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Experimental application of tumor-derived glycopeptide vaccine for treatment of melanoma

Doświadczalne zastosowanie szczepionki przeciwnowotworowej w leczeniu czerniaka

The incidence of melanoma is increasing at a worldwide dramatic rate. Moreover, melanomas represent the third most common primary tumors metastasizing to the brain [5]. Despite recent advances in conventional methods of treatment, innovative strategies are urgently needed for the successful treatment of patients with primary melanoma or brain metastases [2]. One of the more promising approaches for the treatment, of melanoma is the development of cancer vaccines [8]. Over the past two decades the number of cancer vaccine approaches has rapidly grown with progress in the understanding of cancer immunosurveillance and a wide variety of vaccination schdules have been carried out both in animal models and in human trials [2, 8].

The main goal of the present study was to investigate the efficacy of glycopeptide cancer vaccine (gp50) application in mice with melanoma B16.

MATERIAL AND METHODS

C57Bl/6 mice were obtained from vivarium of R.E. Kavetsky IEPOR, NAS of Ukraine (Kyiv, Ukraine) and transplanted with $5 \cdot 10^5$ (subcutaneously – s.c.) or $3 \cdot 10^5$ (intracranial – i.c.) cells of melanoma B16 (B16) obtained from National Bank of Cell Lines and Tumor Strains of R.E. Kavetsky IEPOR, NAS of Ukraine (Kyiv, Ukraine). All procedures of welfare and treatment of animals were conducted under strict ethical conditions according to international recommendations. The glycopeptide cancer vaccine (gp50) was originally prepared as previously described [7]. The gp50 was triply s.c. injected in a dose of 10.000 equivalents of tumor cells to mice. At various times after treatment, non-specific cytotoxic activity of splenic mononuclear cells (MNC) of i.c. B16-transplanted mice were performed according to [1]. To detect MNC infiltration at the site of intracranial tumor cell challenge, the histological examination of mouse brain microscopic sections routinely stained was H&E was performed. Statistical analysis was performed using GraphPad InStat. Survival was determined using Kaplan-Meier method.

RESULTS AND DISCUSSION

Melanoma is one of the most immunogenic tumors and it expresses more than one tumor-associated protein simultaneously. Consequently, B16 was chosen to test the efficacy of gp50 that

employ the composition of B16-derived 50 kDa-glycopeptides. So far Mansour et al. [4] showed that only melanoma-based vaccines containing more than two peptide antigens may improve the therapeutic vaccination outcome. In the present study, the effect of gp50-vaccination on tumor growth and survival of mice with differently transplanted B16 has been investigated. As shown in Fig. 1, there was a significant difference in tumor growth between gp50-vaccinated and control mice during 21st–28th days after s.c. B16 transplantation. Furthermore, the mean survival of gp50-vaccinated mice was 35.4±7.3 days vs 28.7±2.1 days in control mice; however increase in the life span (ILS) was only 23.4% (Table 1). Significantly prolonged mean survival was observed in i.c. transplanted B16 mice after s.c. gp50-vaccinations (27.7±8.0 days in comparison with 13.7±3.8 days in control group; ILS was 102.2%). Moreover, histological examination of all gp50-vaccinated mice that survived until day 50 revealed no evidence of viable i.c. B16 cells and indicated a complete tumor rejection in 50% mice treated with gp50 (Table 1). Similarly, Sampson et al. [6] also demonstrated a significant improvement in the survival of mice with s.c. injections of GM-CSF-transduced irradiated B16 cells.

Table 1. Effects of gp50 application on survival rates of B16-transplanted C57Bl/6 mice

Group	Challenge mode		Median survival, days	ILS, %	No./% Long term survivors
	tumor	gp50			
Control (n=10)	s.c.	s.c.	28.7±2.1	–	0/0
gp50 (n=19)			35.4±7.3*	23.4	1/5
Control (n=6)	i.c.	s.c.	13.7±3.8	–	0/0
gp50 (n=6)			27.7±8.0*	102.2	3/50

*P < 0.05 compared with respective control

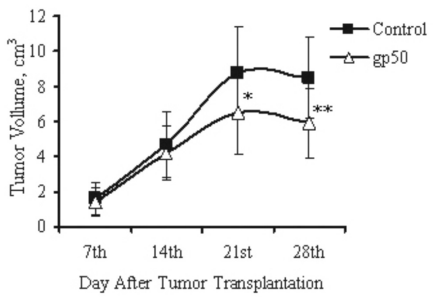

Fig. 1. Dynamic of tumor growth in B16-transplanted mice vaccinated by gp50; *P<0.05, **P<0.01 compared with respective day in control group

To examine if gp50-vaccination indeed exerts an immunomodulatory effect, the non-specific cytotoxic activity of MNC obtained from the spleens of immunized and non-immunized mice with established i.c. B16 was studied. Control B16-bearing mice demonstrated the changeable MNC cytotoxic activity which was significantly decreased on the ninth day after i.c. B16 transplantation (Table 2). In contrast, MNC obtained from gp50-vaccinated mice exhibited a gradual increase of cytotoxic activity. After the third vaccination (on ninth day post i.c. B16 transplantation) cytotoxicity index of MNC was substantially increased as compared with un-treated control mice and the results observed after both previous gp50-administrations (P<0.01; Table 2). Histological analysis of the brain microscopic sections of gp50-treated mice showed separate regions of dystrophy and necrosis of melanoma cells and local peritumoral MNC infiltrates. However, quantitative analysis of the areas of inflammatory infiltrates revealed a significant decrease of MNC in mouse brain after the

second gp50-vaccination as compared to control (Table 3). In contrast, Liu et al. [3] showed that s.c. immunizations after i.c. tumor cell challenge does not result in a significant difference in the local peritumoral MNC infiltration.

Table 2. Cytotoxic activity[1] of mononuclear lymphocytes in gp50-vaccinated C57Bl/6 mice with i.c. transplanted B16

Group	Injection No. / Day after intracranial tumor challenge		
	1st/3rd	2nd/6th	3rd/9th
Intact mice (n=5)	24.3±2.5		
Control B16-bearing mice (n=15)	15.0±0.5	24.5±7.6*	13.3±2.6
gp50-treated mice (n=15)	14.9±1.9	21.2±3.4**	22.9±3.2**/##

*P<0.05, **P<0.01 compared with 1st injection; ##P<0.01 compared with respective injection in control tumor-bearing mice; [1]expressed by cytotoxic index

Table 3. MNC infiltration in gp50-vaccinated C57Bl/6 mice with i.c. transplanted B16

Group	Injection No. / Day after intracranial tumor challenge		
	1st/3rd	2nd/6th	3rd/9th
Control B16-bearing mice (n=15)	16.8±5.5		
gp50-treated mice (n=15)	18.8±2.5	6.8±1.6**/##	10.4±2.1*/##

*P<0.05, **P<0.01 compared with control; ##P<0.01 compared with 1st injection

CONCLUSIONS

These data are partially consistent with the results demonstrated in our earlier survival experiments [7] and support the suggestion that vaccination with gp50 can be a perspective strategy for the treatment of brain-localized metastases of melanoma.

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SUMMARY

The application of melanoma-derived gp50 was more effective in mice with i.c. transplanted melanoma B16; however, mice with s.c. transplanted tumors demonstrated an insignificant increase in survival. Spleen mononuclear cells obtained from i.c. B16 transplanted mice showed enhanced cytotoxic activity after the third injection of gp50, while histological examination of brain tissue specimens obtained in gp50-vaccinated mice after i.c. B16 cell challenge demonstrated a quantitative increase of tumor-infiltrating mononuclear cells. The results provided the evidence that gp50 can be effective for melanoma treatment.

STRESZCZENIE

Podanie szczepionki gp50 z komórek czerniaka było bardziej skuteczne u myszy z wewnątrzczaszkowo (i.c.) wszczepionym czerniakiem B16, zaś u myszy z podskórnie (s.c.) wszczepionym nowotworem stwierdzono nieznaczny, nieistotny wzrost przeżycia. Komórki jednojądrzaste śledziony uzyskane od myszy z i.c. wszczepionym czerniakiem B16 wykazywały zwiększoną aktywność cytotoksyczną po trzecim podaniu gp50, podczas gdy badanie histologiczne tkanki mózgowej wykazało wzrost ilości jednojądrzastych komórek infiltrujących guz. Wyniki badań wskazują, że szczepionka gp50 może być skuteczna w leczeniu czerniaka.