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Effect of the type of application of Newcastle disease virus on the Ehrlich ascites tumor

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Newcastle disease virus (NDV) has been shown to have an inhibitory effect on the tumours. Most authors use peritumoral application of virus. The purpose of our study was to compare the effects of the ip in contrast to sc application of the virus on the ip and sc transplanted Ehrlich ascites tumor (EAT) in CBA/H mouse. We measured the length of survival, the tumor cure rates, the metastatic rate, and the frequency of ascites and sc tumors in the site of ip EAT injection.

Prolongation of survival after the therapy with NDV in ip transplanted EAT was found. The average time of survival in control group was 70.5 days, and 107 and 79.9 days with ip and sc NDV virus therapy respectively. The differences were significant only between control group and the group treated with ip application of NDV. Tumor cure rates were: ipNDV group 30%, scNDV group 20 % and control group 5 %. NDV therapy in sc transplanted EAT prolonged the time of survival; in control group it was 63.3 days, and 75.2 and 65.9 days with ip and sc NDV therapy respectively.

NDV therapy inhibited metastatic rate of ip transplanted EAT. Inhibition was more effective with ip application of NDV. Virus therapy also lowered the frequency of appearance of ascites and sc tumour in the site of ip EAT injection. In sc transplanted EAT ip application of NDV inhibited the metastatic rate while in sc applied NDV some stimulation of metastasation was found.

Ip application of NDV was found to be superior in contrast to sc application in all its therapeutic effects against EAT. Our results show that the tumor inhibition of NDV, in the system we used, has the characteristics of the biological response modifiers.

Key words: Carcinoma, Ehrlich tumor; Newcastle disease virus; survival rate; mice

Introduction

Newcastle disease virus (NDV) is a paramyxovirus pathogenic to birds and only slightly to men.[1, 2] As an inhibitor of tumor growth it has commanded continuous attention ever since the '50s until recently. The tumour inhibition was found in *in vitro* systems, on experimental animals, and in men after incidental infection,[3] or in therapeutic trial.[4, 5] Reichard et al.[6] have found the selective effect on tumor in contrast to normal cells of live NDV *in vitro*.

In *in vivo* experiments the authors use various modes of virus application but no study compares the effect of different modes. In our experiment we tried to find out if there is a difference in the effect between sc and ip virus application on sc and ip transplanted EAT of mouse. In the case that the differences in the effect are found it would to some degree explain the mechanism of NDV's tumor inhibition.

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Abbreviations: BRM-biological response modifier(s), EAT Ehrlich ascites tumor, ipEAT-intraperitonealy transplanted Ehrlich ascites tumor, scEAT-subcutaneously transplanted Ehrlich ascites tumor, NDV-Newcastle disease virus, ipNDV-intraperitonealy applied Newcastle disease virus, scNDV-subcutaneously applied Newcastle disease virus.

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Materials and methods

Experimental animals

We used 120 inbred mice, 8 to 10 weeks old, males of CBA/H strain, which were obtained from the Institute Ruđer Bošković, Zagreb. The animals were

provided pelleted Knapka food and tap water *ad libitum*. The light regime was natural.

Experimental tumor

We used EAT, composed of predominantly hyperdiploid cells, in ascitic form. EAT was transplanted ip to a donor animal 14 days before. Tumor cells were counted in a hemocytometer with Trypan blue exclusion test.

The same number of tumor cells was implanted in both experimental and control groups. For ip transplantation we used 7.9 x 10^3 cells in 0,5 ml of sterile 0.9% NaCl while for sc transplantation we used 18.9 x 10^4 cells in 0.3 ml of sterile 0.9% NaCl per animal. Tumor cells were inoculated into the right inguinal region.

Virus

Wild type NDV strain was used. Virus was obtained and titrated by the Viral laboratory, Faculty of Veterinary Medicine, Ljubljana. It was cultured in chorioallantoic fluid of 10-day old embryonated SPF chicken eggs, EID_{50} was $10^{7.5}$. It was stored at -70°C until application. Before application it was diluted in Hanks' solution in the ratio 1:15. For ip and for sc application 0.2 ml of viral solution was used. Sc application in scEAT groups of animals was in the peritumoral region.

Experimental course

We used 120 mice which were divided in 6 groups, each consisting of 20 animals. EAT was transplanted ip to group 1, 2 and 3 and sc to group 4, 5, and 6. The first and the fourth group were control, and the other 4 were experimental groups, which received NDV either ip or sc (Table 1).

The therapy of the groups with ipEAT started on the 7th day after transplantation, and of scEAT groups when the tumor reached the average diameter of 8 mm. The therapy was applied twice a week, during the total length of the experiment. Mice died spontaneously until 149th day when we finished the experiment. The animals which survived were sacrificed by the method of cervical dislocation.

Morphological Techniques

All the animals were autopsied to check the presence, site, and location of the tumor growth and presence of ascitic fluid. The organs, except the brain, were removed and fixed in 10 % buffered formalin for macroscopic evaluation of tumor growth in fixed tissues and for the histologic examination. The presence of tumor tissue was confirmed histologically in all animals in at least one specimen. In the cases where animals were sacrificed and the tumor was not found macroscopically, we examined histologically all the organs.

Statistical Methods

The result were statistically evaluated with computer statistical package SOLO (BMDP), and Log-rank test.

Results

The effect of the site of NDV application on survival

In ipEAT groups evident differences in survival of animals were found (Fig.1). In control group the average survival was 70.15 days, in ipNDV group 106,15 days and in scNDV group 79.9 days. The difference between control group and ipNDV group is statistically significant (p=0.008), but there is no such difference between control group and scNDV group (p=0.36).

The influence of application site is also reflected in the number of animals which survived the whole length of experiment (149 days) and no tumor was found at morphological analysis: there were 30% such animals in ipNDV group, 20 % in scNDV group and 5 % in the control group.

Table 1. Groups of CBA/H mice and experimental design of NDV treatment.
* 7.9 x 10^3 EAT cells for ip and 18.9 x 10^4 EAT cells for sc transplantation were used per animal. The original virus titer was EID $10^{7.5}$; 0,2 ml of NDV diluted 1:15 with Hanks' solution was applied per animal twice weekly.

Group No., (20 mice in each)	Mode of EAT transplantation	Solution applied	Mode of solution application
1 (control)	ip	0,9% NaCl	ip
2	ip	NDV	ip
3	ip	NDV	sc
4 (control)	sc	0,9% NaCl	sc
5	sc	NDV	sc
6	sc	NDV	ip

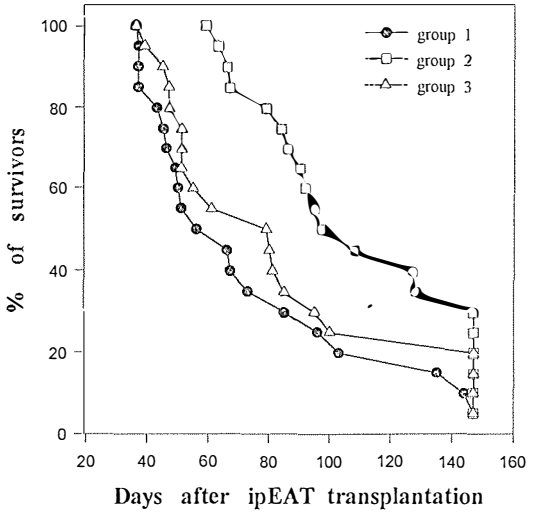

Figure 1. Survival of ipEAT groups (group 1-control, group 2-ipNDV, group 3-scNDV). The animals that survived 147th day were sacrificed and showed histologically no tumor growth. 7,9 x 10^3 EAT cells were transplanted per animal. The original virus titer was EID $10^{7.5}$; 0,2 ml of NDV diluted 1:15 with Hanks' solution was applied per animal twice weekly.

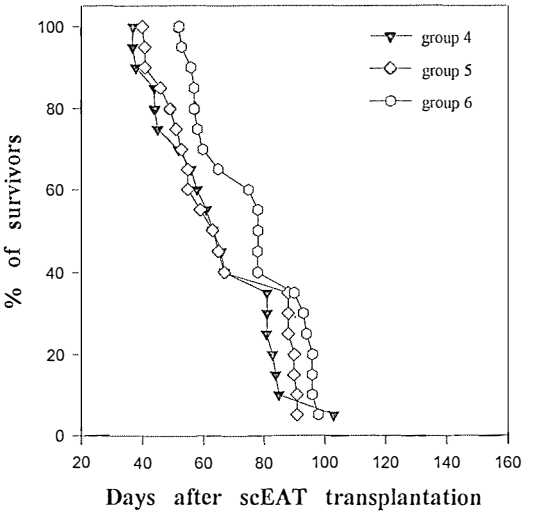

Figure 2. Survival of scEAT groups (group 4-control, group 5-ipNDV, group 6-scNDV). The animals died spontaneously. 18,9 x 10^4 EAT cells were transplanted per animal. The original virus titer was EID $10^{7.5}$; 0,2 ml of NDV diluted 1:15 with Hanks' solution was applied per animal twice weekly.

Among scEAT groups the differences in survival were smaller than in ipEAT groups (Fig. 2). The average time of survival in control group was 63.3 days, and in ipNDV group 75.2 days and in scNDV group 65.9 days. The differences are not significant. All the animals died spontaneously with tumour by 103rd day of the experiment.

The effect of the site of NDV application on the number of metastases

In ipEAT the greatest number of tumors was found in mesentery, pancreas and respiratory diaphragm, and smaller number in organs of small pelvic cavity, kidneys, suprarenal glands and liver. Metastases outside abdominal cavity were found in the lungs, and the lymph nodes (inguinal, axillary). The total number of tumors and metastases found by groups was: control 44, ipNDV 25 and scNDV 34. Ascitic fluid was found in 60 % of animals in the control group and in 25 % and 45 % of animals in ipNDV and scNDV group respectively. The sc tumor in the place of ip EAT injection was found in 70 % of animals in control group and in 45 % and 60 % of animals in ipNDV and scNDV group respectively.

In scEAT groups most of the metastases were in the abdominal organs, while in lungs and lymph nodes they were rare. The total number of metastases per groups are: control 22, ipNDV 16 and scNDV 32.

Discussion

The *in vivo* tumor therapeutic effect of live NDV has been found to depend on many factors of which the virus dose, the virus strain, the regime of application, and the tumor mass seem most known. The authors have also found that the tumor inhibitory effect was best if the NDV was injected into the tumor.[4]

The mechanism of tumor inhibition by NDV has been studied quite extensively. One of the first ideas was that virus incorporates into the membranes of tumor cells in the process of budding and in this way changes antigenicity of tumor cells.[7] On the other hand, there is no objective evidence, except in tumor-adapted NDV strain,[8] that NDV multiplies in tumor cells. The most argued findings are NDV's effects on the immune system generally through interferon induction,[9] TNF induction and the sensibilization of tumor cells to TNF.[10] Some authors have found a selective cytotoxic effect of NDV on tumor cells *in vitro*.[6]

Usually a few millions of cells are used in the experiments with ipEAT. We used only a few thousands of cells to prolong survival, to allow appearance of more metastases and to obtain a more sensi-

ble model for therapy testing. The regime of NDV application was that proposed for the biological response modifiers (BRM),[11] because viruses are also treated as BRMs.[12]

Our finding is that ip NDV application has stronger tumor inhibitory effects than sc application. Because virus is a diffusible particle, which is absorbed after application by mesothelial pores and endothelial capillary cells into blood system, and the blood supply area of peritoneum is much larger than subcutaneous area, we can expect that in ip application there is much higher concentration of virus in the blood. This is probably why the influence on the involved mechanisms of tumor inhibition is stronger.

The length of survival of experimental groups in ipEAT was longer than in scEAT. This is most probably a consequence of bigger tumor masses in the begining of the virus therapy in scEAT groups. Other authors have found the same effect of tumor mass, using NDV[4] or TNF therapy.[13] It is also the guideline for the use of BRMs that they should not be used for advanced neoplastic diseases.[14]

According to our results, NDV inhibits tumor metastatic rate which is also reflected in the reduced incidence of ascites appearance. Both could be the consequence of the reduced tumor mass on the peritoneal surfaces, as a consequence of NDV influence. It was found *in vitro* that NDV activates peritoneal macrophages which showed a strong cytostatic effect against tumor cells.[7, 15] It was also found that the reduction of ascites appearance is an effect of increased immunologic potency.[16] In the scEAT group we found increased metastatic rate after scNDV. This is probably the result of repeated peritumoral injections where we pricked the tumour cells and introduced them into the vessels.

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