Targeting the Peritoneum with Novel Drug Delivery Systems in Peritoneal Carcinomatosis: A Review of the Literature

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Abstract. The Peritoneal cavity is a well-known metastatic site for several intra-abdominal malignancies, such as stomach, colon, pancreas and rectal cancer. For long, it was thought that treatment with curative intent was impossible but that was challenged by the introduction of cytoreductive surgery (CRS) and heated intraperitoneal chemotherapy (HIPEC). Although their effectiveness has been proven both experimentally and clinically, there is need for improvement. Firstly, a significant proportion of patients develop recurrent disease. Secondly, HIPEC demands presence of dedicated perfusion devices not readily available in most hospitals. Since intraperitoneal administration of chemotherapy is thought to play a crucial role, new modalities to deliver effective chemotherapeutics to the peritoneum are developed. The current review aims to present an overview of the experimental data on new drug delivery systems (DDS) in peritoneal cancer.

The peritoneal cavity is a well-known metastatic site for various malignancies. Intra-abdominal malignancies (stomach, colon, pancreas, rectum) have the highest probability to metastasize to the peritoneum but also relatively remote primary malignancies, such as lung and breast cancer occasionally cause peritoneal metastases. These metastases typically consist of small nodules (2-5 mm) scattered over the entire peritoneal surface. Tumor growth eventually results in bowel obstruction and the formation of massive amounts of ascites. This clinical entity is often referred to as peritoneal carcinomatosis (PC).

PC is known to have a poor outcome, with reported median survival rates depending on the primary tumor but typically in the range of only a few months if left untreated (1). For long it was thought that treatment with curative intent was impossible for these patients. This was challenged by the successful introduction of a multimodality treatment including cytoreductive surgery (CRS) followed by heated intraperitoneal chemotherapy (HIPEC) about two decades ago (2, 3). The rationale behind this treatment is surgical removal of all macroscopic tumors followed by sterilization of microscopic disease by exposing the peritoneal cavity to high levels of chemotherapeutic agents. As a result of the barrier between the peritoneal cavity and the blood stream, systemic toxicity is limited (4). The addition of heat is thought to increase the cytotoxicity of chemotherapeutics and to make malignant cells more vulnerable (5). The effectiveness of this combined treatment was proven both in the experimental and clinical setting (6, 7). Therefore, HIPEC is currently the treatment of choice for selected patients with PC of colorectal cancer and its effectiveness is currently investigated in other malignancies, such as gastric and ovarian cancer (8).

In spite of these achievements, there is need for improvement in the current application of intra-peritoneal chemotherapeutics. Firstly because a significant proportion of patients develop recurrent disease after CRS and HIPEC suggesting insufficient efficacy of the intra-peritoneal chemotherapy (9, 10). Secondly because the current practice
of performing CRS and HIPEC demands the presence of dedicated perfusion devices to heat and circulate the chemotherapy in the peritoneal cavity. These devices are not readily available in most hospitals and, as a result, it is estimated that only a minority of patients that may benefit from HIPEC is currently offered treatment.

Ideally, effective treatment for PC patients should be readily available whenever PC is diagnosed. Since the intraperitoneal administration of chemotherapy is thought to play a crucial role in the entire treatment, new modalities to deliver effective chemotherapeutics to the peritoneum are developed (11). These so called “Drug Delivery Systems” (DDS) do not rely on the presence of advanced machinery and may represent a potential solution for the treatment of many PC patients worldwide. The current review aims to present a synopsis of the experimental data on the DDSs in peritoneal cancer.

Materials and Methods

Inclusion/exclusion criteria. Studies concerning DDSs used for treatment of peritoneal carcinomatosis of gastro-intestinal origin were included. Furthermore, follow-up after treatment should be at least 24 hours. Exclusion criteria were as follows: Experiments using “traditional” intraperitoneal chemotherapy without the use of additional delivery systems and experiments without tumor inoculation.

Search. Searches were conducted in PubMed to identify studies describing animal models using DDSs for the treatment of PC. There was no restriction on language. Searches were carried out using Medical Subject Heading (MeSH) and free-text words. Search terms included were nanoparticles, micelles, microspheres and hydrogels. All references of relevant articles were hand-searched to identify further relevant studies.

Results

In total, 17 articles fulfilled the previously defined inclusion criteria (Tables I-III).

Animals and induction of peritoneal carcinomatosis. All experimental studies used mice as test animals, most commonly BALB/C mice (14/17). Nine out of seventeen groups used female mice only, 5/17 males and only 2/17 used both genders.

Experimental PC was most often induced by injecting murine colon adenocarcinoma CT26 cells intraperitoneally (8/17) but the amount of cells varied from $1 \times 10^5$ to $1 \times 10^6$. In two models, CT26 cells were combined with Luc cells. After injection, cancer cells were typically allowed to grow 4 to 10 days. In Hyoudou et al.’s set up, the intervention preceded the application of cancer cell (Table I).

Five groups modeled PC by using gastric cancer cells (gPC); either MKN45P gastric cancer (2-3 $\times 10^6$), TMK1 human gastric cancer (1 $\times 10^7$), or HSC44Luc human gastric cancer (1 $\times 10^6$) were used, all injected intraperitoneally and additionally subcutaneously (1 $\times 10^6$) in two articles. Inoculation time was 3 (HSC33Luc) or 7 days (TMK1 and MKN45P group) (Table II).

Four experiments were performed using different tumor lines. Bajaj et al. and Vassileva et al. used SKOV-3 ovarian cancer (1 $\times 10^7$, i.p.) with an inoculation time of 14 and 7 days respectively (12, 13). Reddy et al. injected Dalton lymphoma cells intraperitoneally in Balb/c mice and waited 6 days prior to drug application (14). Hagiwara used B-16 PC melanoma (1 $\times 10^6$) on BDF1 male mice injected intraperitoneally to mimic PC and applied the DDS after 4 days of inoculation (15) (Table III).

DDS treatment and results. Various DDSs were investigated in the reviewed experiments. The majority used a variant of a hydrogel system to deliver chemotherapy into the peritoneal cavity, some in combination with microspheres and three with microspheres only. Three articles used nanoparticles, one used beads and one liposomes as carriers for different treatment regimens. Chemotherapeutics that were administered were camptothecine, 5-fluorouracil (5-FU), doxorubicin, mitoxantrone, paclitaxel, docetaxel, cisplatin and etoposide. Other compounds used were ECatelse and $^{111}$In-labeled vinorelbine.

Colorectal peritoneal carcinomatosis models. Fan et al. treated colorectal peritoneal carcinomatosis with docetaxel PLLA-L121-PLLA porous microspheres, docetaxel only, microspheres (MS) only or no treatment (16). The mice treated with both MS and docetaxel had an increased median survival compared to the other groups. Moreover, docetaxel with microspheres significantly induced tumor cell apoptosis, inhibition of tumor angiogenesis and suppression of tumor cell proliferation.

Liu et al. used a biodegradable DDS consisting of camptothecine (CPT)-loaded polymeric microspheres in a thermosensitive poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) (PCEC) hydrogel (17). They reported on differences in survival between mice treated with CPT+MS+hydrogel, CPT-MS, free CPT, blank MS/hydrogel and a “no therapy group”. Additionally, a group was used to determine the number and weight of tumor nodules 20 days after application. Combining chemotherapy with microspheres and hydrogel increased survival significantly (50 days vs. 38, 33, 24 and 23 days in previously mentioned groups, respectively) and showed a dramatically decreased number and weight of tumor nodules indicating that not only growth was inhibited but also the process of metastasizing itself was inhibited.

Gunji et al. performed in vitro and in vivo experiments using gelatin microspheres loaded with cisplatin (18).
<table>
<thead>
<tr>
<th>Author</th>
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<th>Tumor line</th>
<th>Treatment</th>
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<td></td>
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<tr>
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<td>Gunji (18)</td>
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<td>Keese (21)</td>
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</tr>
<tr>
<td>Emoto (28)</td>
<td>2012</td>
<td>Female</td>
<td>MKN45P gastric cancer</td>
<td>Paclitaxel 20 μg/g body weight</td>
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<td>7, 14 and 21</td>
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<td>Decreased number and weight of tumors, enhanced penetration in nodules and longer retention in circulation</td>
</tr>
<tr>
<td>Soma (29)</td>
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<td>Paclitaxel 20 μg/g body weight</td>
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<td>7, 14 and 21</td>
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Among the two in vivo PC models, the first experiment showed that combining microspheres with cisplatin could significantly reduce tumor weight after 10 days. The second experiment showed a prolonged survival of the aforementioned combination compared to free cisplatin (74 vs. 23 days, \( p < 0.05 \)), microspheres only or no treatment. Also, cisplatin-loaded microspheres were associated with less nephro- and hematotoxicity.

One group performed a survival experiment in mice administrating micelles containing paclitaxel (PTX) with FU-loaded thermosensitive hydrogel (19). Besides showing an inhibition of tumor growth and metastasis, they showed the possibility to simultaneously administer hydrophilic and hydrophobic drugs. Additionally, an increased survival was observed when combining PTX and FU with a hydrogel compared to free drugs.

Wang et al. used 5-FU loaded into a biodegradable temperature sensitive PEG-PCL-PEG triblock copolymer hydrogel (20). Compared to the no-treatment, hydrogel only or 5-FU only group, the number and size of tumor nodes were significantly decreased after 20 days. Interestingly, 42.5% of mice treated with 5-FU+hydrogel were tumor-free at autopsy. Moreover, they showed that by using hydrogel, the release and uptake of methylene blue was increased, thereby proving that the application also improved the permeability of the peritoneum without affecting body weight, behavior or feeding. However, leukocytes were higher in the 5-FU-hydrogel group compared to the 5-FU group, possibly due to hematological toxicity.

Only one group used doxorubicin as a chemotherapeutic to treat colorectal PC. Keese et al. combined doxorubicin with mitoxantrone delivered through drug eluding beads (21). Administrating doxorubicin three times was lethal to all mice, whereas using beads was well-tolerated by the animals. Moreover, an effective tumor volume reduction was seen compared to the control group.

Two groups experimented with alternative methods for chemotherapy to prevent peritoneal spread from occurring or progressing. Lin et al. experimented with 111In-labeled VNB-PEGylated liposomes in a colorectal cancer ascites model (22). Vinorelbine (VNB) is known to have an inhibitory effect on cell proliferation through disruption of microtubules. They combined this treatment with nanoparticles with the rational in mind that they have enhanced permeability and retention in neoplastic lesions. They achieved higher areas under the curve (AUCs) in ascites and tumor in the intraperitoneally administrated group compared to the intravenous group. Finally, Hyoudu et al.’s group combined ethylenediamine-conjugated catalase (ED-catalase), which has previously shown to inhibit cancer recurrence through tumor cell adhesion inhibition (23) with a biodegradable hydrogel of acidic gelatin. By first injecting saline, ED-catalase only or ED-catalase-hydrogel followed by administration of CT26, they proved the latter to have a superior effect in inhibiting tumor adhesion after 21 days and a significantly increased survival. A possible role for this type of treatment could be administration after surgical removal of GI cancers, thus preventing any peritoneal spread.

Gastric peritoneal carcinomatosis models. Five articles used a gastric cancer cell line to induce PC. Emoto et al. used cisplatin in a cross-linkable hyaluronic acid (HA)-based hydrogel (24). Instead of only applying the gel once, they chose to administer it on day 7, 14 and 21 after inoculation. After 28 days, combining gel with cisplatin significantly decreased peritoneal nodule weight. Since there is evidence of renal toxicity due to prolonged exposure to cisplatin (25), renal function was measured showing no statistical difference between cisplatin + hydrogel or gel alone.

Bae et al. used a thermosensitive hydrogel with conjugated linoleic acid coupled pluronic F-127 (Plu-CLA)
to achieve controlled release of docetaxel (26). After 28 days, F-fluorodeoxyglucose (FDG) uptake was significantly lower. After sacrifice, tumor nodule growth was inhibited and the number of nodules was significantly reduced. Also, mean peritoneal fluid volume (ascites) was less, although this did not reach statistical significance. Median survival was only increased in the docetaxel + hydrogel group.

In the experiment performed by Yu et al., a thermosensitive polymeric hydrogel was loaded with paclitaxel and injected into the peritoneal cavity three days after inoculation (27). Tumor activity was decreased directly in the first two weeks by measuring luciferase activity in the HSC44Luc cells. Side effects were only seen in the group receiving paclitaxel.

In a previous animal experiment by Emoto et al., PC was treated with paclitaxel in micellar nanoparticles (28). The nanoparticles were applied both 7 and 14 days after inoculation and all animals were sacrificed after 19 days. The paclitaxel nanoparticles induced a significant decrease in both number and weight of tumor nodules. Moreover, penetration into the nodules was enhanced and longer retention of paclitaxel was detected in the systemic circulation compared to paclitaxel in cremophor.

In the last gastric model, Soma et al. used paclitaxel in a water-soluble polymer (30W) (29). Treatment was administered three times, after 7, 14 and 21 days. Animals sacrificed at 28 days had a significant growth inhibition of tumor nodules. Moreover, the concentration of paclitaxel in the remaining nodules was higher. In a second experiment, overall survival increased compared to paclitaxel in cremophor, 30W only or cremophor only.

**Alternative models.** The first model describes paclitaxel delivery in an ovarian cancer-induced PC in female mice (12). Using a hyaluronic acid-based hydrogel, intraperitoneal paclitaxel concentrations remained higher 14 days after treatment. However, no significant tumor reduction was found compared to free paclitaxel, Taxol or Taxol gel, possibly due to the limited dissolution of PTX. Therefore, it was postulated that, besides spatial availability, temporal availability is important to elicit an effect.

Vassileva et al. implanted paclitaxel-loaded nanoparticles in mice inoculated with intraperitoneal ovarian cancer (13). Mice that were treated with these nanoparticles on day 7 after inoculation had a complete tumor inhibition. Eighty-five % of cells were found to be apoptotic. Moreover, their newly-developed DDS allowed a higher paclitaxel dosage with no observable toxicities.

Reddy et al. used etoposide in a mouse model in which PC was induced using intraperitoneally administered Dalton’s lymphoma cells (14). Incorporation of etoposide, a topo-isomerase inhibitor, in a solid lipid nanoparticle had a significant effect on the cell cycle, cytogenetic damage and survival compared to etoposide or nanoparticles alone.

Finally, Hagiwara et al. experimented using microspheres loaded with 5-FU (15). Being one of the first experimenting with new drug delivery systems in PC, they showed that slow release creates a maintained 5-FU concentration for a longer period of time with less systemic toxicity compared to 5-FU only. By using microspheres, the anti-cancer activity of 5-FU was prolonged and increased.

**Discussion**

This manuscript summarizes animal models for intraperitoneal chemotherapy application by drug delivery systems. In the last 2 decades, focus of experimental research in PC has been to study animals with different intraperitoneal chemotherapy regimens with different types of traditional chemotherapeutics and varying duration and temperature, with or without surgical intervention. The major disadvantage of traditional chemotherapy is the high interstitial pressure in solid tumors preventing drugs from penetrating into the tumor (30, 31). In 1986, Matsumara et al. reported that the usage of nanodrugs could increase the enhanced permeability and retention (EPR) effect by making use of tumor tissue characteristics, such as immature lymphatic drainage, incomplete vascular structures and hyper-permeability of tumor vessel walls (32). However, little attention was given to these particular types of treatment modalities until the late nineties. In the mean time, CRS and HIPEC has been introduced into clinical practice either during surgery (HIPEC) or directly after surgical intervention for several days (EPIC).

Although treatment protocols and patient selection is not standardized globally (33), CRS and intraperitoneal chemotherapy have currently established an important role in selected patients with peritoneal surface malignancies (34-36), providing the only potentially curative option in this, otherwise, fatal disease. Unfortunately, only a small proportion of patients diagnosed with PC actually benefit from this treatment and up to 60% of patients treated will have recurrent local or distant disease (10). Therefore, there is an urgent need to improve the treatment of peritoneal cancer even further.

Recently, new modalities have been evaluated as potential targeted DDSs. In experimental settings, nanoparticles, micelles, microspheres and hydrogels have been used as carriers in the treatment of peritoneal metastasis of gastrointestinal origin to improve solubility, exposure time and reducing toxicity of chemotherapeutic (37, 38). Several authors report promising results using different chemotherapeutics to substantiate tumor growth inhibition and increased survival in animal models. The rationale behind the additional effect of a carrier includes an increase in intraperitoneal chemotherapy concentration for a longer duration of time without additional systemic toxicity (39). Moreover, a greater intratumoral

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exposure, due to increased extravasation from vessels to the interstitium of tumors, is beneficial for the effect of nanodrugs (32, 40). These phenomena resulted in a decrease in number of tumor nodules and tumor weight. Moreover, the incorporation of naturally-occurring proteins and cell recognizable ligands in gels provides a more bio-mimicking environment for sustained release and could also stabilize bioactivity of the surrounding tissues (41).

Several authors in this overview showed an increased survival using DDSs loaded with chemotherapy compared to chemotherapy-alone. Other implications postulated in the literature are the prevention of peritoneal spread after resection of primary gastrointestinal tumors or application of DDSs in the treatment of malignant ascites (42, 43). Additionally, using DDSs might prove to have practical advantages over traditional IPC. DDS application does not require any additional machinery and is less time-consuming since no perfusion or heating is required. Therefore, in the event that PC is diagnosed during an intervention in a non-HIPEC center, a DDS loaded with chemotherapy could directly be applied.

Due to the limited amount of experiments performed on this subject, a large heterogeneity exists regarding choice of treatment and type of DDS. Little rationale is given to support choice of chemotherapy and the used concentrations, despite the fact that this has been shown to be of the utmost importance in treating PC (44). Another major difference with regular HIPEC treatment is that heat was not applied in the current experiments. The application of heat is believed to further enhance the effectiveness of chemotherapeutics. Although in vitro experiments have proven that heat increases tissue penetration, Klaver et al. showed that the addition of heat itself does not result in a longer survival in an in vivo rat model (45). Furthermore, no experiments in this review used any surgical techniques to treat PC. In contrast, up to 25% of animal models for standard intraperitoneal chemotherapy involved CRS (46), believed to be a necessity for curative intent in PC (47). Although implementing surgery can complicate an experiment and provoke unwanted effects (8), a well-constructed experiment combining the promising results of alternative DDSs with CRS might provide new insights for further development of treatment of PC. This would also more closely mimic current clinical practice in which surgery is already implemented worldwide. However, surgical procedures in mice are difficult due to their size and, therefore, most surgical animal studies are performed on rats inoculated with CC-531 syngeneic colorectal carcinoma cells (46). No experiments to date use alternative DDSs to treat this specific cell line.

**Conclusion**

This overview summarizes alternative ways to apply IPC in peritoneally-disseminated cancer. Promising features, such as tumor growth inhibition, delayed metastasis and increased survival reveal that drug delivery systems, such as microspheres and/or hydrogels loaded with chemotherapeutics, could potentially be a new treatment option to improve oncologic outcomes in this disease entity. Moreover, DDS treatment has potential practical advantages, such as being less time consuming and not requiring any additional machinery. Further experiments are required to evaluate their true clinical potential.

**References**


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