Development of A Cellular Hepatocarcinoma Animal Model for the Evaluation of Pt(acac)₂/TiO₂ Nanoparticles Effect

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Abstract

Because of the worldwide increasing incidence of cancer new alternative therapies are needed. The use of nanotechnology for clinical applications is constantly growing. The objective of this work was to evaluate the effect of the administration of platinum inorganic nanoparticles over livers from Wistar rats. In order to reach this goal cellular Hepatocarcinoma was first induced with N-nitrosodiethylamine (NDEA) and Carbon tetrachloride (CCl₄) injections. Subsequently, Pt(acac)₂/TiO₂ nanoparticles prepared by the sol-gel method, were injected in the portal vein to assess its effects by means of an animal model. After treatment, animals were sacrificed and histopathological studies of the extracted organs (liver mainly) were carried out to determine the morphology of the cells and therefore the presence or absence of cancer. Earliest results confirm the presence of nanoparticles located in the liver but also in small intestine epithelium. We have determined that the effect of a route of systemic administration of the nanoparticles should be tested in later studies.

Keywords

Nanomedicine, Hepatocarcinoma, Titania nanoparticles, N-nitrosodiethylamine, Carbon tetrachloride, HCC
effect of nanoparticles of silicon and titanium oxides has been studied as a treatment to counteract or cure some kinds of cancer [6,7]. This new approach is known as nanomedicine and it has become a new alternative that consists in the synthesis of materials at nanometric level (manometer = 1×10⁻⁹) for medical applications [8-11]. This new technology has its origins in the nanotechnology which has had an important growth in the last years.

The objective of the present study was to investigate alterations in male Wistar rats liver by using N-nitrosodiethylamine (NDEA) and carbon tetrachloride (CCl₄) in order to examine both the development of hepatocellular carcinoma and the effect of platinum nanoparticles, we mainly focused on the incidence of hepatocarcinoma to further address the important roles of platinum nanoparticles in contributing to the anti-cancer therapies.

Materials and Methods

Nanoparticles synthesis

The Pt(acac)/TiO₂ nanoparticles were synthesized by the sol-gel method as described by T. Lopez et al. obtaining particles from 1 to 2 nm, which present micropores and mesopores on its surface [12].

Animal model

Wistar strain male rats with an average weight of 180-220 g were used to develop the HCC model. The rats were placed in aluminum cages, in an air conditioned room with periods of twelve hours light-darkness. They were allowed to freely consumption of food and water. Four groups of fifteen Wistar male rats were formed. Animals from each group were placed in aluminum cages, in an air conditioned room with periods of twelve hours light-darkness. They were allowed to freely consumption of food and water. Four groups of fifteen Wistar male rats were formed. Animals from each group were randomly numbered from 1 to 15. The rats from the one designated as control group, did not received any type of drug or reagent, leaving them with free evolution. Animals designated as group 1, were administered with an interperitoneal dosage of 200 mg/kg of NDEA (Sigma-Aldrich) on the first week, one week later, all fifteen animals received subcutaneous weekly injection of CCl₄, 3mg/kg (REASOL) during six weeks. Animals corresponding to group 2 were administered with three interperitoneal doses of 200mg/kg of NDEA on weeks 1, 3, and 5, but none CCl₄ dosage was administered. Group 3 animals received three interperitoneal injections of 200mg/kg of NDEA on weeks 1, 3, and 5, the animals also received an interperitoneal daily injection of 3mg/kg of CCl₄ for five days on weeks 2, 4, and 6 intercalating this administration between the weeks of NDEA, administration followed the same methodology as group 1, but in this case, CCl₄ was administered by an intraperitoneal route (Table 1) [13-16].

Ten weeks after the beginning of the treatment for the generation of HCC, animals were sacrificed and livers were extracted for its later histopathologic processing and analysis.

Surgery procedure for nanoparticles administration

Ten weeks after the experiment initiation the five rats from the control group that were not sacrificed were randomly chosen and were administrated with an injection of 200 mg of Pt(acac)/TiO₂ nanoparticles suspended in 200 microliters of isotonic sodium chloride solution by means of liver surgery.

All the equipment, instruments, surgery area as well as gauze, and gloves were sterilized before the procedure. During surgery, animals were anesthetized with an interperitoneal injection of a combined dose of 80 mg/kg of ketamine and 10 mg/kg of xylazine. Once anesthetized, the abdominal area of the animals was shaved and disinfected with povidone-iodine. Exploratory laparotomy was realized and Pt(acac)/TiO₂ nanoparticles were infiltrated by the portal vein with an intravenous catheter Radiopaque 16G × 51mm Punzocat® catheter (Figure 1).

Figure 1: Administration of nanoparticles.

Once the whole material was infiltrated, the catheter was removed and bleeding was controlled by applying pressure on the zone, after bleedings were controlled, the incision area was sutured layer by layer. The first layer (muscle) was sutured with anchor technique with 4-0 silk suture. The second layer (skin) with the stitch saturation technique with 4-0 silk suture, and they were supplied with ten units of gentamicin to avoid future infection. The temperature of the animals was monitored and controlled with warm blankets for a two-hour period after the surgery.

After two weeks of initiated the treatment, animals were sacrificed and the organs were extracted.

Histopathologic analysis

All the samples were processed and embedded in paraffin. Organs were sectioned with a steel knife mounted in a microtome to obtain 5-micrometer-thick tissue sections which were mounted on a microscope slide individually and then treated with Hematoxylin-Eosin (H&E) stain. This procedure was realized for all samples to prepare them for its later light microscopy analysis.

Results

Animal model

Significant differences in food and water consumption were observed, among the different groups during the whole study period. Corporal weights of the different study groups are presented in Table 2. A significant weight loss in the three study groups was observed in contrast to the control group.

For group 1 subcutaneous CCl₄ and intraperitoneal NDEA doses were suspended because the animals developed skin ulcers over the area where CCl₄ was administered, therefore they had to be sacrificed before concluding the induction period. For group 3, NDEA was intraperitoneally administered as for group 1, but CCl₄ administration route was also intraperitoneal in contrast to group 1, and any type of skin lesion was developed.

Table 1: Dosing scheme for NDEA and CCl₄.

<p>| Table 2: Summary of the mean weight. |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>172.9</td>
</tr>
<tr>
<td>Week 2</td>
<td>189.4</td>
</tr>
<tr>
<td>Week 3</td>
<td>209.5</td>
</tr>
<tr>
<td>Week 4</td>
<td>230.2</td>
</tr>
<tr>
<td>Week 5</td>
<td>235.5</td>
</tr>
<tr>
<td>Week 6</td>
<td>243.7</td>
</tr>
<tr>
<td>Week 7</td>
<td>253.1</td>
</tr>
<tr>
<td>Week 8</td>
<td>258.9</td>
</tr>
<tr>
<td>Week 9</td>
<td>264.1</td>
</tr>
<tr>
<td>Week 10</td>
<td>268.8</td>
</tr>
<tr>
<td>Control</td>
<td>172.9</td>
</tr>
<tr>
<td>1</td>
<td>189.4</td>
</tr>
<tr>
<td>2</td>
<td>195.5</td>
</tr>
<tr>
<td>3</td>
<td>204.9</td>
</tr>
</tbody>
</table>

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Page 2 of 5
The death rate of group 3 was very high, 73.33% of the animals died before concluding the induction of HCC. The death rate of group 2 was lower, as it is shown in Table 3. One of the probable causes of this differences in the death rate is CCl4 supply, because it might have produced a toxic effect in the liver and kidneys [17,18].

Table 3: Summary of the death rate.

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial No. of Animals</th>
<th>Final No. of Animals</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>15</td>
<td>0.00%</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>8</td>
<td>46.66%</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>8</td>
<td>46.66%</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>4</td>
<td>73.33%</td>
</tr>
</tbody>
</table>

Histopathologic analysis

Even though the animals from group 1 were sacrificed and several from group 3 died before concluding the induction period, livers were extracted and analyzed in order to compare the morphological changes observed in the three groups.

Histopathologic analysis showed that slides from rats in groups 2 and 3 did not present any evidence of neoplasia but they did presented some signs of inflammation in the hepatocytes and fusiform cells, in contrast with the rats in group 1 in which any evidence of neoplasia or hepatocytes inflammation was found (Figure 2).

The diagnosis of hepatocellular carcinoma is based on the confirmation of the histopathologic analysis of the extracted livers. The analysis showed a higher incidence in group 3 with an 80%, meanwhile in group 2 the incidence of hepatic neoplasia was 50% and there was any HCC case in group 1 (Figure 3).

Nanoparticles administration

The organs from five rats of the control group supplied with Pt(acac)2/TiO2 were analyzed by observation with the naked eye to ensure that the infiltration of the nanoparticles was successful. Yellowish areas in the liver were observed, so that confirmed the presence of nanoparticles in the liver (Figure 5). Due to this observation they were sent to further histopathologic investigation to have a microscopic validation of the nanoparticles localization.

Conclusion

Animal models are essential tools for medical research, in this particular case hepatocarcinoma, because they allow us to assess new treatments. The use of Wistar rats gives us a clear idea of how hepatocellular carcinoma is developed. In this work three proposed models were evaluated, and we have determined that model corresponding to group 2 is the most adequate for cancer induction, due to the high incidence of HCC and the low mortality percentage, which allow us to further evaluate the therapeutic effect of nanoparticles. Group 1 was discarded because subcutaneous injection of CCL4 provoked skin lesions and animals had to be sacrificed, animals from group 3 did not present any kind of reaction but the mortality percentage was too high so it was also discarded.

Preliminary results of Pt(acac)2/TiO2 nanoparticles administration showed evidence of the material allocated in the liver, confirming that...
this rout of administration can be used in later studies to evaluate the anticancer effect using the model previously.

References


