CHAPTER 7

ACNP AS ANTICANCER DRUG CARRIER

7.1 Introduction

Cancer is a remarkable heterogeneous disease associated with immune deficiency syndrome and it is major causes of death in humans (The World Health Organization, 2002). Cancer chemotherapy plays a significant role in the treatment of many malignancies, either curative by itself or as an adjuvant to surgery and/or radiation or palliative care, depending upon the specific tumour situation (Carter and Livingston, 1982). The scientist across the world are focusing their attention to develop new drug molecules or new drug delivery systems with existing drug for the better management of the cancer with minimal adverse drug reactions associated with anticancer drugs. It is interesting note that anticancer drugs develop serious, sometimes life-threatening side effects that arise from toxicities to sensitive normal cells because of their non specific reactivity (Allen, 2002, Christina et al., 2004), enhanced proliferative rates of bone marrow, gastrointestinal tract and hair follicles (Allen, 2002). All these things result in the eventual failure of cancer therapy.

The present chapter is aimed to assess the drug carrier /adsorption efficiency of the newly developed ACNP synthesised from the MPAC so as to use the same as a carrier to deliver the drug molecule for a prolonged period of time in the gastro intestinal tract (GIT) during the acute/chronic cancer as well as brain
cancer. The toxicity study with ACNP showed non toxic to rats even at a higher concentration of 32 mg (Chapter 6). Hence, the further studies carried out to assess the adsorption or drug carrier efficiency of the ACNP loaded with breast cancer specific anticancer drug ‘imatinib’ using Scanning Electron Microscopy (SEM) as well as to evaluate the biological activity of ACNP loaded with imatinib by MTT assay using breast cancer cell line MDA-231. The study was conducted in Podicherry centre for biological sciences, Pondicherry. The results are presented in section 7.2-7.4

**7.2 Confirmation of drug loading using SEM**

The adsorption of breast cancer specific anticancer drug imatinib was evaluated using scanning electron microscope as discussed in section 3.1 and the results are given in Figures 7.1-7.2.
Fig. 7.1 The electron microscopic images of ACNP loaded with anticancer drug imatinib (A); without imatinib (B). The images were recorded at × 1000 using scanning electron Microscope.

Fig. 7.2 The electron microscopic images of ACNP loaded with anticancer drug imatinib (A); without imatinib (B). The images were recorded at × 3,300 using scanning electron microscope.

The ACNP with/without loaded with anticancer drug imatinib was observed under the scanning electron microscope at × 1000 and × 3,300. Images were
recorded at randomly chosen fields. From the images it was observed that the ACNP loaded with anticancer drug showed dark coloration due to the loading of drug as compared to the images of the control ACNP samples without the drug. In addition to these ACNP loaded with anticancer drug are appeared like a small aggregates as compared to the ACNP without the drugs since the unique need like shape of the carbon particles enable the drug molecules to attach either covalently or non covalently (Niranjan and Ashwatha, 2014).

7.3. Evaluation of anticancer activity of ACNP

The drug carrier ability of the ACNP was evaluated by MTT assay using breast cancer MDA-231 cell line as discussed in section 3.5 and by assessing the cytotoxic effect of ACNP loaded with imatinib by light Microscopy simultaneously. The results are given in Table 7.1 and Figures.7.3-7.4.

7.3.1 Estimation of cell viability using MTT assay by measuring the OD @ 570nm

The cytotoxic activity of ACNP loaded with anticancer drug “imatinib” at the concentrations of 5, 10, 25, 50 and 100µgm (Test); ACNP without imatinib (Control) on breast cancer cell line MDA-231 using MTT assay was evaluated as discussed in section 3.5. Through this study % viability of cells treated with and without drug “imatinib” and its corresponding optical density (OD) @ 570nm values were measured using ELISA plate reader. The mean triplicate of the results is given in Table 7.1 and Figure 7.3-7.4.
Table 7.1

Evaluation of cytotoxic activity of ACNP loaded with anticancer drug “imatinib” on breast cancer cell line MDA-231 using MTT assay

<table>
<thead>
<tr>
<th>Tested concentrations</th>
<th>OD values at 570nm (Mean ± SEM)</th>
<th>% of cell viability (Mean ± SEM)</th>
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<tbody>
<tr>
<td>5 µg</td>
<td>0.815 ± 0.003</td>
<td>71 ± 0</td>
</tr>
<tr>
<td>10 µg</td>
<td>0.783 ± 0.011</td>
<td>74 ± 1</td>
</tr>
<tr>
<td>25 µg</td>
<td>0.639 ± 0.035</td>
<td>60 ± 3</td>
</tr>
<tr>
<td>50 µg</td>
<td>0.562 ± 0.008</td>
<td>53 ± 0</td>
</tr>
<tr>
<td>100 µg</td>
<td>0.498 ± 0.009</td>
<td>47 ± 0</td>
</tr>
<tr>
<td>Control</td>
<td>1.058 ± 0.001</td>
<td>100 ± 0</td>
</tr>
</tbody>
</table>

Figure 7.3. Evaluation of cytotoxic activity of ACNP loaded with anticancer drug “imatinib” on breast cancer cell line MDA-231 and its corresponding OD values at 570nm using MTT assay
Figure 7.4. Evaluation of cytotoxic activity of ACNP loaded with/without anticancer drug “imatinib” on breast cancer cell line MDA-231 and its % viability of cells using MTT assay.

From the results it was observed that the ACNP with higher concentration of imatinib i.e. 100 µgm showed highly significant maximum cytotoxic effect to the cells i.e. 50% viability of cells observed as compared to the control samples. In addition to these the % viability of cell was in dose dependent manner. These shows the ACNP demonstrated as an efficient drug carrier based on its adsorption characters and it supports the results of scanning electron microscopy.
7.3.2 Assessing the cytotoxic effect of ACNP loaded with imatinib by light microscopy

The cytotoxic activity of ACNP loaded with/without anticancer drug “imatinib” on breast cancer cell line MDA-231 using MTT assay at various concentrations like 5, 10, 25, 50 and 100µgm/ml was carried out as discussed in section 3.5 and the images of the cells were recorded after 24 hours using light microscope at 40x to assess the morphological changes of cells on treatment with ACNP with/without Imatinib using light microscope and presented in Figure 7.5.
Fig 7.5 Evaluation of cytotoxic activity of ACNP loaded with/without anticancer drug “imatinib” on breast cancer cell line MDA-231 at the concentrations of 5 (A); 10(B); 25(C); 50(D); 100µgm(E); MPAC without ‘imatinib’ (Control) (F) using light microscopy.
In the present study the cells were treated with ACNP loaded with imatinib was tested at the concentrations like 5,10,25,50 and 100µgm and the ACNP without imatinib was served as a control, represented in Figure 7.5 (A)-(F) respectively. The results of the study showed in Figure 7.5 reveals that the Figure 7.5 (E), cells treated with the ACNP with higher concentration of imatinib i.e. 100 µgm showed reduced cell numbers as compared to the control and other samples. It reflects results of MTT assay and scanning electron microscopy, these shows the ACNP is acts as an efficient drug carrier based on its adsorption characters.

7.4 Conclusion

The adsorption property of the newly developed ACNP loaded with breast cancer specific anticancer drug “imatinib” was tested using scanning electron microscope, MTT assay as well as light microscopy. The results of the study proved that the drug ‘imatinib’ was adsorbed firmly on the ACNP so that it produced a significant cytotoxic effect (cell death) on breast cancer cell line MDA 231 on treatment with various concentration of ACNP loaded with imatinib. In addition to these the cytotoxic effect observed is directly proportionate to the concentration of the drug. It is interesting to note that the results of the MTT assay perfectly matches with the results of microscopic examination with similar experimental conditions.