

قدرت نابودکنندگی سلولهای LAK/NK

م. منصوری*

ا. م. ای نوری و ال

تی. دی الیور

چکیده:

استفاده از داروهای ضدسرطان در دوزهای مختلف، تأثیر هر یک بر رده‌های مختلف سلول سرطانی و چگونگی تحریک سلولهای تک هسته‌ای خون (M.N.C) فعال شده با اینترلوکین ۲ (IL-2) انگیزه این پژوهش بوده است. اساس آزمایش به استفاده از رده‌های سلول سرطانی (سلول هدف) سلولهای مونوکلئار فعال شده با IL-2 (سلول مؤثر)، داروهای اختصاصی ضدسرطان نظیر *Tamoxifen, cyclohexamide, Pentoxifylline, cisplatin* و روش رنگ‌سنجی *NNT* انجام پذیرفته است.

از نتایج مقدماتی چنین برمی‌آید که داروها در دوز مصرفی یکسان دارای اثرات متفاوت تقویت‌کننده قدرت نابودکنندگی LAK/NK یا بازدارندگی آن خواهد بود. شایان توجه است که در شرایط تضعیف یا بازدارندگی قدرت نابودکنندگی LAK/NK امکان متاستاز و پیشرفت سرطان دور از انتظار نخواهد بود.

ویژگی و اهمیت موضوع، موجبات مطالعات بیشتری را فراهم نموده که برای بررسی نتایج، فرصت بیشتری مورد نیاز می‌باشد.

کلید واژه‌ها: ۱- تومور

۲- سلولهای NK/LAK

۳- تاموکسی فن

۴- پنتوکسی فیلین

۵- سیکلوهگزیمید

* بخش آنکولوژی بیمارستان رویال لندن - انگلستان

The EFFECT OF DIFFERENT PHARMACOLOGICAL AGENTS ON NK/LAK KILLING & TUMOUR CELL LINES PROLIFERATION

M.Mansouri*
M.E.Nouri
L.T.D.Oliver

ABSTRACT

The base of this study has been demonstrated the use of different drugs and dosages on different tumour cell lines and finally the effect of these drugs on natural killer/limphokine activated killer (NK/LAK) Killing. Tumour cells as target , mononeuclear cell activated with inter leukin-2(LAK) as effector, anti cancer drugs like tamoxifen , pentoxifylline , cycloheximide and cisplatin as stimulator or inhibitor of NK/LAK killing and colourimetric MTT₅ assay as evaluation agent. The different drugs with the similar dosages show stimulation or ingibition effect on NK/LAK killing. It is to be taken into concideration the possibility of metastasis of cancer is not unexpected. As the matter is important and needs more profound consideration , more studies and researches are currently being performed .

Key Words: 1) Tumour cell line 2) NK/LAK 3) Tamoxifen
4) Pentoxifylline 5) Cycloheximide

INTRODUCTION

The immune system is the ideal weapon against infectious disease. It eliminates viruses and bacteria that invade the body and kills infected cells, yet it leaves healthy tissue intact. The system is so precise because it responds only to specific targets called antigens, molecules or fragments of molecules that belong to the foreign invaders.

In general, antibody molecules inactivate pathogens and toxins that circulate in body fluids, whereas white blood cells called cytotoxic T lymphocytes destroy (lyse) cells that have been penetrated by viruses⁽¹⁰⁾.

NK activity was first described by Kiessling et al (1975)⁽⁶⁾. Because some tumour cells, such as Daudi were consistently resistant to NK cytotoxicity, it was the discovery that

* *Department of Medical Oncology, The Royal London Hospital.*

IL-2 activated lymphocytes were cytotoxic for Daudi that led to the definition of LAK cells⁽³⁾. It is now thought that LAK represents an activated form of NK cytotoxicity and are involved in protection against experimental animal tumours⁽⁸⁾ and in leukaemia in man⁽²⁾. Critical to the hypothesis of primacy of NK/LAK immunity in resistance to the tumour cells is occurrence of specific drugs and dosage restricted anti-tumour cytolytic T lymphocytes and NK/LAK killing. Most adult tumour patients only show the lymphokine activated killer and natural killer cytotoxicity^(5,9) possibly a reflection of their degree of aberrant drugs and dosage dependency.

Cisplatin is a pharmacological agent which have more studied in the Royal London Hospital.

MATERIALS & METHODS

M.N.C, IL-2 , tumoure cell lines, drugs etc.

The MNCs from normal individuals were separated using density gradient technique (lymphoprep, Nycomed , pharma) ,as described previously⁽⁹⁾. The interface cells were aspirated , washed and stimulated with IL-2 100 u/ml , Biogen) for 72-96h at 37°C. These activated cells, which care known to have both LAK and NK activities, were washed and resuspended at the required density to be used as effector cells (E). Tumour infiltrating lymphocytes (TILS) were

isolated from tumour biopsies as described previously⁽¹⁾. Briefly , suspension of single cells prepared from tumours were prepared immediately after operation and after washing the cells were activated with IL-2(100u/ml) and then cultured . The TIL from successful cases were fed every 2 to 3 days by adjusting the cell number to 0.5×10/ml in RPMI plus 10% foetal calf serum aweum (FCS, Gibco)and IL-2 100u/ml (T).

The target plus drug and ratio of E/T plus drug in 96 wells of microtitre plate and MTT assay as described bellow is the base of this study.

Cytotoxicity using MTT assay

The use of MTT assay for assessment of cytotoxicity has previously been reported⁽⁴⁾. This was carried out using the modified MTT (3- [4,5-dimethyl - tetrazol 2yl]-2,5 diphenyl tetrazolium bromide) assay described by Mosmann(1983)⁽⁷⁾. Exponentially growing cells were treated with trypsin(0.05%)+ EDTA(0.02%) for 5 min., washed resuspended in RPMI containing 10% FCS and plated at 10×10/well (Nunc). Effector cells i.e IL-2 actived M.N.C were added to give efector/target(E/T) ratios of 5/1 , 10/1, 20/1 and were incubated for 4h. at 37. Each 3 replicates of microtitre plate with T,E/T ratio will be with or without specific drugs respectively. After incubation ,plates were washed with fresh medium plus 2% FCS and remaining cells were washed

with fresh medium plus 2% FCS and remaining cells were loaded with 10ul/well of 5mg/ml MTT plus 100ul/well of medium and incubated for 3h. at 37, after the incubation medium was removed and 100ul of acidified (0.04 M HCL) isopropanol was added and the cells were incubated for 30 min.at room temperature, followed by the

reading of the plate by an ELISA reader with 570 nm. filter.

RESULTS

LAK/NK killing against tumour targets in response of different specific pharmacological agents have been investigated , sample of which has shown in table 1.

Table 1. Effect of different pharmacological agents on tumour cell proliferation and their effect on LAK/NK Killing

<i>Pentoxifylline</i>	<i>durg alone</i>	<i>drug + effector</i>	
<i>0.01 ug/ml</i>	2	6	<i>s</i>
<i>0.1</i>	7	0	<i>i</i>
<i>1.0</i>	-2	0	<i>s</i>
<i>10.</i>	1	-8	<i>i</i>
<i>Cycloheximide</i>			
<i>0.01</i>	16	12	<i>i</i>
<i>0.1</i>	43	44	<i>NE</i>
<i>1.0</i>	71	68	<i>NE</i>
<i>Caffeine</i>			
<i>0.01</i>	13	9	<i>i</i>
<i>0.1</i>	7	18	<i>s</i>
<i>1.0</i>	7	6	<i>NE</i>
<i>10.</i>	2	-5	<i>i</i>
<i>Tamoxifen</i>			
<i>0.01</i>	6	12	<i>s</i>
<i>0.1</i>	9	18	<i>s</i>
<i>1.0</i>	8	6	<i>i</i>
<i>10</i>	11	12	<i>NE</i>
<i>Colchicin</i>			
<i>0.01</i>	62	71	<i>s</i>
<i>0.1</i>	69	65	<i>i</i>
<i>1.0</i>	72	74	<i>NE</i>
<i>Cisplatin</i>			
<i>0.01</i>	37	24	<i>i</i>
<i>0.05</i>	42	18	<i>i</i>
<i>1.0</i>	38	50	<i>s</i>
<i>50.</i>	58	68	<i>s</i>

Result are expressed in percent killing, s, i and (NE) represent stimulate (s), inhibit(i) and little or no effect (NE)

Investigation of correlation between the level of specific pharmacological agents and susceptibility of LAK/NK killing

In order to establish whether the intensity of pharmacological agents on tumour targets affects their susceptibility to LAK/NK killing, parallel killing experiments were carried out on several tumour cell lines.

As can be seen from table 1, there was a

varying degree of LAK/NK killing in response of tumour targets plus drugs, there are significant correlation between activity of LAK/NK and target to target dependency to varying dosages of drugs. As can be seen from cisplatin study, testis tumour cell lines have shown more susceptibility than bladder tumour cell lines in response to cisplatin (Figure 1).

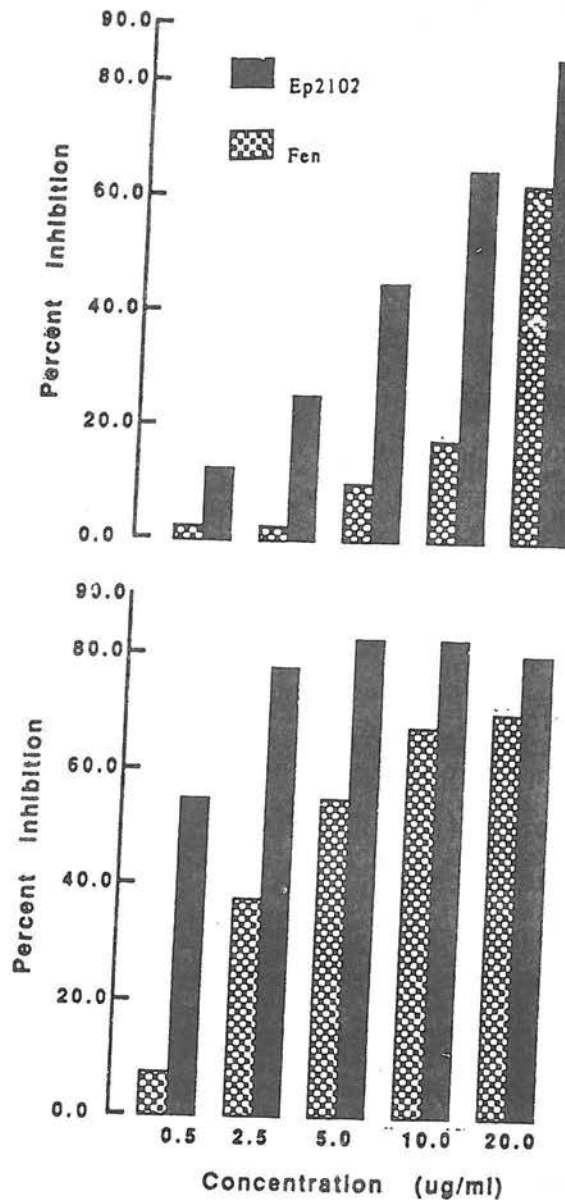


Figure 1. Inhibitory activity of Cisplatin on two tumour cell lines after different times of exposure

Table 2. Effects of Cisplatin on metabolic activities
of human cell lines derived from bladder and testis tumours

ug/ml	Tera I	Tera II	Ep2102	Mean±SD	Wil	5367	T24	Mean±SD
20.0	74.1	86.4	84.0	81.5±1.0	83.0	50.1	77.0	70.0±17.5
2.0	60.4	63.4	79.1	67.6±10.0	25.0	41.1	66.0	44.0±20.6
0.1	8.8	6.3	5.7	6.9±1.6	14.1	1.0	13.0	9.3±7.2

Results are expressed as percent inhibition. The values were calculated using optical densities of cells (100/000 cells / well, three replicates/treatment) of treated with drug over the values of the untreated cells and cultured for 48 hrs. Tera I,II and Ep2102 are testis and the remaining lines are bladder lines.

DISCUSSION

The result of this investigation has demonstrated, there was a large variation in the LAK/NK killing of different individuals in response to drugs treatment of varying tumour cell lines.

Cisplatin is a specific anti-tumour drug which has been more studied in the department of medical oncology of The Royal London Hospital. DNA chains cross link is the mechanism of tumour activity

inhibition. As can be seen the result of testis and bladder tumour cell lines , table 2, there are significant variation between LAK/NK killing and drug efficiency in tumour treatment.

To better clarify our uncertainty about the role of LAK/NK in vivo, more work including specific blocking agents and augmentation experiments in vitro and in vivo are needs to identify the target recognition molecules.

REFERENCES

1) A.M.E, Nouri, , . Hussein, R.F. Dossantose, A.V.L. Mansouri, M. & Oliver, R.T.D. (1993). Intensity of class I antigen expression on human tumour cell

lines and its relevance to the efficiency of non-MHC-restricted killing. Br.J.cancer. 67,1223-1228.

2) Archimbaund , E, Bailly,.M & Dore, JF. (1991). Inducibility of lymphokine

activated killer (LK/NK) cells in patient with acute myelogenous leukemia in complete remission and its clinical relevance. *br. J. Haemat.*, 77, 328-334.

3) Grimm, E.A., Masumder, H.Z & Rosenberg, S.A. (1982). The lymphokine activated killer cell phenomenon: lysis of NK resistant fresh solid tumour cells by IL-2 activated autologous human peripheral blood lymphocytes. *J. Exp. Med.* 155, 1823-1841.

4) Hussein, R.F., Nouri, A.M.E. & Oliver L.T.D. (1992). a new approach for measurement of cytotoxicity using colorimetric assay. *J. Immunol. Methods.* 160, 89-96.

5) Itoh, K., Platsoucas, C.D. & Balch, C.M. (1988). Autologous tumour specific cytotoxic T lymphocytes in the infiltrate of human metastatic melanomas: Activation by interleukin-2 and autologous tumour cells and involvement of the T cell receptor. *J. Exp. Med.*, 168, 1419-1441.

6) Kiessling , R , . Klein, E. &

Wigzell, H. (1975). Natural killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *Eur. J. Immunol.*, 5, 112-115.

7) Mosmann , T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assay. *J.Immunol.*, 65, 55-63.

8) Mule, J.J., Shu, S., Schwars., S.L. & Rosenberg, S.A.(1984). Adoptive immunotherapy of established pulmonary metastases with LAK cells and recombinant interleukin-2. *Science* 225, 1487-1489.

9) Nouri, A.M.E. Bergbaum, A., Lederer, E., Crosby, D., Shamsa, A. & Oliver, R.T.D. (1991). Paired tumour infiltrating lymphocyte (TIL) and tumour cell line from bladder cancer. A new approach to study tumour immunology invitro. *Eur. J. Cancer*, 27,608-612.

10) Thierry Boon,. (1993) Teaching the Immune System To Fight Cancer. *Scientific American*, 32-39 March.