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Evaluation of Mammary Carcinoma per se on Cognitive Function

9.1. Introduction
Most of the animal models exploring chemobrain potential of cytotoxic agents have utilized healthy male rodents and none of the studies have made an attempt to make use of cancer animal models which can most probably reflect the relevant animal model for studying the chemofog complications that are noticed clinically in cancer survivors.

As the chemobrain complications are highly reported in BC survivors, we particularly focused on breast cancer relevant animal models of cancer, i.e. mammary carcinoma in female rats. Hence we established the mammary cancer model in female rats in the previous chapter.

However, it was reported in earlier studies that, cancer by itself may have deleterious effects on behavioural dysfunction and in particular on cognitive deterioration (Mandelblatt et al., 2014). Cancer survivors may experience cognitive deterioration well before the chemotherapy, but once cancer diagnosis is made, this can be attributed to the emotional distress involved in being diagnosed of lethal cancer disease and moreover to the depressive stages experienced by cancer patients (Bergouignan et al., 2011).

To explore the precise chemobrain-inducing effects of chemotherapeutics, it is ideal to overrule this confounding influence, i.e. cancer per se effects on cognitive behaviour. Hence we have planned for a study which can explore the influence of mammary cancer per se on cognitive functioning in female mammary cancer bearing rats.

With the established protocols for mammary carcinoma induction as mentioned in previous chapters, we could also controlled the age factor as the cancer induction takes place within 2-3 months of carcinogen, i.e. NMU administration, i.e. at the age of 4 months.

9.2. Objectives

Primary objective
To evaluate the influence of mammary cancer per se on cognitive function for episodic and spatial memory components using ORT and MWM tasks.

Secondary objective
To explore whether locomotor and general activity is influenced by induction of mammary cancer.
9.3. Materials and Methods

9.3.1. Animals

Female SD rats separated after weaning period and aged 30-35 days weighing 50-80 g were procured from CARF of Manipal University, Manipal. All the experimental procedures were approved by IAEC (IAEC/KMC/17/2013).

9.3.2. Chemicals

N-nitroso, N-methyl urea (NMU) was acquired from Sigma Aldrich chemicals, USA and stored at -20° C until use. Acetic acid (Merck Chemicals, Mumbai) was used to maintain the acidic pH of 4.0. All other chemicals used in this study were of reagent grade.

9.3.3. Experimental design

Total of two groups (n=9 each) of animals were used in this study. Group 1 was normal healthy age controlled group whereas, Group 2 was tumor control (n=9 tumors). Both the groups were treated with normal saline once in every 5 days at a dose volume of 2 ml/kg, i.p. Following 10 cycles of saline administration, animals were subjected to behavioural analysis for episodic and spatial memory functional assessment.

9.3.4. Formulations and treatments

NMU was prepared and the procedure followed to make the carcinogen was similar to the method mentioned in chapter 8, page 76 and 77. Both the groups received normal saline at a dose volume of 2 ml/kg, by i.p. once in every 5 days up to 50 days.

9.3.5. Effect of mammary carcinoma on cognitive behaviour

9.3.5.1. Effect of mammary carcinoma on episodic memory in ORT

Animals were subjected to object recognition test following the intraperitoneal saline administrations. The procedure followed was same as that mentioned in chapter 4. We have used the established ITI of 2 h (Grandhi et al., 2016) as we used in chapter 4.

9.3.5.2. Effect of mammary carcinoma on spatial memory in MWM task

Following the completion of ORT task, rats were subjected to Morris water maze test which is widely used to assess the spatial working memory. The procedure followed was same as that mentioned in chapter 6. We used the protocol having 4 days of acquisition trials and one probe trial on day 5.

9.3.6. Effect of mammary carcinoma on locomotor activity using open field test

To assess the effect of mammary carcinoma on spontaneous locomotor activity, open field test was used. Distance travelled and mean speed was assessed using ANY-maze video tracking system. The procedure followed was same as that of chapter 4.
9.4. Results

9.4.1. Effect of mammary carcinoma on cognitive behaviour

9.4.1.1. Effect of mammary carcinoma on episodic memory in ORT

We found that, healthy control rats and tumor control animals have spent significantly more time exploring the novel object as compared to familiar object which indicates that, healthy and cancerous animals were able to remember the familiar objects equally at an ITI of 2 h. Further, we did not observe any significant difference in recognition or discriminative indices between healthy and tumor control groups (Fig. 9.1).

![Exploration time](image)

![Recognition index](image)

![Discriminative index](image)

Fig. 9.1. Effect of mammary carcinoma per se on episodic memory in ORT. Data represents mean ± SEM of (a) Exploration time of individual familiar or novel objects, ***p<0.001 vs. familiar object, (b) Recognition index and (c) Discriminative index (n=9).

9.4.1.2. Effect of mammary carcinoma on spatial memory in MWM task

During the acquisition trials, healthy and tumor control groups have performed equally well and were able to find the platform over the 4 days of acquisition training. No significant difference was noticed for acquisition measures, viz., latency to reach target, path length taken and also the swim speed. This revealed that, mammary cancer per se does not have deleterious effects on spatial acquisition memory (Table 9.1).
Further, during the retention or probe trial, no significant difference in Q4 latency or Q4 time was noted among the healthy and tumor control groups which indicates that, mammary carcinoma also does not influence spatial retention memory (Fig. 9.2).

Table 9.1. Effect of mammary carcinoma per se on spatial acquisition learning during MWM test.

<table>
<thead>
<tr>
<th>Day</th>
<th>Group</th>
<th>Target latency (s)</th>
<th>Path length (m)</th>
<th>Swim speed (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Healthy control</td>
<td>58.34 ± 1.66</td>
<td>7.61 ± 0.68</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Tumor control</td>
<td>56.84 ± 3.18</td>
<td>7.18 ± 0.62</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>Healthy control</td>
<td>50.80 ± 4.51</td>
<td>9.02 ± 0.93</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Tumor control</td>
<td>48.69 ± 4.32</td>
<td>8.67 ± 0.91</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>Healthy control</td>
<td>51.15 ± 4.34</td>
<td>8.97 ± 0.69</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Tumor control</td>
<td>49.33 ± 5.37</td>
<td>8.28 ± 0.93</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>4</td>
<td>Healthy control</td>
<td>42.66 ± 6.18</td>
<td>7.10 ± 0.99</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Tumor control</td>
<td>41.73 ± 4.96</td>
<td>6.81 ± 0.72</td>
<td>0.16 ± 0.01</td>
</tr>
</tbody>
</table>

Notes: Data represents mean ± SEM of escape/target latency, path length and swim speed (n=9).

Fig. 9.2. Effect of mammary carcinoma per se on spatial retention memory during probe trial of MWM test. Data represents mean ± SEM of (a) Time spent in target quadrant (Q4 Time) and (b) Time taken to first entry of the target quadrant (n=9).
**9.4.2. Effect of mammary carcinoma on locomotor activity using open field test**

Distance travelled, mean speed was found to be almost similar among the healthy age controlled and tumor control groups and no significant differences were noted. This indicates that, treatments or tumor growth does not influence the locomotor activity in these animals which would otherwise become a confounding influence during the assessment of cognitive functioning (Fig. 9.3).

![Graph showing distance travelled and mean velocity](image)

**Fig. 9.3. Effect of mammary tumor on locomotor activity. Data represents mean ± SEM of (a) Mean distance travelled and (b) Mean velocity using an open field task (n=9).**

**9.5. Discussion**

Earlier reports have shown that, cancer survivors can have deteriorated cognitive functioning before start of the chemotherapy well in advance. This is because of the emotional distress involved in being diagnosed with cancer, social stigma etc. Hence it may difficult to draw conclusion for the negative effect of chemotherapy *per se* on memory.

While developing a most relevant animal model for chemobrain complications, it is very essential to exclude this interfering factor, i.e. influence of mammary cancer *per se* on behavioural function. Hence in this study, we tried to explore the deleterious influence of mammary carcinoma on cognitive functioning for episodic and spatial memory components by using ORT and MWM tasks respectively. We have used two experimental groups of age matched healthy control group and tumor control groups. Normal saline was given by *i.p.* route, every 5 days up to 50 days following which behavioural testing was carried out.

We found no worsening effects of mammary carcinoma on neuro-cognitive functioning in female rats either in ORT or MWM tasks as we found insignificant differences for the cognitive parameters monitored for episodic and spatial memory evaluation.
Furthermore, it was found that, there was no difference in the locomotion measures, i.e., distance travelled or mean velocity. Hence, mammary carcinoma did not negatively influence the locomotor activity which further supports the validity of using cognitive functional tests, i.e. ORT and MWM tasks in these animals.

With the above experimental findings, one can confirm that, mammary cancer does not have deleterious effects on cognitive functioning, however there exists a possibility that, it could be the chemotherapy, a major differentiating factor which can produce negative impact on neurocognitive functioning, thereby the associated chemobrain complications.

Therefore, in the coming chapters, we have focused on assessing the effect of DOX chemotherapy on cognitive functioning in mammary carcinoma bearing rats to conclude the most suitable and relevant animal model for chemobrain complications.

**9.6. Conclusion**

Mammary cancer does not influence the neurocognitive function with negative impact, at least for episodic and spatial memory components. Hence, it would be the DOX chemotherapy which can most probably impact the cognitive function negatively in cancer survivors.
Bibliography

