CHAPTER 7

PHOTODYNAMIC ACTIVITY ON
HUMAN EPITHELIAL TUMOUR CELL LINES, He-La AND HEp-2

In the previous chapter, we have discussed about the PDA on rat fibrosarcoma, a fast growing tumour by employing different lasers in conjunction with different sensitizers in order to determine the optimal operational parameters. Besides, a study of PDA with the above operational parameters to assess their effect on the tumour of different origin, it is relevant to view the dynamics of PDA in a better perspective. This is important because there are certain controversies on this aspect among the leading scientists working on PDT. Our investigation is similar to that of Andreoni et. al. [84]. Andreoni et. al., found the differences in PDA effectiveness between normal and transform thyroid cells in culture by employing laser in conjunction with HPD. In contrast, we have compared the effectiveness of PDA on fibrosarcoma with two different types of epithelial tumour cell lines, He-La (cervical epithelial cell line) [64] and HEp-2 (Human larynx epidermoid cell line) [65] under identical experimental conditions.

7.1 PHOTODYNAMIC ACTIVITY ON He-La CELL LINE

As in the case of fibrosarcoma, the cell survival curves of He-La, due to nitrogen laser irradiation in conjunction with DHE and HPD were drawn and are shown in Figure 7.1. From the Figure 7.1, it can be seen that the LD50 values for HPD and DHE are 0.568 J/cm² and 0.442 J/cm².
Figure 7.1 The percentage of viable cells (He-La) as a function of irradiation time at 337.1 nm for HPD and DHE (10μg/ml)
respectively; i.e., DHE is 1.3 times more effective than HPD at 10 μg/ml concentration at 337.1 nm. In terms of Dq value also i.e., fluence required to produce irreparable damage, DHE needs 1.5 times less fluence than HPD.

At a fixed fluence of 0.47 J/cm², the concentration required for 50% cell damage was also estimated from the Figure 7.2. The LC₅₀ for HPD and DHE are 20.0 μg/ml and 7.0 μg/ml respectively. Here also, the DHE is more effective than HPD.

Similarly, the experiment was carried out using He-Ne laser of power 15 mW. We found that DHE requires only 9.7 J/cm² to produce 50% cell damage whereas HPD requires 10.7 J/cm² to produce the same effect and the Dq values for HPD and DHE are 3.8 J/cm² and 2.4 J/cm² respectively (Figure 7.3). Figure 7.4 shows the percentage of cell viability as a function of concentration at a fixed fluence of 7.1 J/cm². It is found that HPD requires 30.0 μg/ml of drug concentration and DHE requires only 23.0 μg/ml of concentration i.e., DHE is 1.3 times more effective than HPD. These results are given in Table 7.1.

7.2 PHOTODYNAMIC ACTIVITY ON HEp-2 CELL LINE

In the second case, HEp-2 human larynx epidermoid carcinoma cell line (HEp-2) obtained from the Virology Department, King Institute for Preventive Medicine, Madras, was irradiated with Ar ion laser at two different wavelengths, 514.5 and 480.0 nm.

Figure 7.5 shows the percentage of cell viability as a function of fluence for HPD, DHE and Eosin Y (EY) each at 20 μg/ml concentration. This has been shown for a wavelength
Figure 7.2 The percentage of viable cells (He-La) as a function of concentration (µg/ml) at 337.1 nm (for 30 minutes exposure)
Figure 7.3 The percentage of viable cells (He-La) as a function of irradiation time at 632.8 nm for HPD and DHE (10μg/ml).
Figure 7.4 The percentage of viable cells (He-La) as a function of concentration (µg/ml) at 632.8 nm (for 30 minutes exposure).
Figure 7.5 The percentage of viable cells (HEp-2) as a function of irradiation time at 514.5 nm for DHE, HPD and EY.
Table 7.1 The values of $D_q$, $LD_{50}$ and $LC_{50}$ for the dyes HPD and DHE at 337.1 nm and 632.8 nm

<table>
<thead>
<tr>
<th>Dye</th>
<th>$N_2$ laser</th>
<th>He-Ne</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$D_q$</td>
<td>$LD_{50}$</td>
</tr>
<tr>
<td>HPD</td>
<td>0.221</td>
<td>0.568</td>
</tr>
<tr>
<td>DHE</td>
<td>0.150</td>
<td>0.442</td>
</tr>
</tbody>
</table>
of 514.5 nm. At this wavelength LD\(_{50}\) for DHE, HPD and EY are 33.6 J/cm\(^2\), 51.6 J/cm\(^2\) and 57.6 J/cm\(^2\) respectively and their corresponding D\(_q\) values are 10.8 J/cm\(^2\), 14.4 J/cm\(^2\) and 19.2 J/cm\(^2\) respectively.

As in the case of N\(_2\) laser and He-Ne laser, here also the dependency of PDA on concentration of the drug at a fixed fluence 72 J/cm\(^2\) was calculated from the Figure 7.6. The concentration required for 50% cell damage for DHE, HPD and EY are 5.5 \(\mu\)g/ml, 7.5 \(\mu\)g/ml and 13.5 \(\mu\)g/ml respectively.

From Figure 7.6, it can be seen that LC\(_{50}\) is least for DHE, next comes HPD and the highest for EY. From Figures 7.5 and 7.6, one can infer that the PDA effectiveness for HPD and EY are comparable, whenever DHE is decisively better. While studying the effect of N\(_2\) gas bubbling, in order to find the difference in PDA between the aerobic and subaerobic condition, we found that DHE and HPD have greater dependency on oxygen when compared to EY (Figures 7.7-7.9).

In order to see the PDA differences between 514.5 nm and 488 nm, the samples were irradiated at 488 nm, at the same irradiance of 40 mW/cm\(^2\). Here also it can be seen from Figure 7.10 that at 488 nm also DHE is more effective than HPD and EY. The LD\(_{50}\) for DHE, HPD and EY at 488 nm are 28.8 J/cm\(^2\), 39.6 J/cm\(^2\) and 66.0 J/cm\(^2\) respectively. Their corresponding D\(_q\) values are 9.6, 14.4 and 26.4 respectively. Table 7.2 shows the comparison of the values of D\(_q\) and LD\(_{50}\) for 514.5 nm and 488 nm.

7.3 DISCUSSION

This chapter gives not only the effectiveness of the PDA on two different epithelial cell lines but also the
Table 7.2 The values of \( D_{q} \), \( LD_{50} \) and \( LC_{50} \) for dyes HPD, DHE and EY at 514.5 and 488 nm

<table>
<thead>
<tr>
<th>Dye</th>
<th>514.5 nm</th>
<th>488 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( D_{q} )</td>
<td>( LD_{50} )</td>
</tr>
<tr>
<td></td>
<td>J/cm²</td>
<td>J/cm²</td>
</tr>
<tr>
<td>DHE</td>
<td>10.8</td>
<td>33.6</td>
</tr>
<tr>
<td>HPD</td>
<td>14.4</td>
<td>51.6</td>
</tr>
<tr>
<td>EY</td>
<td>19.2</td>
<td>57.6</td>
</tr>
</tbody>
</table>
Figure 7.6 The percentage of viable cells (HEp-2) as a function of concentration at 514.5 nm for DHE, HPD and EY (μg/ml)
Figure 7.7 The percentage of viable cells (HEp-2) as a function of irradiation time at 514.5 nm for HPD both at aerobic and reduced oxygen conditions.
Figure 7.8 The percentage of viable cells (HEp-2) as a function of irradiation time at 514.5 nm for DHE both at aerobic and reduced oxygen conditions.
The percentage of viable cells (HEp-2) as a function of irradiation time at 514.5 nm for EY both at aerobic and reduced oxygen conditions.

Figure 7.9 The percentage of viable cells (HEp-2) as a function of irradiation time at 514.5 nm for EY both at aerobic and reduced oxygen conditions.
Figure 7.10 The percentage of viable cells (HEp-2) as a function of irradiation time at 488 nm for HPD, DHE and EY (20μg/ml)
photosensitivity comparison of three different tumour cell lines. From Figure 7.1 it can be seen that the PDA effectiveness due to $N_2$ laser is 1.3 times higher in the presence of DHE than with HPD. In terms of $LC_{50}$ and $D_q$ also, DHE is 1.4 and 2.6 times more efficient than HPD respectively. In the case of He-Ne laser, the $LD_{50}$ is 1.1 times higher in its efficiency for DHE than HPD. In terms of $D_q$ value also, DHE requires 1.6 times lesser energy density than HPD and in terms of $LC_{50}$ also DHE requires 1.5 times less concentration to produce 50% cell damage at 7.10 $J/cm^2$. This indicates that among the two lasers, Nitrogen and He-Ne, Nitrogen laser has higher photodynamic activity than He-Ne laser. This may be due to the high absorption cross-section of DHE and HPD at 337.1 nm than at 632.8 nm. Besides that, there is a chance of photo-ionization due to two photon absorption when irradiated with pulsed laser (delivering higher energy density within a short duration of time) [85].

For example, the mean lethal dose ($LD_{50}$) for DHE due to $N_2$ laser is 18.8 times less than that due to He-Ne laser. Similarly for HPD, the $LD_{50}$ is 21.9 times less for Nitrogen laser than that for He-Ne laser. In the case of $LC_{50}$, $N_2$ laser-DHE combination requires 2.86 times less concentration at 15 times less energy density, than those of He-Ne laser.

If we compare the PDA effectiveness between rat fibrosarcoma and He-La cell line for these two lasers, He-Ne and Nitrogen, it is found that fibrosarcoma has higher photosensitivity than He-La cell lines. For example, fibrosarcoma is 1.2 times more sensitive than He-La at $LD_{50}$ due to $N_2$ laser. In terms of concentration also fibrosarcoma requires 2 times less amount than He-La for DHE. In the case
of HPD, fibrosarcoma requires 1.2 times less dose for LD$_{50}$ than He-La. In terms of LC$_{50}$ also fibrosarcoma requires 2.5 times less concentration of HPD than He-La. Similar results have been observed in the case of He-Ne laser also.

The effect of Ar ion laser in conjunction with DHE, HPD and EY were studied on HEp-2 cell line. At 514.5 nm of Ar ion laser, it can be seen from the Table 7.3 that DHE is more effective than other two sensitizers HPD and EY in terms of all parameters like D$_q$, LD$_{50}$ and LC$_{50}$. It can be seen that DHE requires 1.5 times less LD$_{50}$ than HPD and 1.7 times less LD$_{50}$ than EY at a concentration of 20 $\mu$g/ml. To produce irreparable damage to the cellular system, DHE requires 1.3 times less dose (D$_q$) than HPD and 1.8 times less than EY. At a fixed fluence, the concentration required for DHE to produce 50% cell damage is 1.36 times less than that for HPD and 2.45 times less than that for EY.

In the case of dependency of oxygen, it is seen that due to N$_2$ gas bubbling, the cell survival rate is considerably increased. Among the three sensitizers, DHE has higher percentage of cell survival than HPD and EY. It reveals that though PDA depends on oxygen concentration, PDA is present considerably even after N$_2$ gas bubbling. This indicates that these dyes are effective even in the case of hypoxic condition of tumour.

When the effectiveness of PDA for LD$_{50}$ due to Ar ion laser at 488 nm is seen, only DHE and HPD are having higher efficiency and EY has 1.2 times lesser efficiency at 488 nm than at 514.5 nm. This increase in PDA at 488 nm for HPD and DHE may be due to high absorption capability of DHE and HPD at 488 nm than at 514.5 nm and the reason for reduced PDA
due to EY at 488 nm can be attributed to the relatively reduced absorption at 488 nm.

If we look into the difference in the photosensitivity between the two cell lines fibrosarcoma and HEp-2 at 514.5 nm, fibrosarcoma has higher photosensitivity than HEp-2. From the Tables 7.3 and 6.3, it can be seen that the ratio of LD$_{50}$ of HEp-2 to that of fibrosarcoma for DHE is 1.3, for HPD, it is 1.4 and for EY, it is 1.1. In the case of D$_{q}$, fibrosarcoma requires 1.3 times less dose for DHE than HEp-2, 1.3 times for HPD and 1.4 times for EY.

In terms of concentration (LC$_{50}$) also, PDA on fibrosarcoma requires 1.1, 1.0 and 1.28 times less amount for DHE, HPD and EY respectively than in the case of HEp-2. Thus, from our results it is found that fibrosarcoma has greater photosensitivity than He-La and HEp-2 for all the dyes and for all the wavelengths, which means photosensitivity is also depended on tumour origin as in the case of radiotherapy [86].

Though EY has higher D$_{q}$ value than at HPD, they are having almost same LD$_{50}$ value. This reveals that, one can use EY for photodynamic therapy of tumours instead of HPD. This is because, though HPD and DHE are having good photodynamic efficiency, by considering their drawbacks which were mentioned earlier in Chapter 6, it is better to use EY at 514.5 nm.

From the results presented in the Chapter 6 and Chapter 7, one can draw the following conclusion:

i. PDA strongly depends upon the nature of laser and sensitizers used
ii. Among the two classes of sensitizers compared, Xanthene exhibits comparable, though marginally better, PDA effectiveness.

iii. Among porphyrins, DHE is about 1.4 times better PDA effect than HPD. This is independent of the nature of laser, tumour origin and the cellular environment.

iv. Among xanthenes, the PDA of RB and EY are comparable, though both are better than FL.

v. PDA for all these lasers and sensitizers strongly depends upon oxygen. In this respect DHE exhibit greater sensitivity and EY and FL least sensitivity.

vi. Considering the above factors in addition to high cost, photoallergies, dark toxicity and neurotoxicity of HPD and non-availability of pharmacological data for DHE, use of EY or RB is a judicious step for clinical trials, particularly for tropical countries like India.

vii. Of the three types of tumours studied, the fibrosarcoma exhibits greater photosensitivity than epithelial cell lines (He-La and HEp-2)

This led us to go for in-vivo study on rat fibrosarcoma, human oral cancer and leukoplakia with EY as sensitizer. The results of the study form the next chapter.