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PREVENTIVE STUDY OF GASTRIC CANCER PERITONEAL MICROMETASTASIS IN NUDE MICE WITH ^{188}Re-LABELLED MONOCLONAL ANTIBODY 3H11

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Abstract. In advanced gastric cancer, especially when the serosa is invaded, the implantation of cancer cells in the peritoneum is common, and it affects patients' survival time severely. Based on successfully ^{labelled} monoclonal antibody 3H11 with ^{188}Re, we investigated the effect of RIT (radioimmunotherapy) with ^{188}Re-3H11 on preventing the establishment of gastric cancer cell peritoneal micrometastasis in nude mice. After 1×10^6 BGC - 823, gastric cancer cells were injected into the peritoneal cavity of each mouse, 45 BABL/C nude mice were divided into 9 groups. Each group received the various doses of ^{188}Re-3H11 or ^{188}Re-IgG or saline I.P.16 hours postoperation. The injected volume of each mouse was 1.0 mL. The results showed that the survial time depended on injected doses from 0 to 37MBq. The survival time was 170 ± 25.3 days after 37MBq ^{188}Re-3H11 were treated . It was over 5 times that of the saline group and about 3 times that of the 74MBq ^{188}Re-IgG group ($p<0.05$). The mice hemograms were reduced to lowest after injection 14 days, but they recovered after 28 days. Conclusion: Through properly injected doses, early postoperative ^{188}Re-3H11 I.P. is effective and safe in the prevention of intra-peritoneally injected gastric cancer cells from surviving, growing and disseminating in nude mice.

1. INTRODUCTION

In advanced gastric cancer, especially when the serosa is invaded, the implantation of cancer cells in peritoneal is common and it affects patients' survival time severely. So it is an important to prevent the plantation of cancer cells in peritoneal in order to increased 5 years survival ratios in patients with gastric cancer.

Dr Lu reported that 131I-3H11 I.P. postoperatively was effective and safe in the prevention of intra-peritoneally injected gastric cancer cells from surviving, growing and disseminating in nude mice and effectively reduce the incidence of liver metastases and the number of metastatic nodules in nude mice , with a prolongation of survival.

^{188}Re (β;Emax, 2.12MeV; γ;155keV, abundance of 15%) is a very attractive isotope for radioimmunotherapy, since it is obtained from a ^{188}W/^{188}Re generator in a carrier-free form on a daily basis. So it is a better therapeutic isotope than ^{131}I and ^{90}Y in aspect of RIT [1].

On the basis of successfully ^{labelled} monoclonal antibody 3H11 with ^{99m}Tc and ^{188}Re[2,3], we investigated the effect of RIT with ^{188}Re-3H11 on preventing the establishment of gastric cancer cells peritoneal micrometastasis in nude mice.

2. MATERIALS AND METHODS

2.1 Materials

^{188}W/^{188}Re Generator (Kexin Company, Shanghai China), SnCl2, A.R. (Sigma , American), UV (Sweden), 2-ME, A.R (American), ICON SPECT (Siemens, Germany)

2.2 Preparation of ^{188}Re-radio^{labelled} -3H11

Intact anti-gastric cancer monoclonal antibody 3H11 was reduced with 2-mercaptoethanol for 15min at room temperature and purified from excess thiol on a Sephadex G50 Column eluted with 0.05mol/L ABS (previously described). The reduced antibody 3H11 (1.0mg)was mixed with 1.0×10^{-3}g $SnCl_2$ and 15×10^{-3}g glucoheptonate and 7.0×10^{-3}g tartrate and 1.1×10^9Bq $Na^{188}ReO_4$ .The final

pH of mixed solution was about 50–5.5. It reacted 1.5–2.0h at room temperature. And then the product was purified by Sephadex G50 Column again [1,3].

3. ANIMAL TESTS

3.1. Biodistribution

The animal models of gastric cancer were established in nude mice by injecting 5×10^6 of 823 cells (0.1ml) subcutaneously into the axilla. When the tumour were approximately 0.3–0.4cm in diameter, the animals were given injection of the radio[labelled] Mabs 7.4 MBq by tail-vein. Five mice in each group were sacrificed by cervical dislocation at 24 h, 48 h and 72 h postinjection, respectively. Samples of tumour, blood and normal tissues were weighed, and then counted in an automatic γ well counter as well as a sample of the injection. The percentage of injected dose/g of radioactivity (i.d.%/g) in tissue were calculated. Tumor/normal tissue (T/NT) location ratios were determined from the cpm in tumourous and normal tissues.

3.2. RIT in nude mice

After 1×10^6 BGC -823 gastric cancer cells were injected into the peritoneal cavity of each mouse., 45 BABL/C nude mice were divided into 9 groups. Each group received the various doses of ^{188}Re-3H11 or ^{188}Re-IgG or saline I.P., 16 hours postoperation. The injected volume of each mouse was 1.0 mL. The injected doses of each group were saline, 7.4 and 37 MBq ^{188}Re-IgG, 7.4 MBq, 18.5 MBq, 37 MBq, 55.5 MBq and 74 MBq ^{188}Re-3H11 respectively. They were observed through hemogram, tumour formation and survival time.

4. RESULTS

The chemical purity of 3H11 was more than 95%,Kd was $5.68 \times 10^9 M^-$. The radiolabelling yield was more than 90%. The immunoreactivity of ^{188}Re-3H11 was over 70%.

TABLE I. BIODISTRIBUTION OF 188RE-3H11 IN NUDE MICE BEARING 823 GASTRIC CANCER XENOGRAFTS. DATA ARE MEAN ±S.D. OF FIVE ANIMALS AT EACH TIME POINT.

Time	24h		48h		72h	
Organ	id%/g	T/NT	id%/g	T/NT	id%/g	T/NT
Blood	6.29 ± 0.41	1.15 ± 0.03	3.28 ± 0.54	2.45 ± 0.55	2.06 ± 0.17	2.71 ± 0.53
Heart	1.37 ± 0.21	5.52 ± 0.98	0.95 ± 0.15	8.59 ± 0.47	0.70 ± 0.05	7.52 ± 0.28
Liver	2.20 ± 0.61	3.50 ± 0.81	1.39 ± 0.05	5.55 ± 0.95	0.81 ± 0.08	6.93 ± 0.62
Spleen	2.85 ± 0.78	2.67 ± 0.57	1.79 ± 0.50	4.40 ± 0.44	0.75 ± 0.09	7.06 ± 0.69
Kidney	4.03 ± 0.14	1.80 ± 0.11	3.55 ± 0.46	2.17 ± 0.20	2.18 ± 0.45	2.48 ± 0.41
Lung	1.95 ± 0.19	3.73 ± 0.15	1.55 ± 0.21	4.97 ± 0.32	0.95 ± 0.19	5.53 ± 0.95
Stomach	0.89 ± 0.06	8.21 ± 0.67	0.77 ± 0.13	10.4 ± 3.17	0.52 ± 0.06	10.2 ± 0.87
Intestine	0.65 ± 0.06	11.3 ± 1.06	0.58 ± 0.08	13.6 ± 3.90	0.42 ± 0.09	12.6 ± 1.27
Muscle	0.63 ± 0.10	11.8 ± 0.63	0.59 ± 0.07	16.8 ± 4.58	0.24 ± 0.05	21.8 ± 4.63
Bone	0.82 ± 0.07	8.91 ± 1.08	0.98 ± 0.21	8.22 ± 2.81	0.92 ± 0.17	5.71 ± 1.28
Tumour	7.60 ± 0.24		7.73 ± 1.36		5.25 ± 0.41	

The biodistribution results (Table 1) in nude mice demonstrated that ^{188}Re-3H11 was fast cleared from the blood and given rise to good T/NT ratios at 24 to 72h postinjection. The tumour uptake appeared to reach a peak at 24h postinjection and fell slowly thereafter. The id%/g of tumour was 7.60 ± 0.24 and 5.25 ± 0.41 at 24h and 72h postinjection respectively. The T/NT ratios were increased from 24h to 72h postinjection.

The mice hemogram were reduced to lowest 14 days after injection when the doses were 37 MBq and 55.5 MBq, respectively. But they recovered after 28 days. The mice hemograms were not significantly decreased when injected doses were less than 18.5MBq.

Two weeks after tumour implantation without therapeutically injected drugs, over a hundred cancerous nodules were found in serosa of each mouse in control group of three nude mice.

The relation between the survival time and therapeutic doses was shown in Figure 1. It showed that the survial time depended on injected doses during 0 to 37 MBq. The survival times of saline group, 7.4 MBq and 18.5 MBq, 37 MBq ^{188}Re-3H11group was 33.5 $\pm$ 3.3 days, 37.5 $\pm$ 4.2 days, 45.2 $\pm$ 6.8 days and 170 $\pm$ 25.3 days, respectively. But when injected doses was 55.5 MBq and 74 MBq, the survival time was significantly reduced. The animals died of internal hemorrhage 18 days after injection, and of intestinal liquefacient ulcer 5days after injection, respectively.

The survival time of injected 7.4 MBq ^{188}Re-3H11 group was more than 5 times than saline group and about 3 times than of 7.4M Bq ^{188}Re-IgG group ($p < 0.05$).

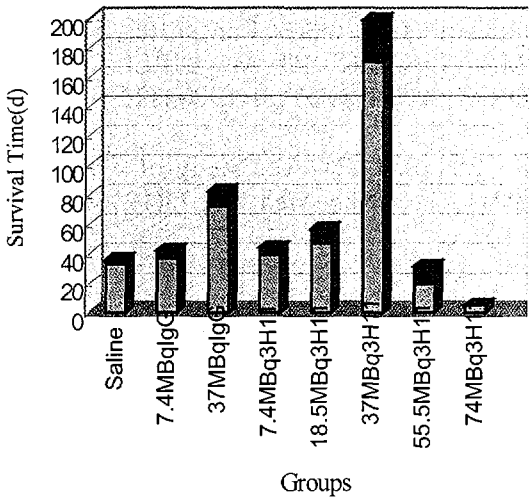

FIG. 1. The relation between therapeutic doses & survival time

5. DISCUSSION AND CONCLUSION

Currently, in most of clinical RIT in solid tumour, the percentage of injected pharmaceutical dose in tumour is less than 0.0001/g. The effective rate is less than 20%. There are two main reasons for the poor effect. In one hand, most of patients anticipated in the clinical test are in advanced stage. In this stage, the tumour's volume is large, its interstitial pressure is high and the absorbed dose of pharmaceutics to tumour is low, so the therapeutic effect is limited. In the other hand, the administration route of pharmaceutics in present is always by i.v.. The pharmaceutical dose to the tumour site is not enough to kill the tumour spheroid thoroughly.

So we think: (1) In the future, the real prospect of RIT is to kill the micrometastasis of tumour postoperation in order to prevent its recurrence; (2) The administration route should be changed to reduce the barriers to the tumour in order that the MoAb could be touched with tumour directly, and more MoAbs are absorbed by tumour and less are present in other organs and tissues. Based on the research work and considering the problem of RIT in the clinic, we think the real prospect of RIT for gastrointestinal conditions is to kill the micrometastasis of tumour postoperation.

The radioactivity was well concentrated in tumour from 24 h to 72 h after injection of ^{188}Re labelled 3H11. It is possible to make a study of RIT with ^{188}Re-3H11 on preventing the establishment of gastric cancer cell peritoneal micrometastasis in nude mice.

The survival time is related to injected doses. When the injection doses ranged from 0 to 37 MBq, the survival time was prolonged with raised injection doses. However, when the doses were over 55.5 MBq of ^{188}Re-3H11, the survival time was rapidly reduced. Thus it is very important to determine the proper dose range in order to effectively prevent micrometastasis form and prolong the survival time in nude mice.

Conclusion: Through properly injected doses, early postoperative ^{188}Re-3H11 I.P. is effective and safe in the prevention of intra-peritoneally injected gastric cancer cells from surviving, growing and disseminating in nude mice.

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