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Local And Systemic Effects Of Injection Of Lytic And Non-lytic Strains Of Newcastle Disease Virus On Regression Of Cancer

تأثير الحقن الموضعي و الجهازي لعتر فايروس نيوكا سل الحاَلَّة وغير الحاَلَة في ضمور السرطان

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Abstract

In this paper report the regression effects of NDV(lytic and non-lytic strains) adiministered by either the intratumoral (I.T) route or by the systemic (intraperitoneal , I.P) route to treat murine mammary adenocarcinoma transplanted subcutaneously in mice . For (I.T)treatments,mice were randomized in to treatment groups and given a five doses I.T injection of lytic strain of NDV ($ELD_{50}10^9$) or non-lytic strain ($EID_{50}10^9$) ,vehicle (allantoic fluid) .Significant tumor size regression(70%) was seen in the group treated with lytic strain and (54%) was seen in the group treated with non-lytic strain compared with tumor size at the start of the treatment. For I.P treatments with lytic and non-lytic strains ,shown increase in the relative tumor volume but less than increased will be occur in the groups treated with allantoic fluid and group without treatment. In groups ,tumors treated I.T or I.P with allantoic fluid or negative control group displayed rapid tumor growth. These data shown that the ingection of NDV I.T is an effective more than when injection I.P,and the lytic strain causes regression of tumor size more than will causes by non-lytic strain.

الخلاصة :

تمت دراسة تأثير فايروس نيوكاسل في ضمور سرطانة الغدة اللبنية المغروس تحت الجلد في الفئران عن طريق حقنه سواء داخل الكتلة السرطانية(موضعيا) او داخل تجويف الخلب(جهازيا) .اوضح العلاج الموضعي للفايروس من ضمور حجم الورم النسبي بشكل مهم احصائي وبنسبة (70%) مقارنة مع حجم الورم عند بدء العلاج عند استخدام العترة الحالَّة وبنسبة (54%) عند استخدام العترة غير الحالَّة مقارنة مع حجم الورم عند بدء العلاج.اما العلاج الجهازي للورم فقد اوضح زيادة في حجم الورم النسبي ولكنها اقل مقا رنة مع الزيادة الحاصلة في المجموعة التي عولجت بالسائل الآلنتوي فقط والمجموعة التي لم يتم علاجها.كذلك اظهرت المجاميع التي عولجت بالسائل الآلنتوي فقط سواء عند حقنه داخل الكتلة السرطانية او داخل تجويف الخلب وكذلك المجموعة التي لم يتم علاجها زيادة في حجم الورم النسبي مقارنة مع حجم الورم عند بدء العلاج.

أظهرت الدراسة ان العلاج الموضعي سواء بالعترة الحالَّة او غير الحالَّة كان اكثر تاثيرآ من العلاج الجهازي وان العلاج بالعترة الحالَّة كان اكثر فعالية من العلاج بالعترة غير الحالَّة.

Introduction

Breast cancer is the most commonly diagnosed cancer in women (1).In the US in 2001,breast cancer accounted for 192.200 new diagnosis of cancer and nearly 42.000 death in women(2).In Iraq, the breast cancer has remained the commonest tumor , a mong females it accounts for more than 30% of the registered female cancers, with a sharp increase in the incidence of this tumor in younger age groups. Patients under 30 years age forms about 5% and 75% of the cases is between 40 and 50 years age group(3).

The avian paramyxovirus, newcastle disease virus (NDV) has steadily increased in recent years, as exemplified and reviewed by (4).The virus preferentially replicates in and kills tumor cells and appears to be safe and to varing degrees effective in phase II-clinical studies in the US and in Europe (5). The NDV treatment of cancer patients have resulted in various modes of application e.g. a-Use of oncolytic virus strains such as NDV 73T for production of oncolysates as tumor vaccines (6).b- Use of non-lytic virus strains such as NDV Ulster for production of live virus –modified tumor cell vaccine (7). c- Use of veterinary vaccine

strains such as MTH-69 as inhalation vaccine for systemic treatment and non – specific immune stimulation(8).

Material And Methods

Newcastle Disease Virus(NDV).

The lytic strain of NDV (Iraqi virulent strain) was obtained from department of Pathology and Poultry Disease , College of Vet. Med. Baghdad University .A stock of infectious virus was propagated in emberyonated chicken eggs (9-11days) harvested from the allantoic fluid,purified from debris by cold centrifugation (3000 rpm , 30 min , $4c^{o}$) and the non-lytic strain of NDV (attenuated LaSota virus) was obtained from Kendy Company for veterinary vaccines production .Amplification of the original stock was done by inoculation through (10day) old chick emberyos . Four days after inoculation ,virus was harvested from the allantoic fluid by centrifugation (30 min , 3000 rmp, $4c^{o}$)(9). NDV was quantified by the hemagglutination and hemagglutination inhibition test (10).The measurement of Emberyonated Lethal Dose 50% (ELD_{50}) of lytic strain and Emberyonated Infected Dose (EID_{50}) of non-lytic virus was conducted according to the Karber method(11).

Tumor cells and transplantation.

The tumor cells were obtained from live mice housed in Iraqi center of cancer and medical cytogenetics . It was transplanted previously the S/C with murine mammary adenocarcinoma (fig.1) .after sterilization of the outside of tumor nodule , the tumor cells were aspirated by needle gage (18mm) , suspended in phosphate buffer saline (PBS),centrifugation (1000rpm,10min.,$20c^{o}$), the supernatant was left and the sediment was resuspended in PBS and transplanted of tumor cells were done S/C in mice (12).

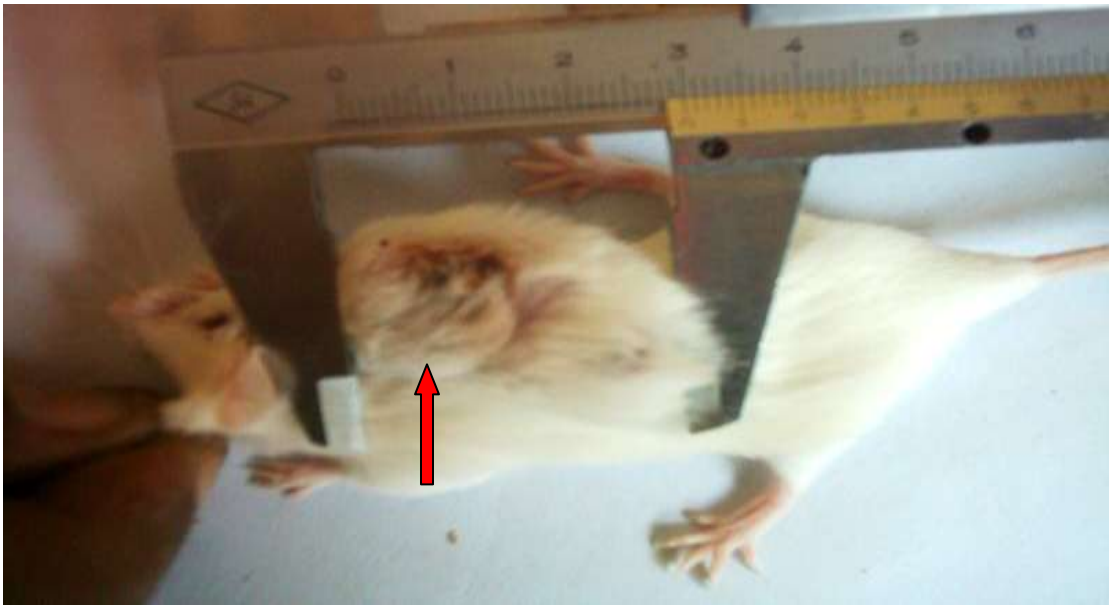

Fig.1:Tumor nodule transplanted subcutaneously in mice.

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Determination of tumor volume(T.V).

The tumor volume of treated mice and tumor bearing mice was estimated according to Grote ,*eta*l (13).Tumor volume=$A.B^{2}$ \2= mm^{3}

A=Length B=Wide

Determination of Relative Tumor Volume (R.T.V)

The determination of R.T.V. was followed according to Phuangsab ,*etal*(14).

$$R.T.V.\% = \frac{T.V(\text{day X})}{T.V(\text{day 0})} \times 100$$

Laboratory animals.

Female balb \C mice (24gm) (10 weeks) were obtained from Iraqi Center of Cancer and Medical Cytogenetics .They were housed in boxes in a control ed environment (temp.25C^{o}) with standard laboratory diet and water . The animals were classified in to seven groups each of them contain six mice. All of them were injected subcutaneously by (0.25)ml suspension of tumor cells. When the tumor nodule growth S\C reached about (9-12 mm) ,all animals were subjected to different treatments as follows .

Group I : The mice were treated with injection of the lytic strain of NDV ($ELD_{50}10^{9}$) directly in the tumor (Intratumoral) (I.T) five doses, three days intervals between doses.

Group II : **:** The mice were treated with injection of the non- lytic strain of NDV ($EID_{50}10^{9}$) directly in the tumor (Intratumoral) (I.T) five doses, three days intervals between doses.

Group III: Similarly treated as in the group I, but injection of the lytic strain of NDV ($ELD_{50}10^{9}$)was done Intraperitonealy(I.P) . .

GroupIV: Similarly treated as in the group II , but injection of the non- lytic strain of NDV ($EID_{50}10^{9}$)was done Intraperitonealy .

Group V : The mice were treated with injection of (0.1 ml) allantoic fluid (virus-free) directly in the tumor(I.T).five doses, three days intervals between doses. This group is considered a positive control group for group I and group II.(C+VE).

Group VI : Similarly treated as in the group IV but injection of allantoic fluid was done Intraperitonealy .Positive control group for group III and group V . **Group VII :** The mice were injected with tumor cells only without treatment. A negative control group to all groups(C-VE).

Statistical analysis:

Programe of SAS(2001) to analysis the result of study by Least Significant Differences(LDS) was used in this study (15).

Results:

Intratumoral(locally)treatment with NDV.

In the first group, six mice with subcutaneous murine mammary adenocarcinoma are treated with lytic strain of NDV($ELD_{50}10^{9}$), shows regression in the tumor size after three days from the start of the treatment and the percentage of relative tumor volume at the end of experiment was (30%) which was statistically significant($P<0.0001$)compared with the tumor size at the start of the treatment,that means (70%) of tumor size was regressed(fig.2).

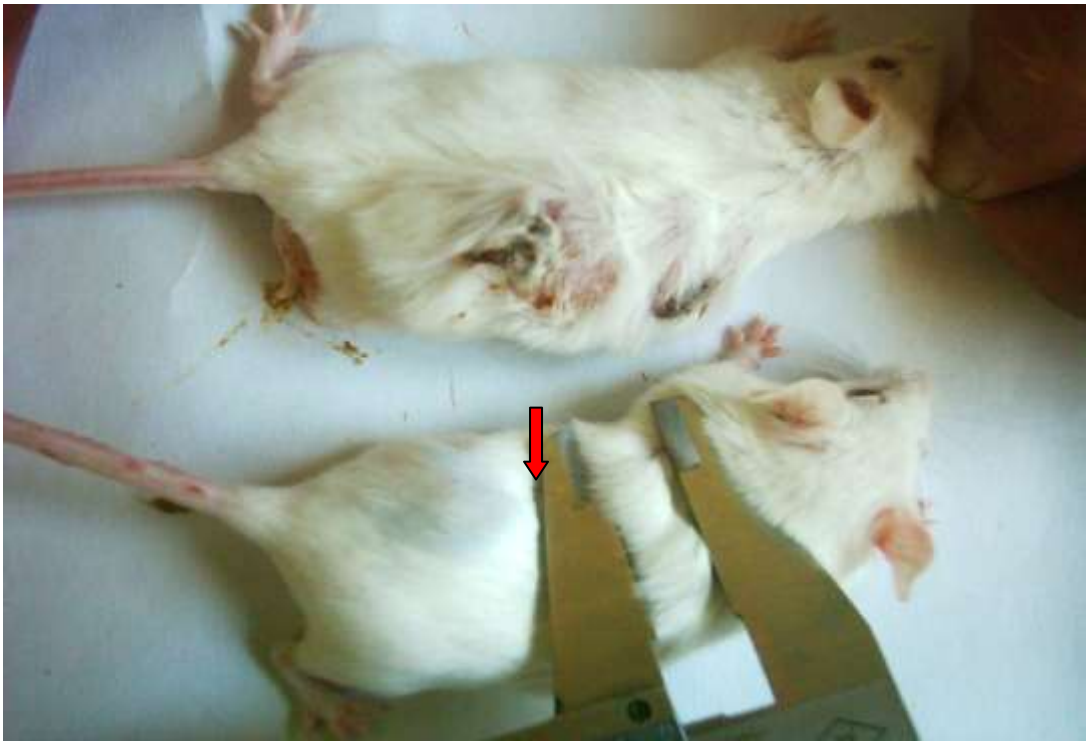

Fig.2: Regression of tumor size after treatment with lytic strain of NDV (I.T) compared with untreated mice (negative control).

The second group, which was treated with non-lytic strain of NDV (I.T) ($EID_{50}10^9$) Shows a regression in the tumor size and the percentage of relative tumor volume at the end of experiment (15 days post treatment) (46%) statistically significant ($P<0.001$) compared with the tumor size at the start of the treatment, that means (54%) 0f tumor size was regressed. While the positive control group for group I and II, which was treated with allantoic fluid only (I.T) and negative control group (without treatment) shows rapid growth in the tumor size such that the relative tumor volume increased 2-fold and 2.7 –fold respectively by 15 days post treatment fig.3).

The regression of tumor size in the group treated with lytic strain of NDV was more than($P<0.1$)regression will occured in the group treated with non-lytic strain of NDV.

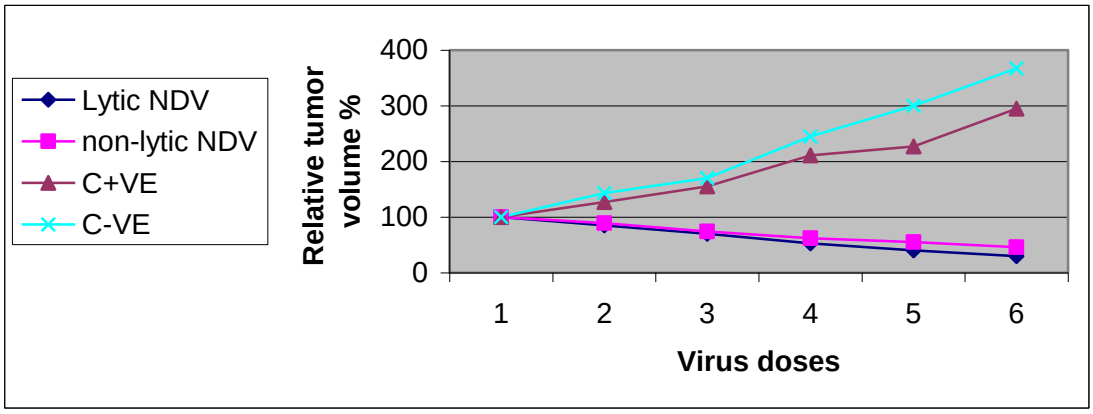

Fig.3. The effects of treatment with lytic and non-lytic strains of NDV(I.T).

Systemic (I.P)treatment with NDV.

To evaluate the efficacy of systemic treatment, NDV was injected intraperitonealy in to mice carrying established subcutaneous murine mammary adenocarcinoma. Tumor from mice treated with allantoic fluid only (I.P) ,positive control group for group (III and IV) and negative control group(without treatment) grew rapidly such that the relative tumor volume increased 2.5 -2.7 fold respectively ,by 15 days post treatment compared with tumor size at the start of the treatment. While ,mice given five doses($ELD_{50}10^9$ – $EID_{50}10^9$) of lytic and non-lytic of NDV in the groups (III and V) respectively ,shown increase in the relative tumor volume (0.8 -0.9) fold respectively, by 15 days post treatment compared with tumor size at the start of the treatment (fig.4)

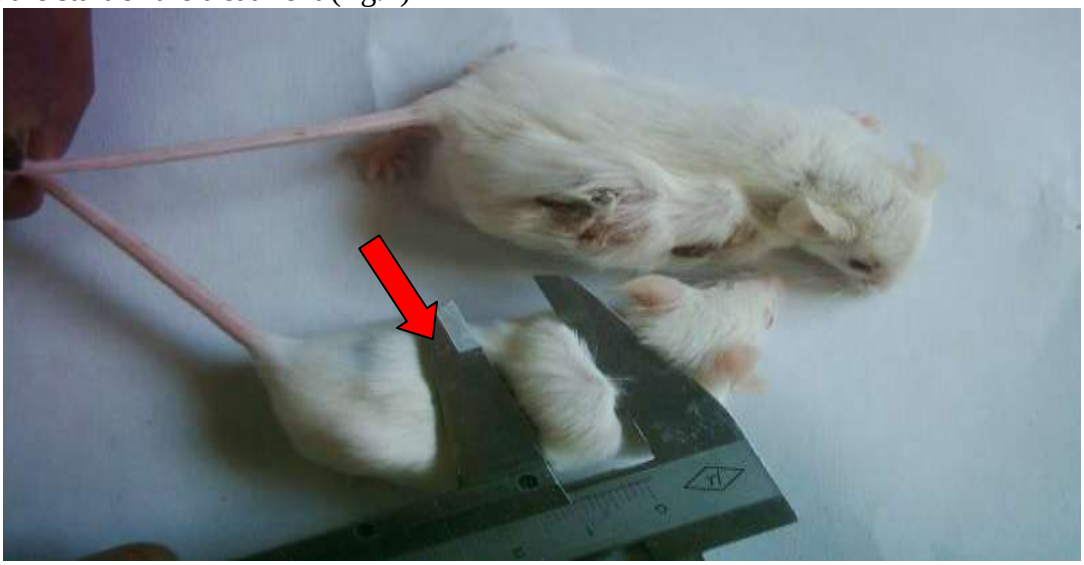

Fig.4: The effect of treatment (I.P) with non lytic strain of NDV.

The differences in the increase of relative tumor volume between groups treated with NDV and positive and negative control groups, that means the virus effective in the regression of tumor growth .

The regression of tumor size in the groups treated with NDV (I.T) is more than statistically significant ($P<0.001$) compared with regression will occur in the groups treated with NDV(I.P).(fig.5).

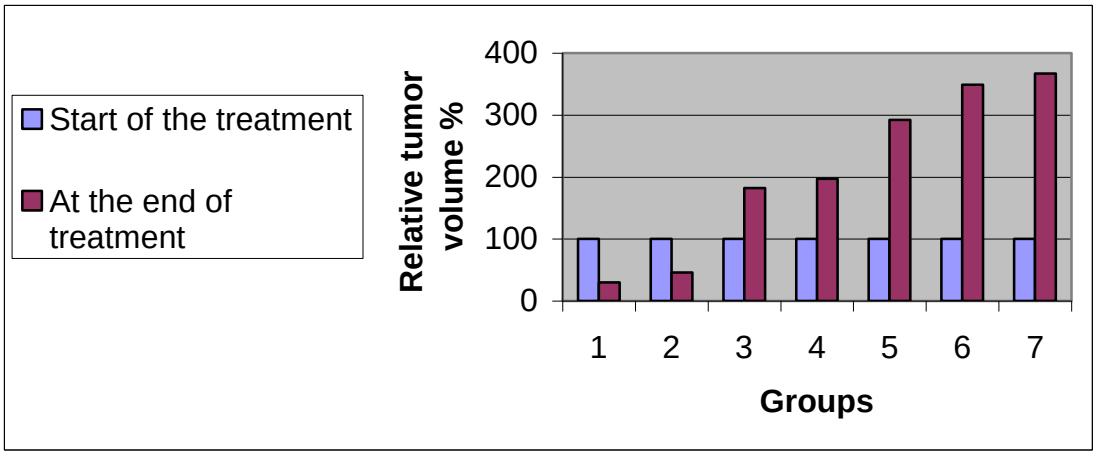

Fig.5: The effect of treatment with NDV locally(I.T) and systemically(I.P).

Discussion

According to my results , the lytic strain of NDV (Iraqi strain) and non-lytic strain (LaSota attenuated strain) had a good anti-neoplastic effects through the reduction of relative tumor volume when given by both local (I.T) and systemic (I.P) routes. Lytic strain was selected for study since it has been previously given to humans by a variety
of routes and had demonstrated a good safety profile at the doses used (16). The ability of NDV to replicate efficiently in human cancer cells has been demonstrated in both laboratory and animal studies (17). Several of these studies have provided much of the evidence that lytic strains of NDV are also oncolytic (18). Strain 73-T,
which is lytic ,has been shown to kill the following types of human cancer cells :
Fibrosarcoma , osteosarcoma, neuroblastoma, and bladder carcinoma (19) . Lytic strain Italien has been shown to kill human squamous cell lung carcinoma ,melanoma, breast carcinoma and larynx carcinoma (20). NDV strain Ulster, which is non-lytic ,has also been shown to replicate efficiently in human cancer cells, including cells of the following types of human tumors : colorectal carcinoma ,gastric carcinoma, and pancreatic carcinoma(8).

The regression of tumor size in the mice treated with lytic strain of NDV more than regression which occured in the mice treated with non-lytic strain . There are many different strains of NDV ,and they have been classified as either lytic or non-lytic for human cells Lytic and non-lytic strains both appear to replicate much more efficiently in human cancer cells than they do in most normal human cells (21) and viruses of both strain types have been investigated as potential anti-cancer agents. One major difference between lytic strain and non-lytic strain is that lytic strain are able to make infectious progeny virus particles in human cells,while non-lytic strain are not(22).
This difference is due to the ability of lytic strain to produce activated hemagglutinin-neuraminidase and fusion protein molecules in the outer coat of progeny viruses in human cells(23).The progeny virus particles made by non-lytic strain contain inactive versions of these molecules(24).Another major difference between lytic and non-lytic strains is that, although they both have the potential to kill infected cells , the mechanisms by which they accomplish this result are different. The production of infectious progeny virus particles by lytic strain gives them the ability to kill host cells fairly quickly (25). The budding of progeny viruses that contain activated hemagglutinin-neuraminidase and fusion protein molecules in their outer coats causes the plasma membrane of NDV-infected cells to fuse with the plasma membrane of adjacent cells, leading to the production of large, inviable fused cells known as syncytia (26).The more efficiently a lytic strain can replicate inside a host cells, the more quickly it can kill that cells .In contrast, non-lytic strain of NDV kill infected cells more slowly, with death apparently the result of viral disruption of normal host cell metabolism(27).

Anti-tumor effects of NDV were much stronger when applied locally
(I.T) than systemically (I.P). The regression of tumor size was occured in the group treated with virus (I.T) through reduction of R.T.V and in the group treated with virus(I.P) through the increase of R.T.V but less than increased which occured in the positive and negative control groups. A major concern about the effectiveness of treating cancer patients by repeated administration of NDV is the possibility that the immune system will produce virus- neutralizing antibodies . These antibodies would prevent NDV from reaching and infecting malignant cells,thereby blocking oncolysis (28).Impairment of NDV infection would also limit the ability of cytotoxic T cells that target virus antigens to kill virus-infected cancer cells (29). The Hungarian investigators have shown that anti- NDV antibodies are produced in MTH-68 strain treated patients(8).However, the recent observation that immune system tolerance to viruses can be induced by repeated oral administration of virus proteins (7).

In conclusion, this study has shown that the lytic and non-lytic trains of NDV is preferentially killing tumor cells and the local route is more efficient than systemic route . NDV may therefore be recommended for anti-tumor activity.

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