قدرت نابودکننده‌گی سلول‌های LAK/NK

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چکیده:

استفاده از داروهای ضدسرطان در دروزهای مختلف، تأثیر هریک بردههای مختلف سلول سرطانی و چگونگی تحریک سلول‌های تک هسته‌ای خون (M.N.C) فعال شده با اینترولوکین 2 (IL-2) امکان ایجاد پروتئز فیبراسیون می‌دهد. اساس آزمایش به استفاده از داروهای سلول سرطانی (سلول هدف) سلول‌های مونوکلینار فعال شده با 2-IL (سلول مؤثر) و روشن Tamoxifen, cyclohexamide, Pentoxifylline, cisplatin داروهای اختصاصی ضدسرطان نظیر رنگ‌سنجی نتایج پذیرش‌های انجام پذیرفته‌است.

از نتایج مقدماتی جنینی بر می‌آید که داروها در دروزه‌های مصرفی یکسان دارای اثرات متفاوت تقویت کننده قدرت نابودکننده LAK/NK یا بازدادرنگی آن خواهند گذاشت. شاید توجه است که در شرایط تضعیف یا بازدارنگی قدرت نابودکننده LAK/NK امکان می‌باشد. پیشرفت سرطان دور از انتظار نخواهد بود.

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

کلید واژه‌ها: 1- تومور

NK/LAK 2- سلول‌های 3- نابودکننده

LAK/NK 5- سیکلوهیپسید

6- پنتوکسی فلین

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

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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, mononuclear 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 MTT assay as
evaluation agent. The different drugs with the similar dosages show
stimulation or inhibition effect on NK/LAK killing. It is to be taken
into consideration 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 eliminate 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 fragment 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)\(^{(16)}\). Because some tumour cells,
such as Daudi were consistently resistant to
NK cytotoxicity, it was the discovery that
IL-2 activated lymphocytes were cytotoxic for Daudi that led to the definition of LAK cells\textsuperscript{(3)}. It is now thought that LAK represents an activated form of NK cytotoxicity and are involved in protection against experimental animal tumours\textsuperscript{(8)} and in leukaemia in man\textsuperscript{(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\textsuperscript{(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\textsuperscript{(9)}. The interface cells were aspirated, washed and stimulated with IL-2 100 u/ml, Biogen) for 72-96h at 37\textdegree 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\textsuperscript{(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 0.5x10^6 in RPMI plus 10% foetal calf serum (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\textsuperscript{(4)}. This was carried out using the modified MTT (3-[4,5-dimethyl - tetrazol 2yl]-2,5 diphenyl tetrazolium bromide) assay described by Masmann(1983)\textsuperscript{(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 10x10/well (Nunc). Effector cells i.e IL-2 activated 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>
<thead>
<tr>
<th>Pentoxifylline</th>
<th>Drug alone</th>
<th>Drug + effector</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 ug/ml</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>0.1</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
<td>-2</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>-8</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>0.01</td>
<td>43</td>
<td>44</td>
</tr>
<tr>
<td>0.1</td>
<td>71</td>
<td>68</td>
</tr>
<tr>
<td>Caffeine</td>
<td>9</td>
<td>i</td>
</tr>
<tr>
<td>0.01</td>
<td>13</td>
<td>44</td>
</tr>
<tr>
<td>0.1</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>1.0</td>
<td>2</td>
<td>-5</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>0.01</td>
<td>9</td>
<td>18</td>
</tr>
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<td>0.1</td>
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<td>-5</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colchicin</td>
<td>62</td>
<td>71</td>
</tr>
<tr>
<td>0.01</td>
<td>69</td>
<td>65</td>
</tr>
<tr>
<td>0.1</td>
<td>72</td>
<td>74</td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>37</td>
<td>24</td>
</tr>
<tr>
<td>0.01</td>
<td>42</td>
<td>18</td>
</tr>
<tr>
<td>0.05</td>
<td>38</td>
<td>50</td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

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 results 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 needed to identify the target recognition molecules.

**REFERENCES**


