THE EVALUATION OF IN VITRO EFFECT OF DAUNORUBICIN AND TAMOXIFEN IN EHRlich ASCITES TUMOUR (EAT) CELLS

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In the most countries, breast cancer is still the most important cancer among women. It is known that Ehrlich Ascites Tumour is experimental breast cancer model in animal. The cells used in the study are hyperdiploid line of Ehrlich Ascites Tumour (EAT) cells, initially provided to us from Institute of Pathology, Koln University. In the present study, an hyperdiploid line which is estrogen receptor positive was used.

An anthracycline-derived antibiotic, Daunorubicin (DNR, Cerubidine) is one of the clinically used anticancer drugs. DNR has been used alone or in combination with other cytotoxic agents against a variety of animal and human tumours. In vitro cell culture studies show that DNR enters the cell nuclei, inhibits nucleic acid synthesis, and arrest cell division. Tamoxifen (TAM, Nolvadex) is a semi-synthetic estrogen antagonist, used in the management of pre and post menopausal breast cancer. This drug bind to intracellular estrogen receptors, and prevents endogenous estrogens from binding to their own receptors.

In the present study, changes on ³H-thymidine labelling index (³H-TdR LI) were examined applying in vitro optimum doses DNR, TAM and DNR+TAM to EAT cells. The results of the study, show a significant decrease in the ³H-TdR LI values (p< 0.01). The findings reveal that treatments of DNR+TAM lower the percentage of the cells at S phase, while combined treatments of DNR+TAM gives succesful results. Thus, the results of our study seem to be concordant with the above mentioned studies, suggesting that combinations of drugs are superior to single agents.

The incidence of breast cancer has been increasing in Europa and United States throughout the past decade; the incidence of metastatic breast cancer is therefore also expected to be a growing therapeutic problem.

It is known that Ehrlich Ascites Tumour is experimental breast cancer model in animal. The cells used in the study are hyperdiploid line of EAT cells initially provided to us from Institute of Pathology, Koln University. In the present study, an hyperdiploid line which is Estrogen Reseptör (+) was used. Estrogen Reseptör levels were studied by the methods of Lippman and Huff and Raynaud et al. with minor modifications. Estrogen Reseptör activity as demonstrated by dextran-coated charcoal technique is closely correlated with the clinical ability of Tamoxifen to inhibit tumour growth.
The two most important modalities for breast cancer are hormonal manipulations and chemotherapy. The antracyclines represent the most active class of drugs for the treatment of breast cancer. In vitro cell culture studies show that an antracycline-derived antibiotic Daunorubicin enters the cell nuclei, inhibits nucleic acid synthesis, and arrests cell division. Randomized trials have demonstrated that essentially all hormonal interventions exert a similar therapeutic effect in terms of response rate and response duration; the only substantial differences relate to pattern and severity of side effects. Today, most oncologists select an antiestrogen, such as Tamoxifen, as the first-line hormonal intervention.

In this study, changes on \(^{3}\text{H}\)-thymidine Labelling Index were examined applying in vitro optimum doses DNR, TAM, DNR+TAM to EAT cells. These doses have found 0.01 \(\mu\text{g/ml}\) for DNR and 2.00 \(\mu\text{g/ml}\) for TAM.

For Labelling Index determination, cells were than incubated with M-199 (Gibco Lab) containing 5 \(\mu\text{Ci/ml}\) \(^{3}\text{H}\)-thymidine (Amersham). Both control cells and treated cells were labelled for 1 hour. After slides were rinsed with 2 percent perchloric acid twice at 4°C for 30 minutes to remove dissolved radioactive material. They were than coated with K2 emulsion gel (Ilford) kept at 4°C for 3 days and developed. Labelling Index was calculated by counting 3000 cells stained with Giemsa for 3 minutes. Student-t test was used to evaluate the results.

Table and Figure indicate Labelling Index values of Control, DNR, TAM and DNR+TAM groups after an application of 0, 2, 4, 8, 16, 32, 64 hours.

Treatments of DNR and TAM lower the percentage of the cells at S phase (relative to control), but combined treatment of DNR+TAM gives successful results, being statistically significant.

![Figure: Labelling Index values of EAT cells after drug application.](image-url)
### Table: Labelling Index values of EAT cells after drug applications, given in mean±SD

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Control</th>
<th>DNR</th>
<th>TAM</th>
<th>DNR+TAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>13.23±0.21*</td>
<td>13.20±0.17</td>
<td>13.21±0.13</td>
<td>13.22±0.18</td>
</tr>
<tr>
<td>2 h</td>
<td>13.22±0.19</td>
<td>13.21±0.11</td>
<td>13.20±0.16</td>
<td>13.18±0.12</td>
</tr>
<tr>
<td>4 h</td>
<td>13.23±0.26</td>
<td>13.02±0.16</td>
<td>12.76±0.14</td>
<td>11.65±0.09</td>
</tr>
<tr>
<td>8 h</td>
<td>12.28±0.11</td>
<td>11.88±0.14</td>
<td>11.56±0.10</td>
<td>8.58±0.07</td>
</tr>
<tr>
<td>16 h</td>
<td>12.11±0.14</td>
<td>10.99±0.13</td>
<td>8.89±0.09</td>
<td>5.16±0.04</td>
</tr>
<tr>
<td>32 h</td>
<td>10.85±0.21</td>
<td>9.95±0.10</td>
<td>8.23±0.05</td>
<td>4.67±0.02</td>
</tr>
<tr>
<td>64 h</td>
<td>7.06±0.11</td>
<td>6.80±0.03</td>
<td>5.52±0.02</td>
<td>3.63±0.02</td>
</tr>
</tbody>
</table>

* Standard Deviation (SD)

*In vitro* studies show that DNR has maximal cytotoxic effects in the S and G2 phases. TAM is a competitive inhibitor of Estrodiol binding to the Estrogen Receptor which can cause G1 arrest in sensitive cell lines.

It was apparent that combination therapy was more effective than single agent treatment in this setting. We think that in this experiment, some interactions between cytotoxic agents and Tamoxifen is synergistic and additive.

The challenge for all clinical investigators is to determine, in the most efficient manner, how these new cytotoxic agents should be incorporated into the optimal management of breast cancer. To meet this challenge, strategies are being developed to evaluate innovative combinations, including new and old drugs, to compare the best new combinations with the best "old" combinations, and perhaps to develop innovative approaches to treatment, such as the use of non-cross-resistant combinations in a fixed cross over pattern or use of sequential high dose single agent regimen.

### REFERENCES