The Relative Tumor Volume of Murine Mammary Adenocarcinoma Treated With Levamisole and Virulance Newcastle Disease Virus.

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Abstract

The Relative tumor volume (R.T.V) of mice treated orally, with levamisole in addition to a multiple injection of virulence Newcastle disease virus intratumoral (I.T), was shown a significant reduction ($p<0.05$) at the compared wih the tumor size pretreatment treatment, that means (95%)of the tumor size was regressed . Whereas in the group of mice treated with levamisole and injected with Newcastle disease virus intraperitonealy (I.P) ,the R.T.V was increased significant but a less than those in the negative and positive control groups.

Key words: levamisole , Adenocarcinoma , Virulance Newcastle Disease Virus

Introduction

Levamisole is a white to almost white crystalline powder which is almost odorless and is freely soluble in water [1]. Levamisole, a well known antihelminthic agent with immune stimulating activity to conventional antibiotic therapy , would improve the energy against Brucella[2].Levamisole is a nonspecific immunomodulator that is used in the treatment of colon cancer in combination with fluorouracil (5-Fu) after surgery[3]. Newcastel disease virus(NDV)was first described in the early 1900s as the contagious agent of the fatal avian disease known as chicken pest [4].NDV has a strong cytotoxic potential against different tumor cells [5]. NDV is one of the few oncolytic viruses that do not naturally infect hums [6]. According to the previous studies [3,4 and 11]the levamisole and NDV, has been used in the treatment of mammary adenocarcinoma.

Material and methods

Levamisole.

Levamisole HCL,Bp98,Working stander,Assay-99.74%, Reference stander , Germany Dr.Ehrenstarfer. Levamisole powder soluble in water and treated the mice orally with dose (10mg/kg,B.W) through out the experiment[7].

Newcastle disease virus(NDV).

The velogenic strain(Iraqi strain) of NDV was obtained from pathology and poultry disease department,College of Vet.Med.Baghdad.University. Amplification of the original stock was done by passage through (9 days) old chick emberyos.Two days after inoculation,virus was removed from the allantoic fluid by centrifugation for (30min.4000rpm.4c^{o})(8).The measurement of Emberyonated Leathal Dose (ELD_{50}) and Hemagglutination Unit(HU) according to Karber method[9].

Cell line and transplantation.

The tumor cells were obtained from the Iraqi center of cancer and medical cytogenetic and transplanted the mice subcutaneously according to[10].

Relative tumor volume(R.T.V).

Determination of R.T.V. according to [11].

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

Laboratory animals.

Thirty six female balb/c mice(22gm,10wks) were obtained from the Iraqi Center of cancer and medical cytogenetic .They were housed in a control environment and classified into six groups each of them contain six mice.All of them were injected with S/C by(0.25ml) suspended of tumor cells. After 10 days from injection when the tumor nodules growth S/C was (9-12mm) [11].The animals were subjected to different treatments as follows.

Group I: The mice were treated with levamisole orally through out the experiment with dose (10mg/kg.B.W)and injection of(0.1ml) from virulance NDV(128HU,$ELD_{50}10^{10}$)intratumoral(I.T),six doses, three days intervals between doses.

Group II: Similar treatment to that of group I but the injection of virus was intraperitoneal (I.P).

Group III: The mice were treated with levamisole orally through out the experiment (10mg/kg.B.W).

Group IV: The mice were treated with levamisole orally and injection (0.1ml) with allantoic fluid (fluid without viruses) (I.T). six doses, three days intervals between doses. Positive control group(C+VE) for group I.

Group V: Similar treatment to that of group IV but was injected with allantoic fluid (I.P).Positive control group for group II.

Group VI: The mice were injected with tumor cells only without treatment. Negative control group(C-VE) for all groups.

Histopathological examination.

At the end of the experiment, all animals were killed and the liver,lung and spleen were removal for histopathological examination.The tissues were Fixed in buffer formaline(10%),sections were routinely prepared and stained with eosin and hematoxylin(H&E)for microscopic examination[12].

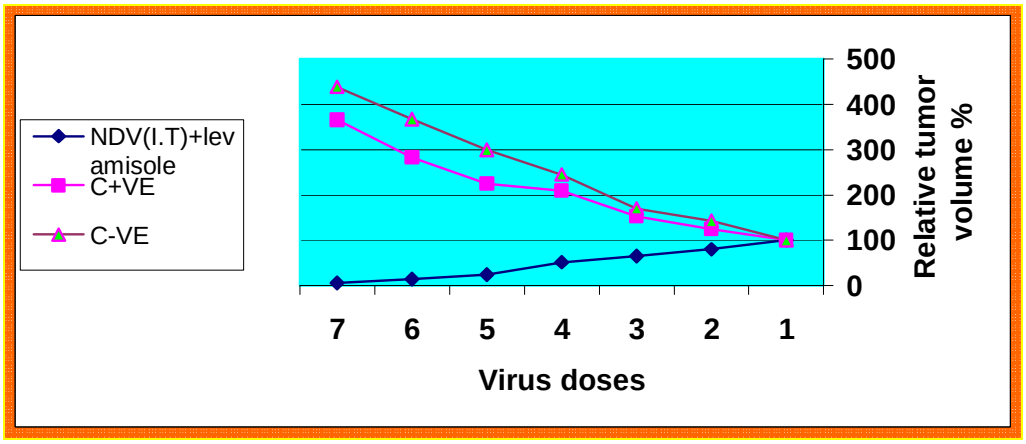
Statistical analysis.

Analysis of the differencesis shown in the results by the Least Significant Differences(LSD)method.

Results

Relative Tumor Volume(R.T.V).

The first group,which was treated with levamisole orally and multiple injection of NDV(I.T) shows a reduction of R.T.V after three days of the first dose of virus injection.These reduction was continuous to the end of the experiment(18 days after started the treatment).The percentage of R.T.V. at the end of the treatment was (5%)statistically significant($P<0.0001$)compared with the tumor size before the start of treatment, that means (95%) of tumor size was in regression.(Fig.1).



Fig(1).The effect of treatment by NDV(I.T)& levamisole on R.T.V. through out the experiment.

The second group , which was treated with levamisole orally and multiple injection of NDV(I.P)and the third group,which was treated with levamisole only,show an increase in the R.T.V. compared with tumor size before the start of the treatment ,but this increase was less than those occurred in the negative and positive control groups.(Table 1).

Table(1).The effect of treatment on R.T.V.in all groups through out the experiment.

Groups	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	significance
I	100%	80	65	51	23	13	5	P<0.0001
II	100%	102	119	133	147	179	203	P<0.0001
III	100%	134	159	211	251	305	371	P<0.0001
IV	100%	124	153	209	225	283	366	P<0.0001
V	100%	140	168	238	264	337	412	P<0.0001
VI	100%	143	170	245	300	367	439	P<0.0001

Histopathological examination.

Lung: Shows, emphysematous lesions in all groups and thickening of interalveolar septa due to infiltration of inflammatory cells such as lymphocytes and macrophages and congestion of blood vessels(fig.2).

Liver: Shows a congestion of central veins and the sinusoid were narrow and infiltrated by lymphocytes(fig.3).

Spleen: Shows, an increased in the number of lymphocytes in the white and red pulp(fig.4).

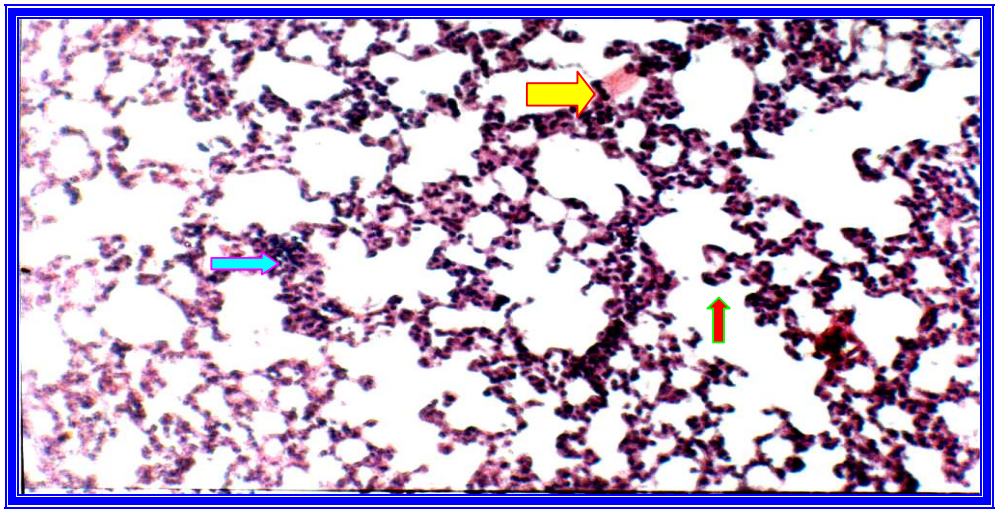


Fig.2.Lung ,shows ↑ emphysema and → thickening of interalveolar septa and ↘ inflammatory cells. Group III.(H&E,100 X).

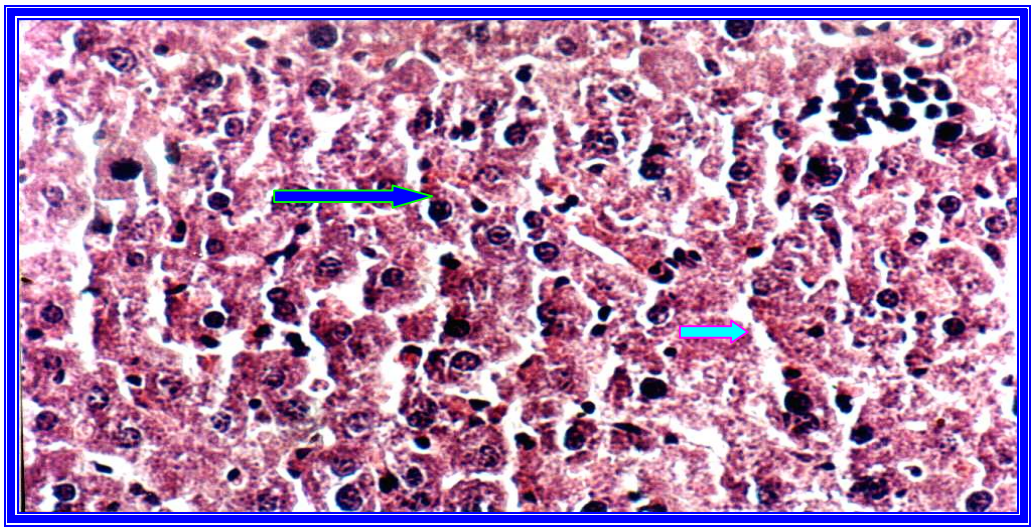


Fig.3.Liver,shows a congestion of capillaries of sinusoid and infiltration of Lymphocytes.Group II.(H&E,200X).

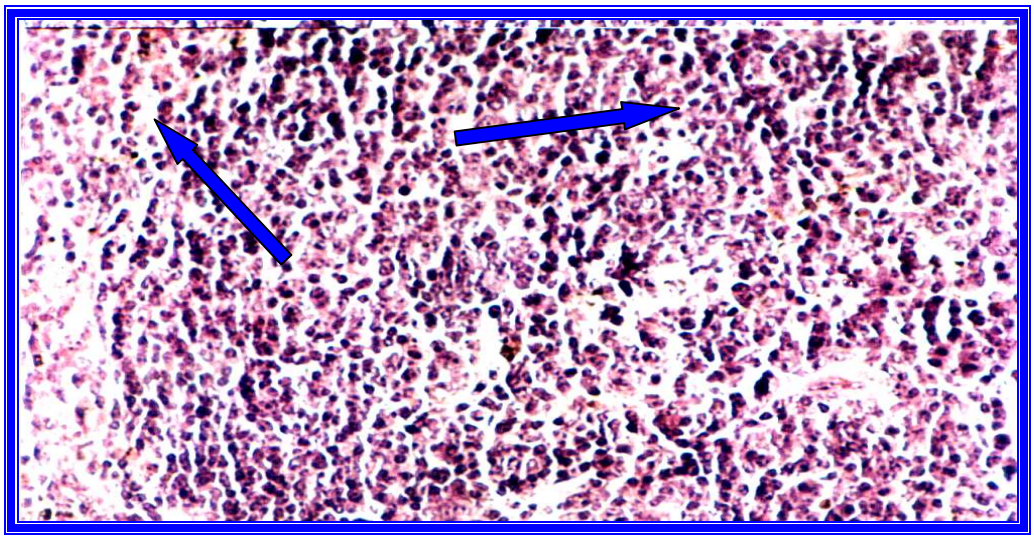


Fig.4.Spleen, shows an increased of lymphocytes in white and red pulp. Group I.(H&E,200X)

Discussion

According to present study, the R.T.V. was reduced and there was increase in the number of lymphocytes in the spleen of the first group, which was treated with levamisole orally and multiple injection of virulence NDV intratumoral .Levamisole can increase delayed hypersensitivity and / or T-cell mediated immunity and augment macrophage chemotaxis and phagocytosis[2].Its mechanism of action is unknown,but it seems to help in maintaining microtubule integrity which is essential for macrophage and lymphocyte adequate functioning[13].

The activated phagocytic index was significantly increased after levamisole treatments in asthmatic patients with phagocytic deficiency [14].Levamisole can stimulate formation of antibodies to various antigens [15], enhance T-cell responses by stimulating T-cell activation and proliferation ,potentiate monocyte and macrophage functions including phagocytosis and chemotaxis , and increase neutrophile mobility, adherence and chemotaxis[16].Besides its immunomodulatory function, levamisole has other mammalian pharmacologic activities, including inhibition of alkaline phosphatase and cholinergic activity[1].

NDV is an oncolytic virusesthat are replicating microorganisms that have selected or engineered to grow inside tumor cells and kill them. NDV specifically target cancer cells because they are able to exploit the very same cellular defects that promote tumor growth[17]. NDV can also play a role in the induction of interferons.Interferon induction was one possible mechanism of enhancement of tumor-specific host immune responses[18].The interferon-gamma was the most effective at suppressing the growth of murine mammary adenocarcinoma previously implanted intra dermally [19].

The interferons are incompatible with efficient tumor evolution [20], this probably because interferons have a crucial role in cancer immunosurveillance-aprocess where by the adaptive immune system recognizes tumors as foreign entities and eliminates them[21].

Anti-tumor effects of NDV were much stronger when applied locally (I.T)than systemically(I.P).The inhibition of tumor growth occurred 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 a less than increased will occur in the positive and negative control groups. The differences between local effectivity and systemic ineffectiveness of NDV in this study are most likely due to tumor targeting of NDV upon systemic application.The virus doses were close to maximal tolerated doses and caused already detrimental effects on overall survival[22].

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حجم الورم النسبي لسرطانة الغدة اللبنية الفآري المعالج بالليفاميزول وفايروس نيوكاسل الضاري

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الخلاصة

انخفض حجم الورم النسبي في المجموعة المعالجة بالليفاميزول عن طرق الفم والحقن المتعدد لفايروس نيوكاسل الضاري داخل كتلة الورم عند نهاية التجربة الى (5%) و بمستوى احصائي مقارنة مع حجم الورم قبل بدء العلاج وهذا يدل على ان(95%) من حجم الورم قد تقهقر بعد العلاج.اما المجموعة المعالجة بالليفاميزول عن طريق الفم والحقن المتعدد لفايروس نيوكاسل الضاري داخل تجويف الخلب فقد اظهرت زيادة في حجم الورم النسبي ولكن هذه الزيادة آقل من الزيادة الحاصلة في مجموعتي السيطرة السالبة والموجبة.