Echovirus-mediated Biotherapy for Malignant Tumours: 40 Years of Investigation

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Mainly two approaches of active tumour immunotherapy, the antigen-loaded dendritic cells and several live non-pathogenic Newcastle disease virus have been considered by Western scientists as effective in clinical trials. The purpose of the present publication is to initiate scientific society into the 40-year experience of Latvian scientists in another live virus-mediated, *e.g.*, ECHOvirus-mediated biotherapy of malignant tumours. In the early studies (1960–1965), 72% of the 60 proven human enteroviruses, mainly the ECHO group, appeared to be oncotropic or oncolytic in various human tumours grown in short-time tissue cultures, or as heterotransplants. Malignant melanoma (MM) appeared to be the most susceptible tumour. It gave hope for the development of active ECHOvirus-mediated biotherapy in oncology. One of the ECHOvirus strains with the most stable oncolytic properties has been attenuated, repeatedly adapted to malignant melanoma (MM) tissue and labelled as Rigvir (Riga virus). In experimental tumour-host systems Rigvir exerted significant immunomodulatory features. This resulted in a promotion of the tumour regression and an adjuvant effect with surgery and/or low-dose chemotherapy. Rigvir elevated expression of differentiation antigens on MM cells and caused a full regression of hetero-transplants in 7–8 days along with abundant infiltration of regressing tumours by lymphocytes, macrophages and plasma cells. The clinical benefit of Rigvir used as an adjuvant after surgery or chemotherapy was evaluated by 3- and 5-year survival in 750 skin and 77 ocular MM patients, 97 gastric and 60 rectum cancer patients (stage II–IV). Rigvir appeared to have a strong lymphocyte blast-transforming activity *in vitro* and to provoke a radical increase of $CD8^+$ and $CD38^+$ cell number. Patients with regional lymph node metastases revealed a significant 5-year survival benefit (75% as compared with 21% of other therapies). The best results were achieved by regional inoculation of the virus: 80.9% in Rigvir-treated patients versus 42.5% without Rigvir. A stable increased $CD8^+$ cell number during Rigvir therapy was associated with advanced but slow regression of MM metastases. Rigvir improved the 5-year survival of ca rectum patients: 77.5% *versus* 41% by only surgery.

Key words: ECHOvirus, immunotherapy of melanoma, immunotherapy of gastric cancer, immunotherapy of cancer rectum, tumour-associated antigens, differentiation antigens

INTRODUCTION

Recent advances in the understanding of the molecular mechanisms of antigen processing and presentation and the identification of tumour-associated antigens in melanoma and other cancer cells have pointed to a role for immunotherapy in the treatment of cancer. New generations of cancer vaccines have been developed. Nevertheless, only two approaches, the antigen-loaded dendritic cells (1) and live Newcastle disease (NDV) virus strains (2, 3) have been considered as effective in clinical trials. Unfortunately, Western scientific society is not familiar with another virus, the human ECHOvirus strain Rigvir. Rigvir has been developed in Latvia under the leadership of Dr. Med. A. Muceniece. Rigvir exerts a specific immunologic reaction since it pos-

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sesses high antigenic and imunogenic properties. The administration of Rigvir leads to a regression of experimental tumours in different animal tumours. The regression is proceeded against the background of viral immunogenesis (4, 5). Latvian virologists and oncologists have an experience of 40-year investigation of Rigvir-mediated imuno/biotherapy in oncology.

The purpose of the present publication is to initiate scientific society into the 40-year experience of Latvian scientists in ECHOvirus-mediated biotherapy of malignant tumours.

MATERIALS AND METHODS

Interference of Rigvir with MM cell cultures, transplants in nude mice as well as MM, gastric, and rectum cancer disease in patients was evaluated by the growth rate, antigenic pattern, and immune cell response. Clinical benefit of Rigvir used as an adjuvant after surgery or chemotherapy was evaluated by 3- and 5-year survival in 750 skin and 77 ocular MM patients, 97 gastric and 60 rectum cancer patients (stage II–IV). The survival rates in Rigvir-treated patients were compared with those of patients treated with several other immunomodulators (levamisole, *Corynobacterium parvum*, splenin) and after surgery alone. Rigvir was injected intramuscularly in the gluteal region or regionally to the tumour site. The tumour-specific (progression) antigens (MAGE group) as well as the differentiation antigens (gp100, tyrosinase 100) in tumour specimens and MM cell cultures were detected by PCR before and after Rigvir treatment. The systemic immune cell response was evaluated by patient's peripheral blood mononuclear cell (PBMC) sub-populations detected by a FASCalibur laser cytometer in the blood and short lymphocyte cultures before and after Rigvir treatment. The local immune cell response as well as neoplastic cell morphology was evaluated in tumour smears and/or histological sections. For statistical analysis the Student's t test and the cumulative survival analysis (6) were employed.

RESULTS AND DISCUSSION

In the early studies (1960–1965), 72% of the 60 proven human enteroviruses appeared to be oncotropic or oncolytic in various human tumours grown in short-time tissue cultures or as heterotransplants. Malignant melanoma (MM) appeared to be the most susceptible tumour (4) One of the ECHOvirus strains with the most stable oncolytic properties was attenuated, repeatedly adapted to malignant melanoma (MM) tissue and labelled as Rigvir. Rigvir is a human ECHOvirus strain and, being an RNA virus, it does not integrate into the host cell DNA. Rigvir exhibited significant immunomodulatory features both in experimental tumour-host systems and in oncologic patients (7–13). This resulted in a promotion of the tumour regression and an adjuvant effect with surgery and/or low dose chemotherapy (5, 13, 14).

An ideal cancer treatment should be able to eradicate systemic tumours at multiple sites in the body and to discriminate between neoplastic and non-neoplastic cells. A significant role in the antigen processing and presentation belongs to dendritic cells. Activation of antigen-specific T-cell-mediated immune responses allows for killing of tumours associated with a specific antigen (15, 16). Rigvir meets this demand, since it elevates the expression of differentiation antigens on MM cells. The expression of MAGE antigens in tumours of Rigvir-treated patients is depressed (Table 1). Rigvir exhibits a strong lymphocyte blast-transforming activity *in vitro* (2-fold of PHA and 4-fold of LPS blast transforming activity) and provokes a radical increase of $CD8^+$ and $CD38^+$cell number (Table 2), induces maturation of monocytes into dendritic cells.

It has been repeatedly shown that Rigvir causes a direct short-term oncolysis in 6–20% of malignant cells in susceptible human tumours and may provoke subsequent rejection of such tumours (4, 5, 11, 12).

The delay of tumour growth or regression of tumour nodes caused by Rigvir was accompanied by local, regional and systemic cellular reactions: peritumoural, intratumoural perivascular infiltration of T-, B-, and plasma cells (sometimes Mo/Mϕ), output of active T-lymphocytes (CD38) and $CD8^+$. The cellular reactions were observed both in experimental tumour-host systems and patients (4, 5, 7–21).

In clinical studies Rigvir as an adjunct to surgery resulted in an increase of 3- and 5-year survival of patients and in delay of recurrences. The expression of the tumour progression antigens was depressed (Table 1). The 3-year survival rate of Rigvir-

Table 1. **Expression of Progression antigens (MAGE group) in melanoma tissue specimens (percent of positive patients)**

Naevus pigmentosus	0	n = 10
Primary melanoma	58.0	n = 48
Melanoma metastases	85.0	n = 7
Melanoma: stabilization	27.0	n = 8
Melanoma: progression	84.0	n = 13
Treatment:		
• Rigvir	14.2	n = 12
• Placebo	60.0	n = 10
• Surgery only	56.0	n = 62

Table 2. **3-year-survival (%) of malignant melanoma patients**

(A) Treated after surgery with immunomodulators

Trials	Rigvir	Levamisole	C. parvum	C. P., Levamisole, Splenin	Surgery alone
(1983) n = 149	84.0*	78.6	62.0	n.d.	54.5
(1987) n = 156	78.3*	54.5	n.d.	71.0	56.2
(1996) n = 142	77.7*	n.d.	n.d.	n.d.	56.0
(1991) n = 252	78.0*	n.d.	n.d.	70.1	56.5

(B) According to melanoma localisation

Localisation of melanoma	Rigvir	Rigvir, regional inoculation	C. parvum, Levamisol, Splenin
Head and neck	68.0	*87.5**	78.7
Face and mouth	78.0	n.d.	n.d.
Eye	77.0	n.d.	n.d.
Arms	72.1	*100.0**	86.9
Legs	n.d.	*86.0**	46.5
		*94.0**	
Trunk	66.0	n.d.	72.0
	50.0	n.d.	72.0

*Difference from the rate in other columns $p < 0.05$.

treated MM was significantly higher than that of skin MM patients treated with levamisol, *Corynobacterium parvum* or splenin (Table 3A). Patients with regional lymph node metastases showed a significant 5-year survival benefit (75% as compared with 21% of other therapies). The best results were achieved by regional inoculation of the virus (Table 3B). A stable increased $CD8^+$ cell number during Rigvir therapy associated with an advanced but slow regression of MM metastases (7, 9). Rigvir improved the 5-year survival of *ca rectum* patients: 77.5% *versus* 41% by surgery alone (12, 13). The survival improvement by Rigvir is near to that by NDV (17–19), but much better by regional Rigvir application.

Table 3. **Distribution of peripheral blood lymphocyte subpopulations after *in vitro* treatment of peripheral blood mononuclear cells with Rigvir (short lymphocyte culture)**

Subset	Reference range	72 h After Rigvir	3 weeks after Rigvir
CD 3	60–85	80.26	*98.08*
CD 4	35–55	36.04	*51.27*
CD 8	20–32	*47.60*	*42.50*
CD 38	10–20	*26.38*	3.92
CD 16	6–20	14.20	1.10
HLA-DR	10–18	3.80	1.23
CD 95	12–20	*48.25*	3.32

Our clinical trials (1063 oncologic patients) confirmed the parenteral administration of Rigvir, even upon a long-term use, to be completely clinically and epidemiologically safe.

Local and systemic immune response patterns under the action of Rigvir differed at various stages of the tumoural processes and depended upon the course of the disease (slow, rapid) and the immunological background (immune status) at the beginning of the therapy (8, 10).

The Rigvir treatment modified the amount (recruits) of T-lymphocytes, both the total amount and the number of active T (CD38) in the tumour, peritumoural tissue and Ts (CD8) in the circulation (7).

CONCLUSIONS

Clinical trials (1063 oncologic patients, including 824 MM patients, more than 30 years of observation) confirmed the parenteral administration of Rigvir as clinically and epidemiologically safe. Rigvir replication properties show tumour specificity. It is an agent with multiple immunomodulating and/or adaptogenic properties and has been successfully tested in various pre-clinical and clinical studies without causing severe side effects. Rigvir significantly improves 3- and 5-year survival of melanoma and *cancer rectum* patients. Rigvir may be used for post-surgical prevention of metastases, but the best survival benefit has been achieved when inoculation of Rigvir was regional to the site of tumour.

For the action of Rigvir in MM patients our findings suggest the mutual overlapping of:

(1) initial short-term viral oncolysis;

(2) local (in the tumour burden) modulation of the melanoma-associated antigens (MAGE, MART, tyrosinase, gp100) expression that may result (in an appropriate HLA-type patient) in an enhanced immunogenicity by transforming the growing tumour nodule into a tumour vaccine *in situ*. That if followed by recruitment and activation of the host antitumoural cellular and humoral immune reactions with subsequent immunological tumour rejection and tumour cell death via apoptosis;

(3) systemic correction of tumour-altered immunity (withdrawal of tumour escape reactions?) on the background of a complete ECHOvirus immunogenesis.

References

1. Bubenic J. Genetical engineering dendritic cell-based cancer vaccines (review). Int J Oncol 2001; 18(3): 475–8.
2. Schirrmacher V, Haas C, Bonifer R, Ahlert T, Gerhards R, Ertel C. Human tumor cell modification by virus infection: an efficient and safe way to produce cancer vaccine with pleiotropic immune stimulatory properties when using Newcastle disease virus. Gene Therapy 1999; 6: 63–73.
3. Moss RW. Newcastle disease virus vaccine (MTH-68). The Cancer Chronicles 1996; 7: 7–10.
4. Муцениеце АЯ. Онкотропизм вирусов и проблема виротерапии рака. Рига, Зинатне, 1972. 443 с.
5. Фердат АК, Брувере РЖ, Витолинь ЛА, Петровска Р. Механизм иммуномодуляции в противоопухолевом действии энтеровируса ECHO-7. Экспериментальная онкология 1989; 11, 5: 43–48.
6. Березкин ДП. Метод расчета показателей наблюдаемой и скорректированной выживаемости больных. Вопросы онкологии 1982; 11: 12–9.
7. Глинкина ЛС, Брувере Р, Венскус Д, Попена Б, Муцениеце А. Показатели клеточного иммунитета у больных злокачественной меланомой при применении вирусного иммуномодулятора ригвира. Вопросы онкологии 1992; 38(5): 540–7.
8. Глинкина ЛС, Хейселе ОГ, Гарклава РР, Муцениеце АЯ. Показатели гуморального иммунитета у больных злокачественной меланомой кожи при применении вирусного иммуномодулятора ригвира. Вопросы онкологии 1992; 38(5): 540–7.
9. Глинкина ЛС, Брувере Р. Реакция Т-системы иммунитета больных злокачественной меланомой кожи и раком желудка на активную неспецифическую иммунотерапию. Вопросы онкологии 1992; 38(6): 659–66.
10. Ferdats A, Volrāte Ā, Heisele O, Glinkina L, Muceniece A. Induction of alloimmunity to the melanoma associated antigen by enterovirus ECHO-7. Proc Latvian Acad Sci 1993; 5: 65–7.
11. Брувере РЖ, Витолинь ЛА, Гарклава РР, Муцениеце АЯ. Влияние иммуностимулятора вирусной природы на клеточный состав и топографические особенности инфильтрации стромы первичной опухоли рака прямой кишки. Известия АН Латвийской ССР 1980; 7: 137–142.
12. Гарклава Р, Брувере Р Ж, Витолинь Л А, Приедите И Ю, Муцениеце А Я. Морфологические и клинические паралели изменения рака прямой кишки во время комбинированного лечения. In: Иммунологические аспекты вирусного онкотропизма. Рига, 1979: 114–120.
13. Гарклава РР, Приедите ИЮ, Муцениеце АЯ. Отдаленные результаты оперативного лечения больных раком желудка и прямой кишки после иммуностимуляции их апатогенным энтеровирусом. Иммунокомпетентность и иммунотерапия больных злокачественными новообразованиями. Под ред. Городиловой ВВ. Кемерово, 1981: 77–91.
14. Григалинович ГА, Рудзитис МФ, Скудра МП, Попена БА, Десятникова ИП, Гарклава РР. Влияние иммуностимулятора вирусной природы на морфологию меланомы кожи и выживаемость больных. Известия АН Латвийской ССР 1988; 12: 72–75.
15. Boon T, Cerotini JC, Van den Eynde B, Van der Bruggen P, Van Pel A. Tumor antigens recognized by T-lymphocytes. Ann Rev Immunol 1994; 12: 337–65.
16. Chen CH, Wu TC. Experimental vaccine strategies for cancer immunotherapy. J Biomed Sci 1998; 5: 231–52.
17. Cassel WA, Murray DR, Phillips HS. A Phase II study on the postsurgical management of stage II malignant melanoma with a Newcastle disease virus oncolysate. Cancer 1983; 52: 856–60.
18. Plager C, Bowen JM, Fenoglio C. Adjuvant immunotherapy of MD Anderson hospital (MDAH Stage III-B malignant melanoma with Newcastle disease virus oncolysate). Proc Ann Meet Am Soc Clin Oncol 1990; 9: A1091.
19. Csatary LK, Eckhardt S, Bukosza I. Attenuated veterinary virus vaccine for treatment of cancer. Cancer Detection and Prevention 1993; 17: 619–127.