Chapter - VIII

Studies on the antimetastatic activities of anti-inflammatory agents in mice
8.1 INTRODUCTION

The metastatic spread of solid tumours is responsible directly or indirectly for most cancer related deaths. Metastasis occurs as a result of a complex series of interaction between the cancer cell and its surroundings (90).

Tumour cell proliferation, metastatic formation, and tumour-host effects are mediated by complex interactions between cytokines, growth factors and classic hormones (19,28). Prostaglandins are known to be involved in the regulation of cell proliferation and differentiation in a large number of systems (225). Eicosanoids are also important factors in the cascades that determine the balance between growth arrest and tumour progression in experimental and clinical cancer (225). Eicosanoids play a significant role in carcinogenesis (224). Inhibition of prostaglandin synthesis reduced tumour progression and cancer cachexia (108).

Epidemiological evidence shows that patients on long-term aspirin treatment have a lower incidence of colon carcinoma and patients on anti-inflammatory treatment due to rheumatoid arthritis have a lower risk to develop solid tumours. Indomethacin treatment to tumour-bearing rodents prolonged survival and was curative (108).

Curcumin, is reported to inhibit both lipoxygenase and cyclooxygenase and to be effective in acute and chronic models of inflammation (64). It has also been reported to be a potent scavenger of oxygen free radicals (316).

The aim of this study was mainly to show whether the antiinflammatory property of some of the polyphenolic compounds (mainly curcumin and catechin) are responsible for its antimetastatic activity. In
order to confirm our study (previous chapter) some of the clinically administered antiinflammatory drugs, were checked for its antimetastatic activity.

The antiinflammatory agents used for the present study are non-steroidal as well as steroidal antiinflammatory agents. These compounds are clinically administered as analgesics and antipyretics.

8.2 MATERIALS AND METHODS

8.2.1 Animals - C57BL/6 female mice,

8.2.2 Cell line - B16F-10 melanoma cells

8.2.3 Test compounds - Ibubrofen, Betamethazone, phenylbutazone, mefanamic acid, paracetamol.

8.2.4 Effect of antiinflammatory agents in the inhibition of lung tumour nodule formation

1x10^6 B16F-10 melanoma cells were injected through the lateral tail vein into C57BL/6 mice (6 groups, 14/group). Simultaneously the compounds (200 μmoles/kg body weight, suspended in 1% gum acacia) were administered orally to the respective groups for 10 alternate days. One group was kept as the untreated control animals and the other group with vehicle alone (0.5 ml of 1% gum acacia) for 10 alternate days.

All the animals were sacrificed on the 21st day after tumour inoculation, and all internal organs were examined for visible melanotic focii. Blood was collected by heart puncture and the lungs were excised.
Pulmonary metastasis was assayed (a) by counting metastatic focii on the surface of the lungs (b) histopathological analysis (2.2.21) (c) estimation of lung collagen hydroxyproline content (2.2.19) and (d) serum sialic acid content (2.2.20).

A similar set of experiment was performed with five groups (6 animals/group) of C57BL/6 mice for observing the survival rate (2.2.13). The mortality rate of animals was noted. The vital organs were examined for spontaneous metastasis at any other sites. The percentage increase in life span was calculated (2.2.11).

8.2.5 Statistical Analysis

All results are expressed as mean ± standard deviation. Statistical evaluation of the data was done using students t-test (214). The experiment was repeated twice.

8.3 RESULTS
8.3.1 Effect of antiinflammatory agents on the inhibition of lung metastasis

The effect of various compounds on lung metastasis of B16F-10 melanoma cells is given in Table 22. All the untreated tumour bearing animals had massive metastatic nodule formation. For practical purposes, lungs with massive tumour growth that did not permit enumeration of individual nodules were assigned a value of 250. No visible melanotic focii were observed in any other organs.

Only mefanamic acid treatment had a significant \( P < 0.001 \) reduction in the lung tumour nodule formation (56.4%). The other compounds had no effect on the lung tumour nodule formation.
1 x 10^6 B16F-10 melanoma cells per animal were injected through the lateral tail vein of animals. Simultaneously the compounds (200 μmoles/kg body weight/dose) were administered orally, for 10 alternate days. The animals were sacrificed on the 21st day and lung tumour nodules counted.

*P < 0.001, significance from untreated
8.3.2 Effect of antiinflammatory agents on the survival rate of metastatic tumour bearing animals

The rate of survival of metastatic tumour bearing animals treated with the test compounds is given in Table 23. Mefanamic acid treated animals survived 59.67 days with an increase in life span of 43.2% compared to that of control animals (41.67 days). Rest of the compounds did not increase the life span of tumour bearing animals.

8.3.3 Effect of antiinflammatory agents on the lung collagen hydroxyproline content

The effect of compounds on lung collagen hydroxyproline content is given in Table 24. The control tumour bearing animals showed a higher level of lung collagen hydroxyproline (10.48 μg/mg protein) compared to that of normal lung (0.98 μg/mg protein). The vehicle alone treated animals also had high levels of lung collagen hydroxyproline. Mefanamic acid treated animals had lower levels of lung collagen hydroxyproline (5.25 μg/mg protein). While Betamethasone, Phenylbutazone, Ibuprofen and paracetamol treated animals had lung collagen hydroxyproline content similar to that of untreated control animals.

8.3.4 Effect of antiinflammatory agents on the serum sialic acid level

The effect of antiinflammatory agents on the serum sialic acid level is given in Table 24. The control metastatic tumour bearing animals had higher serum sialic acid content (152.4 μg/ml serum) compared to the normal animals (23 μg/ml serum). Mefanamic acid treatment could lower the serum sialic acid content to 68 μg/ml. Other antiinflammatory agents
Table - 23

Effect of antiinflammatory agents on the rate of survival of metastatic tumour bearing animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average number of days survived ± S.D.</th>
<th>% ILS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41.67 ± 4.93</td>
<td></td>
</tr>
<tr>
<td>Mefanamic acid</td>
<td>59.67 ± 7.79</td>
<td>43.2*</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>42.3 ± 5.96</td>
<td>1.51</td>
</tr>
<tr>
<td>Betamethasone</td>
<td>45.0 ± 3.4</td>
<td>7.99</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>43.83 ± 4.2</td>
<td>5.18</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>40.3 ± 2.1</td>
<td>Nil</td>
</tr>
</tbody>
</table>

1 x 10^6 B16F-10 melanoma cells per animal were injected through the lateral tail vein of animals. Simultaneously the compounds (200 μmoles/kg body weight/dose) were administered orally, for 10 alternate days. The animals were observed for their survival.

* P < 0.001, significance from untreated
Table - 24

Effect of antiinflammatory agents on the serum sialic acid and lung hydroxyproline content of metastatic tumour bearing animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum sialic acid level (µg/ml serum) ± SD</th>
<th>Lung hydroxyproline content (µg/mg protein) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>23 ± 2.8</td>
<td>0.98 ± 0.41</td>
</tr>
<tr>
<td>Control</td>
<td>152.4 ± 5.46</td>
<td>10.48 ± 0.86</td>
</tr>
<tr>
<td>Mefanamic acid</td>
<td>68 ± 7.98*</td>
<td>5.25 ± 0.67*</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>148.6 ± 12</td>
<td>8.88 ± 0.9</td>
</tr>
<tr>
<td>Betamethasone</td>
<td>158.7 ± 7.1</td>
<td>10.16 ± 0.47</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>158 ± 7.5</td>
<td>9.27 ± 1.4</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>147.7 ± 2.5</td>
<td>10.17 ± 0.2</td>
</tr>
</tbody>
</table>

1 x 10⁶ B16F-10 melanoma cells per animal were injected through the lateral tail vein of animals. Simultaneously the compounds (200 µmoles/kg body weight/dose) were administered orally, for 10 alternate days. The animals were sacrificed on the 21st day and biochemical assays were carried out.

* P < 0.001 significance from untreated
did not have any effect in reducing the sialic acid levels in the serum of the metastatic tumour bearing animals.

8.3.5 Histopathological analysis

Pathological analysis of lung tissue of control tumour bearing animals showed infiltration of neoplastic cells and necrotic areas. Lung tissue from mefanamic acid treated group showed a reduction in the infiltrating neoplastic cells and tumour mass. Animals treated with other agents showed similar lung architecture as that of untreated tumour bearing animals.

8.4 DISCUSSION

In vivo and in vitro metastatic studies using polyphenolic compounds, showed that some of the compounds possessed antimetastatic activity. Among these compounds, curcumin and catechin possessed antiinflammatory property also. Therefore the antimetastatic activity of some clinically used antiinflammatory agents were analysed in this chapter.

Among these compounds studied only mefanamic acid could partially inhibit B16F-10 melanoma induced lung tumour nodules. The lung hydroxyproline and serum sialic acid levels were also lower in these animals when compared to the untreated metastatic tumour bearing animals. The other agents did not show any reduction either in the lung tumour nodules, lung hydroxyproline content or serum sialic acid levels.

From these results it could be concluded that the antimetastatic activity of the polyphenolic compounds was not due to their antiinflammatory property but by some other mechanisms.