PHOTODYNAMIC THERAPY OF RAT FIBROSARCOMA, HUMAN ORAL CARCINOMA AND LEUKOPLAKIA

In the last three chapters we have presented our results and interpretations of photodynamic activity on various cell lines. Such in-vitro study are only exploratory and preparatory investigation for the clinical trials. The in-vitro study is close to the mathematical or theoretical modeling in many physical and engineering disciplines and the in-vivo study is close to the experimental study in practical condition and hence may be regarded as the touchstone for the concept and conclusion arrived from the theoretical study. In other words, in any biological investigation, the in-vivo study is the culmination and fruit of the labor of an in-vitro study.

In the preceding chapters, we have arrived at the conclusion that fibrosarcoma would exhibit favorable response under Ar ion laser with DHE, RB or EY acting as sensitizers. With this result in the background we investigated the effect of Ar ion laser and Eosin-Y sensitizer combination on the clinically induced rat fibrosarcoma. The encouraging results obtained there has led us to apply the technique for spontaneous human oral carcinoma and oral leukoplakia.

Though DHE is more effective when compared to HPD and xanthene derivatives, there is lack of data regarding the amount of porphyrin needed to produce PDA, and no toxicologic studies have been reported with DHE. Only HPD is
now commercially available for clinical trial [87] and PDT is generally done with Rhodamine B dye laser with typical irradiance level of 200 mW/cm² and with HPD as sensitizer [88,89]. In HPD based PDT, the sensitizer was given i.v., at a dosage of 2-3 mg/Kg, then one has to wait for 48-72 hours to treat the tumour. This i.v., administration of HPD has led to some complication such as large accumulation in lungs and its neurotoxicity. So, topical application is preferred whenever possible [90,91]. Even then it has been reported that topical application of HPD produces phototoxic effects such as edema, erythema and burns. These factors make HPD not quite suitable in tropical countries. This led us to go for EY, the least toxic and photoallergic dye used by us as a sensitizer and this necessitates Ar ion laser capable of delivering 1 W power at 514.5 nm as light source. It must be mentioned here that though N₂ laser is quite effective for PDA, it cannot be used for clinical trial since it has average power of only 1 mW.

8.1 PDT OF RAT FIBROSARCOMA

Albino rats were obtained from King Institute for Preventive Medicine, Madras and were entered into studies at 10 to 11 weeks of age. A rat fibrosarcoma tumour which was previously induced by a chemical carcinogen, the 8-methyl chlonthrene, was obtained from the Department of Microbiology and Tumour biology, Cancer Institute, Madras. This was grown as a subcutaneous mass for serial passage and tumour cell transplantation. Single cell suspensions of fibrosarcoma were obtained by passing minced tumour pieces through 18 gauge sterile needle. Cell suspension of volume approximately 0.2 to 0.3 ml was injected s.c in the right or left hind flank of experimental rats. The volume of the grown tumour was obtained by using the relation
V = 0.4 ab² \hspace{1cm} (8.1)

as determined by Affia and Weiss [92], where "a" and "b" are
the long and short axes of the tumour, respectively. The
axes lengths were measured using a vernier caliper. The
volume estimation was carried out four times in a week.

The rats with tumour were divided into four groups:

i. control (untreated with either sensitizer or laser)

ii. treated with laser alone

iii. treated with topical application of sensitizer (EY
of 50 µg) alone and

iv. treated with laser in conjunction with topical
application of Eosin Y (50 µg/ml)

Before laser treatment or application of sensitizer,
the tumour part was carefully shaved off, cleaned few times
with surgical spirit and dried up. The treatment was given
for at least three rats from each group.

8.1.1 Irradiation method

The rats were irradiated with Ar ion laser at 514.5
nm at different experimental conditions like laser alone and
laser in conjunction with Eosin-Y with an energy density
varying from 150 to 500 mJ/cm² for about 20 days at the rate
of 10 minutes exposure for each sitting.

The laser beam was expanded to an area of tumour.
The rat survival and the tumour volume were noted once in
two days. The growth rate of tumours for different experimental conditions are shown in Figure 8.1

Rats treated with laser alone or sensitizer alone had tumour growth very similar response to the untreated ones. Most of them had an average life span of only 15 days after the appearance of tumour to the above groups. In contrast, the rats of the fourth group, which underwent laser treatment in conjunction with Eosin-Y showed tumour regression. However, a four of them had the growth arrested but there was no recession. But in another three rats, the tumour not only stopped growing but started receding. The former ones died after 22 days without any further growth or regression of tumour, whereas, the latter ones, had complete suppression of tumour, and lived long for about 3 months after the treatment. For the latter set, histopathology study was made, when the rat was alive, which confirmed the disappearance of tumour, indicating the presence of only normal fiber tissues.

A few important observations made during the period of in-vivo PDT trial on rats are as follows:

i. For three rats which had complete remission, swelling started receding after each sitting and surface started peeling off. Figures 8.2 and 8.3 show the rats before and after treatment respectively and Figure 8.4 represents the microscopic appearance of tumour tissue. This part has to be cleaned off, before further topical application of sensitizer and laser treatment to be done (Figures 8.5 and 8.6). This way the laser treatment has to be done layer after layer.
Figure 8.1 The volume of the tumour with respect to the days following treatments
Figure 8.2 The rat with tumour before treatment.

Figure 8.3 The rat with tumour after 10 sitt of the treatment.
Figure 8.4 Microscopic appearance of fibrosarcoma tissue x 50 (H & E stain)
Figure 8.5 The treated rat after three month

Figure 8.6 Microscopic appearance of tissue at treated area indicating the normal fibrous tissues x50 (H & E stains)
ii. Comparing the untreated rats which died in about 24 days (Figure 8.7) and the treated rats which died about 24 days, we found that the former had pus formation on the tumour surface, the latter has clear tumour mass. This indicates that treatment had indeed arrested the growth of the tumour but could not go beyond a certain depth to uproot malignancy. This should have led to the infiltration (metastasis) and consequent death (Figure 8.8). Example, pathological studies on the set of rats which had tumour arrest, without remission, showed that on the surface area tumour growth had been curtailed. However in those regions where laser treatment is inaccessible, proliferation is high as shown in the biopsy reports (Figure 8.9).

iii. The tumour showed random nodules on the hemispherical swelling. These nodules required higher energy density for suppression than the back swelling.

iv. It was found that only when the tumour volume was about 0.5 cm³, our PDT works. That is, the irradiation with topical application of EY employing interstitial therapy (which involves the administration of PS followed by laser beam directed through optical fibers). We have confirmed our findings at this stage with topical application of senescence external beam irradiation and have concluded that interstitial irradiation for future study.
Figure 8.7 Control rat
Figure 8.8 Microscopic appearance of infiltration of tumour cells x 50

Figure 8.9 Microscopic appearance of high metabolic activity of cells
8.2 PDT ON ORAL CANCER PATIENTS

The usual method of treating oral cancer is radiotherapy, either by teletherapy and brachy-therapy. To treat the oral cancer one has to remove all the teeth in order to avoid the scattering of ionizing radiation which are giving unnecessary radiation dose to the normal surrounding tissues and unestimated over dose at the tumour site. Besides this, after the radiation exposure, disfiguring of skin and face may result due to erythema, edema etc. These disadvantageous factors of radiotherapy and experience gained from the animal study, we made pilot study employing Argon ion laser at 514 nm for irradiation and topical application of EY for three cases of carcinoma in situ and on three cases of leukoplakia into clinical trials.

8.2.1 PDT of oral carcinoma

Case I

Patient Name : Neela
Sex and age : Female, 43
Lesion : bucal mucosa (carcinoma in situ)
Area : About 1 cm²
Thickness : About 0.7 cm

To treat this case, the lesion was completely cleaned with sterile cotton gauge and the Eosin-Y was applied topically. After about 10 minutes, lesion was irradiated at an energy density of 150 mJ/cm² for 15 minutes. This was repeated for 15 sittings. At this stage considerable remission of tumour was observed. Since subnodules were persistent even after the continuous treatment
(Figure 8.10), the dose was increased to 420 mJ/cm². At this dose both photochemical and photothermal effects could take place which we not quantify. In another 5 sitting treatments at 450 mJ/cm² complete regression of tumours was achieved (Figure 8.11) and after two months the site acquired healthy coloration (photograph 8.12).

Case II

Name: Krishnasamy  
Sex and age: Male, 66  
Lesion: palate and mucosa  
Area: 1 cm² area 2-3 mm thickness at palate  
0.5 cm² area and 2 mm thickness

As usual, the tumour sites were cleaned with a 0 gauge and the sensitizer EY was applied topically. Irradiation was given at an energy density of 300 mJ/cm² for 10 minutes. After seven sittings, on tenth day the swelling at the palate was completely gone, only nodules were left at the mucosa, the growth had completely disappeared. The nodules which was left after the seven sittings of treatment were given an increased dose of 400 mJ/cm² for 10 minutes. After 10 sittings (totally 17 sittings) the effective area (1cm²) was reduced to 0.3 cm². Before the treatment the patient had difficulty in opening his mouth and after treatment he could do it with comfort.

Case 3

Name: Rani Boy  
Sex and age: Female, 75  
Lesion: Lower lip, diagnosed as premalignant
Figure 8.10 Oral carcinoma in situ before treatment.

Figure 8.11 Treated area after one month.
This patient was given high dose rate of 450 mJ/cm² for 10 minutes, but only part of the tumour was disappeared (in terms of thickness). During the treatment, growth was observed at the adjacent tissues. The treatment was continued at the rate of 1 sitting a day, at the above dosage level. The treatment is still under continuation.

8.2.2 PDT on leukoplakia

Leukoplakia, a disease marked by the development on the mucous membranes of the cheeks, gums, or tongue, appears as white thickened patches which sometimes show a tendency to tissue and to become malignant. A pilot study on the photodynamic therapy of leukoplakia was carried out on three patients.

In the case of leukoplakia a dose rate of 500 mJ/cm² for 10 minutes was given. This is because at low dose rate most of the incident photon dissipated by reflection and scattering due to its milky white color.

To avoid the reflection of photon, the EY was applied like a paste and irradiated at a moderately high dose. In about 6 times after the treatment, blistering have occurred and after repeated treatment necrosis of the skin was observed and after one week the skin at the treatment area pealed off and became normal tissue color. From our experience, it is also found that if the size of the lesion is less than 1 cm², one can treat it within 10 sittings. Beyond this area we have to treat it area by area for long time.
Case reports

Case I

Name : R. Radhakrishnan
Sex and Age : Male, 39 years
Lesion : Leukoplakia at the lower lip.
Area : 1 cm²

Since the patient has very small area of leukoplakia, it was treated with a dose rate of 350 mJ/cm² for 10 minutes. Complete remission was seen after seven sittings.

Case II

Name : Vallasamma
Sex and age : Female, 62 years
Lesion : Leukoplakia at the lower lip
Area : 1 cm²

This patient was given a dose rate of 400 mJ/cm² for 10 minutes. At the end of 6 sittings necrosis was seen and the treatment was continued for 10 sittings. After about 10 days the lesion area acquired the normal skin coloration.

Case III

Name : Valliammi Aathimulam
Sex and age : Female, 63 years
Lesion : Oral mucos membrane
Area : 4 cm²
The patient was given a dose rate of 480 mJ/cm² for 10 minutes for an area of 1 cm². After 15 sittings the lesion was acquired the normal tissue condition at the treatment area. After producing the necrosis, the treatment was shifted to the adjacent area.

The study is confined only to oral carcinoma, since these lesions are easily accessible and could be carried with the facilities available in this laboratory and without the necessity of presence of medical personnel. We also restricted our studies to carcinoma of thin lesion (thickness less than a cm) and which could be interacted with the laser beam without using fiber optics. (We do not have at present, optical fibers, and couplers for efficient delivery of laser beam)

It was noted that during the first five minutes of exposure at a dose rate of 400 mJ/cm², there was a progressive increase in oral tissue temperature which persisted for the whole exposure. Since the patient did not feel much discomfort during this treatment no local anesthesia was given.

The patients were quite satisfactory with the treatment, because it is not producing any side effects and not affecting their normal day to day habits.