bar{x} \pm s$, n = 6).

Group	Indicators	0 d	12 h	7 d	14 d	21 d	28 d
50 ng/kg	CD4/8	20.4 ± 2.6	18 ± 1.2	17.8 ± 1.3	18.4 ± 1.2	17.3 ± 1.4	6.5 ± 0.4
	CD45	75.2 ± 7.5	68.1 ± 5.2	83.3 ± 5.2	79.3 ± 3.7	62.4 ± 5.1	60.3 ± 3.6
	CD56	12.3 ± 1.2	8.7 ± 0.6	11.4 ± 1.3	10.4 ± 0.9	8.2 ± 0.6	6.7 ± 0.5
100 ng/kg	CD4/8	15.4 ± 1.5	18.7 ± 1.2	13.6 ± 1.2	6.1 ± 0.3	5.2 ± 0.4	2.7 ± 0.2
	CD45	84.3 ± 2.6	66.1 ± 3.8	67.9 ± 4.4	69.6 ± 4.6	65.1 ± 3.6	62.2 ± 3.1
	CD56	14.8 ± 1.8	8.9 ± 0.6	17.3 ± 1.5	6.4 ± 4.6	5.9 ± 0.3	6.4 ± 0.4
200 ng/kg	CD4/8	9.6 ± 0.4	8.3 ± 0.61	10.3 ± 0.9	11.03 ± 4.6	10.2 ± 1.2	2.05 ± 0.2
	CD45	64.5 ± 4.2	60.7 ± 4.2	76.6 ± 5.9	74.3 ± 4.6	73.2 ± 5.2	55.9 ± 5.0
	CD56	3.8 ± 0.2	2.8 ± 0.2	10.9 ± 0.9	8.8 ± 0.6	4.9 ± 0.3	1.5 ± 0.1
3000 ng/kg	CD4/8	3.4 ± 0.2	3.2 ± 0.2	4.7 ± 0.3	5.5 ± 0.3	11.4 ± 0.9	10.1 ± 0.9
	CD45	46.5 ± 3.9	40.2 ± 3.2	66.5 ± 3.8	54.9 ± 4.2	50.4 ± 4.9	35.9 ± 3.2
	CD56	3.3 ± 0.2	3.9 ± 0.2	7.3 ± 0.5	3.6 ± 0.3	7.2 ± 0.6	10.2 ± 0.8

before rhIL-12 treatment. However, we found that the lesions size became less on day 0, day 7, day 28 with rhIL-12 treatment in 200 ng/kg (**Figure 4**).

4. Discussion

Liver cancer is one of the most cancers all over the world, accounting for 50% of all the cancers and the fatality rate ranks second of our country. Although the local treatment has achieved the better improve, the effect of treatment is not significant [9] [10]. Thus, it becomes very essential subject to develop a new treatment for liver cancer except surgery, radiotherapy chemotherapy. Interleukin-12 (IL-12), a heterodimeric cytokine with p40

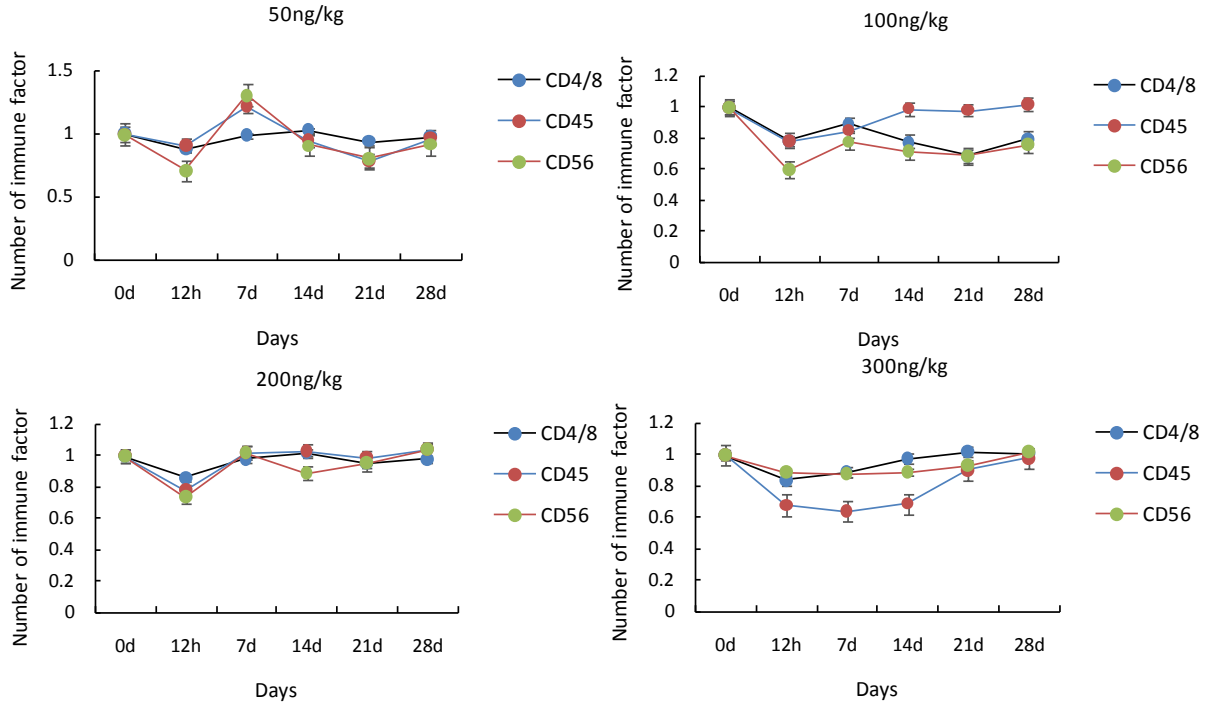


Figure 3. Different group immune factor change.

Table 4. ECOG score comparison.

Prior treatment			After treatment		
Group	**N**	**Symptom**	**Group**	**N**	**Symptom**
0	**2**	**Normal activity**	**0**	**10***	**Normal activity**
1	3	Have symptoms, but can be almost completely normal activity	1	2*	Have symptoms, but can be almost completely normal activity
2	10	Sometimes stay in bed, bed time not more than 50% during the day	2	9*	Sometimes stay in bed, bed time not more than 50% during the day
3	9	Sometimes stay in bed, bed for more than 50% during the day	3	7*	Sometimes stay in bed, bed for more than 50% during the day
4	6	Remain in bed	4	2*	Remain in bed
number	30		Number	30	
Effective rate	50%		Effective rate	70%	
χ^2 value		7.452			
P value		0.025			

*compared with before treatment ($P < 0.05$).

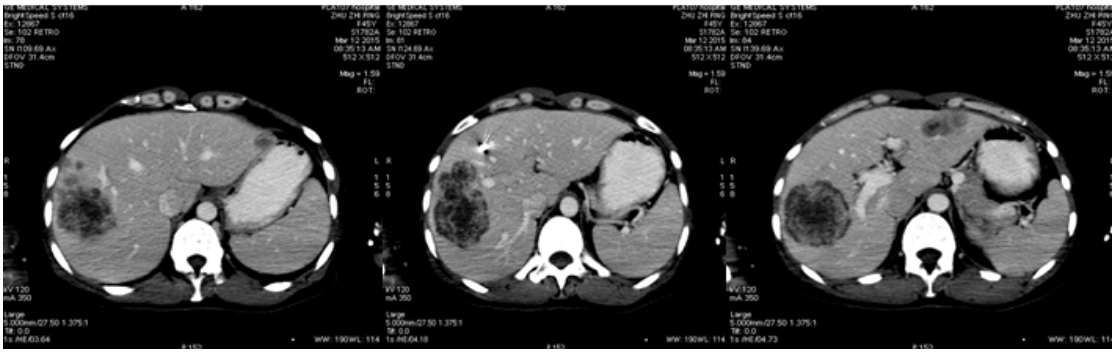
and p35 subunits, is well-known for its pleiotropic effects. Some studies show that IL-12 is capable of hematopoiesis with other cytokines [11] [12]. Meanwhile, IL-12 has been showed to play an essential role in the interaction between the innate and adaptive arms of immunity [13]. So based on its hematopoietic and immunomodulatory activities, a recombinant human IL-12 (rhIL-12) is now under development against the liver cancer [14].

Some researches have verified that the hematopoietic of rhIL-12 could affect the bone marrow stem cells, thereby promoting the proliferation and differentiation of hematopoietic progenitor cells [15]. Our studies showed

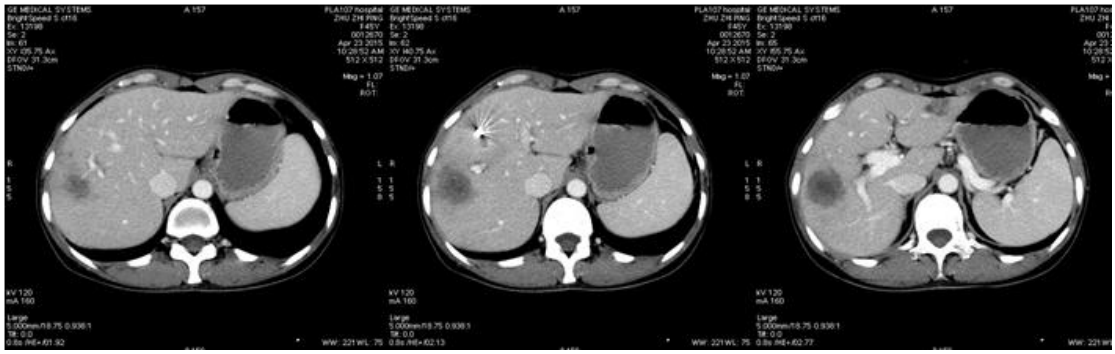
Table 5. WHO curative effect evaluation to compare.

Group	Complete relief	Partial relief	No change	Progress	Number	Effective rate (%)	χ^2 value	P value
Radiotheraphy	7	4	8	2	21	52.4	7.471	0.044
rhIL-12 + radiotheraphy	16	6	2	1	25	88*		

*compared with before treatment ($P < 0.05$).



(a)



(b)

Figure 4. CT revealed that tumor lesions are became decreasing with rhIL-12 treatment. (a) CT before rhIL-12 therapy (2015-01-28); (b) CT afte rhIL-12 therapy (2015-03-11).

that the blood cells of the liver cancer patients had returned to the normal level, while there is a transient decrease at hour 12, with a peak around day 3, recovered from day 7, and then decreased from day 21. These transient hematological changes could be explained on the basis of trafficking and redistribution of cells from the central blood compartment, neutrophil margination and exist into tissues, and lymphocyte redistribution to lymphoid organs. In this study, we also found that the number of blood cells was not restored significantly in the controls, but these were returned to the baseline around day 7. These results suggested that rhIL-12 have the effects that treat the liver cancer patients.

Gately *et al*. have reported a study where livers of IL-12 treated mice contained increased focal mononuclear cell infiltrates [16]. Interestingly, the transient changes in hematological and lymphocyte counts observed in our studies have been reported by others [17]-[19]. The immune factors results showed that rhIL-12 treatment-induced transient decreases in both CD4/8+ T, CD45+ leukocytes, and CD56+ NK cells at 12 hour, with a peak on day 3. At last, the level returned to baseline about on day 7 for the all immune cells. Interestingly, some studies have implicated interferon gamma (IFN-γ), the hallmark of immune activation by IL-12, as an important mediator of antitumor activity [20] [21]. Overall the changes observed in the immune factors studies suggest that the hallmark of rhIL-12 in immunity is its ability to stimulate the production of IFN-γ from NK cells, macrophages and T-cells. Meanwhile, the rhIL-12 could up-regulated adhesion molecules, thus the CD4/8+ T, CD45+ leukocytes, and CD56+ NK cells disappear the transient changes.

However, such changes were not seen without rhIL-12 treatment in controls, we could find that the numbers of blood cells and immune factors of the patients were returned to the normal gradually. Furthermore, studies showed that have five patients developed symptoms of fever, bilirubin increased and liver dysfunction with the dose of 300 ng/kg. So we found that the safe and well-tolerated human dose of 200 ng/kg is within this efficacious range based on exposure parameters through the research.

The observations from these studies indicate that rhIL-12 administered subcutaneously could elicit hematological and immune-mediated effects, resulting in enhancing the cytokine of immune response against liver cancer cells. It implies a potential effective method to treat the liver cancer. However, the exactly mechanism of rhIL-12 in treating liver cancer was still needed to be further explored.

Fund

This study was funded by the major projects Wu Jieping medical foundation (320.6750.14204).

Conflict of Interest

None.

References

[1] Pircher, A., Medinger, M. and Drevs, J. (2011) Live Cancer: Targeted Future Options. *World Journal of Hepatology*, **27**, 38-44. http://dx.doi.org/10.4254/wjh.v3.i2.38

[2] Ferlay, J., Autier, P., Boniol, M., Heanue, M., Colombet, M. and Boyle, P. (2007) Estimates of the Cancer Incidence and Mortality in Europe in 2006. *Annals of Oncology*, **18**, 581-592. http://dx.doi.org/10.1093/annonc/mdl498

[3] Little, R.F., Pluda, J.M., Wyvill, K.M., Rodriguez-Chavez, I.R., Tosato, G., Catanzaro, A.T., Steinberg, S.M. and Yarchoan, R. (2006) Activity of Subcutaneous Interleukin-12 in AIDS-Related Kaposi Sarcoma. *Blood*, **107**, 4650-4657. http://dx.doi.org/10.1182/blood-2005-11-4455

[4] Hamza, T., Barnett, J.B. and Li, B. (2010) Interleukin 12 a Key Immunoregulatory Cytokine in Infection Applications. *International Journal of Molecular Sciences*, **11**, 789-806. http://dx.doi.org/10.3390/ijms11030789

[5] Pertl, U., Luster, A.D., Varki, N.M., Homann, D., Gaedicke, G. and Reisfeld, R.A. (2001) Lode HN:IFN-Gamma-Inducible Protein-10 Is Essential for the Generation of a Protective Tumor-Specific CD8 T Cell Response Induced by Single-Chain IL-12 Gene Therapy. *The Journal of Immunology*, **166**, 6944-6951. http://dx.doi.org/10.4049/jimmunol.166.11.6944

[6] Yao, Q.Y., Zhang, Q.H., Ni, Q.X., *et al*. (2000) The Expression of Serum IL-6,IL-10,IL-12 Level in Gastrointestinal Neoplasm and Its Significance. *Chinese Journal of Gastrointestinal Surgery*, **3**, 3435.

[7] Basile, L.A., Gallaher, T.K., Shinbata, D., *et al*. (2008) Mutilineage Hematopoietic Recovery with Concomitant Antitumor Effects Using Low Dose Interleukin-12 in Myelosuppressed Tumor-Bearing Mice. *Journal of Translational Medicine*, **6**, 26. http://dx.doi.org/10.1186/1479-5876-6-26

[8] Chen, T., Burke, K.A., Zhan, Y., Wang, X., Shibata, D. and Zhao, Y. (2007) IL-12 Facilitates Both the Recovery of Endogenous Hematopoiesis and the Engraftment of Stem Cells after Ionizing Radiation. *Experimental Hematology*, **35**, 203-213. http://dx.doi.org/10.1016/j.exphem.2006.10.002

[9] Zhou, Z.F., Jiang, J.H., Li, J.Y., Chen, Q. and Ye, Y.B. (2013) IL-12 Plays Anti-Tumor Effect by Inducing NK Cell Activation in Hepatic Carcinoma Microenvironment. *Chinese Journal of Cancer Biotherapy*, **20**, 93-98.

[10] Kerkar, S.P. and Restifo, N.P. (2012) The Power and Pitfalls of IL-12. *Blood*, **119**, 4096-4097. http://dx.doi.org/10.1182/blood-2012-03-415018

[11] Hirayama, F., Katayama, N., Neben, S., Donaldson, D., Nickbarg, E.B., Clark, S.C. and Ogawa, M. (1994) Synergistic Interaction between Interleukin-12 and Steel Factor in Support of Proliferation of Murine Lymphohematopoietic Progenitors in Culture. *Blood*, **83**, 92-98.

[12] Jacobsen, S.E., Veiby, O.P. and Smeland, E.B. (1993) Cytotoxic Lymphocyte Maturation Factor (Interleukin 12) Is a Synergistic Growth Factor for Hematopoietic Stem Cells. *The Journal of Experimental Medicine*, **178**, 413-418. http://dx.doi.org/10.1084/jem.178.2.413

[13] Basile, L.A., Ellefson, D., Gluzman-Poltorak, Z., Junes-Gill, K., Mar, V., Mendonca, S., Miller, J.D., Tom, J., Trinh, A. and Gallaher, T.K. (2012) HemaMax, a Recombinant Human Interleukin-12. Is a Potent Mitigator of Acute Radiation Injury in Mice and Non-Human Primates. *PLoS ONE*, **7**, 330-434. http://dx.doi.org/10.1371/journal.pone.0030434

[14] CDER (2009) Guidance for Industry: Animal Models—Essential Elements to Address Efficacy under the Animal Rule.

US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research (CBER).

[15] Dybedal, I., Larsen, S. and Jacobsen, S.E. (1995) IL-12 Directly Enhances *in Vitro* Murine Erythropoiesis in Combination with IL-4 and Stem Cell Factor. *The Journal of Immunology*, **154**, 4950-4955.

[16] Gately, M.K., Warrier, R.R., Honasoge, S., Carvajal, D.M., Faherty, D.A., Connaughton, S.E., Anderson, T.D., Sarmiento, U., Hubbard, B.R. and Murphy, M. (1994) Administration of Recombinant IL-12 to Normal Mice Enhances Cytolytic Lymphocyte Activity and Induces Production of IFN-Gamma *in Vivo*. *International Immunology*, **6**, 157-167. http://dx.doi.org/10.1093/intimm/6.1.157

[17] Trudeau, C., Cotreau, M.M., Stonis, L., Dykstra, K.H., Oestreicher, J.L., Strahs, A., Dorner, A.J., Van Cleave, V.H., Trepicchio, W.L. and Schwertschlag, U.S. (2005) A Single Administration of Recombinant Human Interleukin-12 Is Associated with Increased Expression Levels of Interferon-Gamma and Signal Transducer and Activator of Transcription in Healthy Subjects. *The Journal of Clinical Pharmacology*, **45**, 649-658. http://dx.doi.org/10.1177/0091270005276116

[18] Ohno, R., Yamaguchi, Y., Toge, T., Kinouchi, T., Kotake, T., Shibata, M., Kiyohara, Y., Ikeda, S., Fukui, I., Gohchi, A., Sugiyama, Y., Saji, S., Hazama, S., Oka, M., Ohhashi, Y., Tsukagoshi, S. and Taguchi, T. (2000) A Dose-Escalation and Pharmacokinetic Study of Subcutaneously Administered Recombinant Human Interleukin 12 and Its Biological Effects in Japanese Patients with Advanced Malignancies. *Clinical Cancer Research*, **6**, 2661-2669.

[19] Robertson, M.J., Pelloso, D., Abonour, R., Hromas, R.A., Nelson Jr., R.P., Wood, L. and Cornetta, K. (2002) Interleukin 12 Immunotherapy after Autologous Stem Cell Transplantation for Hematological Malignancies. *Clinical Cancer Research*, **8**, 3383-3393.

[20] Gollob, J.A., Mier, J.W., Veenstra, K., McDermott, D.F., Clancy, D., Clancy, M. and Atkins, M.B. (2000) Phase I Trial of Twice-Weekly Intravenous Interleukin 12 in Patients with Metastatic Renal Cell Cancer or Malignant Melanoma: Ability to Maintain IFN-Gamma Induction Is Associated with Clinical Response. *Clinical Cancer Research*, **6**, 1678-1692.

[21] Gollob, J.A., Veenstra, K.G., Parker, R.A., Mier, J.W., McDermott, D.F., Clancy, D., Tutin, L., Koon, H. and Atkins, M.B. (2003) Phase I Trial of Concurrent Twice-Weekly Recombinant Human Interleukin-12 plus Low-Dose IL-2 in Patients with Melanoma or Renal Cell Carcinoma. *Journal of Clinical Oncology*, **21**, 2564-2573. http://dx.doi.org/10.1200/JCO.2003.12.119