Local ablative therapies for colorectal liver metastases and the immune system

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Summary

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Colorectal carcinoma is one of the most frequently occurring cancers in the Western world. Its prognosis is poor, especially when disease has metastasized. As metastases, mainly to the liver, occur eventually in over 50% of patients, the need for improved or new therapeutic modalities is high.

When metastases are confined to the liver, resection of metastases is currently the treatment of choice. Unfortunately, only 20–30% of patients with liver metastases are eligible for this treatment. In recent years, local ablative therapies have emerged that may offer palliation or even curation to this group of patients. These therapies are based on local tumour destruction by focal generation of heat, cold or oxygen radicals. In radiofrequency ablation (RFA), electrodes inserted in the tumour generate high temperatures, resulting in local tissue destruction. For cryotherapy, probes are inserted in the tumour through which liquid nitrogen circulates, resulting in local freezing of the tissue. By repeatedly freezing and thawing the tissue, the tumour is destructed. Both these local ablative therapies are extensively used in present day clinical practice, with varying results. Local recurrence after inadequate tumour treatment seems to be the main limiting factor in efficacy of these treatments.

A more experimental local ablative therapy is photodynamic therapy (PDT). In this method a chemical substance, the photosensitiser, is administered intravenously and more or less selectively localises in tumour tissue. After a certain interval, optical fibres are inserted percutaneously directly in the liver tumour. Through these fibres, laser light is delivered to the tumour, which activates the photosensitiser. This activation of the photosensitiser is associated with generation of unstable radical oxygen molecules that eventually result in local tissue destruction.

Another treatment option for patients with colorectal liver metastases that are confined to the liver is the application of regional chemotherapy by hepatic artery infusion of cytostatics (HAI) or isolated hepatic perfusion (IHP) with cytostatics. In HAI, chemotherapeutic drugs are administered not systemically, but directly into the hepatic artery. This results in higher liver tumour exposure to the cytostatic drug and lower systemic toxicity. For IHP, circulation of the liver is completely isolated from systemic circulation, and high doses of a chemotherapeutic drug (melphalan is most often used in recent clinical trials) are perfused through the liver for a certain time period (perfusion duration is one hour in most trials). With both HAI and IHP, high local drug doses can be achieved with minimal or no systemic toxicity.

Apart from these locoregional therapies, new systemic approaches include the application of immunotherapy. Clinical trials with tumour vaccination for melanoma have proven to be reasonably effective, but for colorectal cancer results are less encouraging. Experimental studies show that although tumour

specific T cells or antibodies may be generated upon immunotherapy, these are not yet effective against established tumours. Apparently, tumours are equipped with defence mechanisms preventing the immune system from exerting antitumour effects.

In **Chapter 2**, we investigated the limitations of immunotherapy in our CC531 rat colorectal liver metastases model. Wag/Rij rats were inoculated with four liver tumours, by subcapsular injection of CC531 tumour cells. Control rats did not receive liver tumours. Two weeks later, half of the rats from both groups received CC531 tumour cells intravenously (i.v.). Three weeks after this intravenous tumour cell administration, weight of liver tumours and number of lung metastases were assessed. Results showed that rats with pre-existing liver tumours before i.v. tumour cell administration did not develop any lung tumours, whereas rats without liver tumours developed numerous lung tumours. Apparently, rats had developed an immune response following inoculation of liver tumours that enabled them to destroy the circulating tumour cells after i.v. administration. Contrary to this effect on circulating tumour cells, we observed no growth inhibiting effects on the established liver tumours, as weight of liver tumours in rats that received i.v. tumour cells was equal to liver tumour weight in control rats that had not received i.v. tumour cells. We concluded that if a systemic immune response is induced, it might be able to recognize and destruct circulating tumour cells, but not tumour cells that are settled in tissue and have formed tumour nodules.

We hypothesized that these tumour nodules are surrounded by an extracellular matrix, which structure prevents immune effector cells from recognizing tumour associated antigens (TAAs) and, consequently, from destroying the tumour cells. A unique feature of locoregional therapies for liver tumours is that treated tumours remain in situ after treatment and are not removed from the liver. The local destruction of tumour tissue will destroy the protective structure of the tumour nodule and as treated lesions remain in situ, this could lead to increased exposition of TAAs on (parts of) destructed tumour cells to cells of the immune system. Possibly, this results in generation or enhancement of a systemic tumour specific immune response.

In **Chapter 3**, we investigated the effect of PDT of experimental liver tumours on the immune response. Rats were provided with three CC531 liver tumours, as described above. One of these tumours was then treated with PDT and effect on growth of both treated and untreated tumours was evaluated. In addition, immunohistochemical staining of tumours was performed, to identify T cells, NK cells and macrophages in tumours and surrounding liver tissue. These results were in line with those described in chapter 1 and fitted in our hypothesis, as we observed that PDT of one tumour did not affect growth of nearby, established liver tumours. In these untreated tumours we did not see increased amounts of immune effector cells, indicating

that there was no recognition of TAAs by cells of the immune system. We concluded that PDT of one liver tumour did not affect growth of established tumours via systemic immune response. Possibly, a systemic immune response was present but could not recognize TAAs in the established tumour nodule, whereas it may be effective against circulating tumour cells.

To further clarify the effects of locoregional therapies on the immune system another in vivo experiment was performed with RFA, PDT and HAI **(Chapter 4)**. Again, rats were provided with CC531 liver tumours, two tumours in each rat. One of these tumours was treated with RFA or PDT, or liver of rats was treated with HAI with the cytostatic drug melphalan. Liver tumour bearing control rats did not receive any treatment. Twelve days after tumour treatment, both treated and control rats were (re)challenged with CC531 tumour cells either locally, by renewed subcapsular tumour cell administration at a third location in the liver, or systemically, by administration of tumour cells in the femoral vein. Two weeks after this (re)challenge, growth of treated and untreated initial liver tumours was assessed, as well as outgrowth of a third liver tumour following local rechallenge or outgrowth of lung tumours following systemic rechallenge. Results indicated that an adequate systemic immune response was induced by RFA, PDT and HAI, as in these rats no outgrowth of a third liver tumour was observed, whereas control rats with untreated liver tumours all developed a third liver tumour upon (re)challenge. Similarly, rats from treatment groups did not develop any lung metastases whereas rats in control groups did. We concluded that a systemic tumour specific immune response was induced by locoregional therapies, but that this response again was not able to affect established tumour nodules.

Having now shown the presence of an immune response in experimental studies, we wanted to evaluate the situation in patients that were treated with locoregional therapies as well **(Chapter 5)**. As we could not copy the experimental study, we used another parameter for the presence of a systemic immune response, namely the presence of antibodies directed against a wide range of TAAs. According to our hypothesis, the presence of antibodies would possibly increase following locoregional therapy of liver metastases but would not increase when liver tumours were surgically removed. We collected sera from patients with liver metastases from colorectal carcinoma that were treated with RFA, PDT, hepatic resection or isolated hepatic perfusion. Sera were taken directly before and within 4 weeks after the procedure. All sera were then analyzed for the presence of antibodies directed against TAAs in 6 different human coloncarcinoma cell lines, as these 6 cell lines presented a wide array of TAAs that would partly also be present in the in vivo situation of the different individuals. We found that the presence of antibodies directed against human

coloncarcinoma TAAs decreased after hepatic resection of liver tumours, whereas antibodies increased following IHP or, to a lesser extent, RFA of liver tumours. In patients treated with PDT there was also a slight increase in antibodies but this was not significant. These findings are only circumstantial evidence for the induction of a systemic immune response but combined with the results of our and other experimental studies, we suggest that locoregional therapies do have a positive effect on the systemic antitumour immune response. Such an effect would offer new clues for further development of these therapies that may enhance their clinical efficacy.

In **Chapter 6**, we report the results of a phase I clinical trial concerning PDT of colorectal liver metastases. Twenty-four patients were treated with percutaneous PDT and safety, technical feasibility and antitumour efficacy were assessed. PDT proved to be a safe procedure, with no mortality and only two serious complications reported, that could be avoided in future trials by minor treatment adjustments. Occurrence of skin phototoxicity however did occur in three patients, who suffered mild skin reactions resembling sunburn. Transient hyperpigmentation along the trajectory of the injection vein was seen in 4 patients and in two patients, face and hands were hyperpigmented following sunlight exposure. Total tumour necrosis was achieved in all but one patient, and at 1 month after PDT, 27 tumours were stable and 4 showed progression. PDT can therefore be considered a safe and useful new treatment modality for liver metastases.

A technique that is already widely used in a clinical setting is RFA of liver metastases. In **Chapters 7 and 8**, the experience with this technique in the Netherlands is described. **Chapter 7** reports on the safety of this treatment and describes complications in 122 patients following RFA treatment of liver tumours. RFA is concluded to be a less safe procedure than expected, with RFA related morbidity of 9.8% and major complications occurring in 10 patients. We therefore propose that RFA procedures should performed in specialized centres by a multidisciplinary team. In **Chapter 8**, local success rates of RFA treatment of 199 colorectal liver metastases in 87 patients are discussed. They show that although RFA is now widely used in patients that do not qualify for hepatic resection, its results are disappointing in terms of local disease control, as our study showed local failure of RFA treatment in 47.2% of treated lesions. To further identify factors influencing this high rate of local treatment failure, we performed multivariate statistical analysis and reviewed local failure rates in other clinical RFA trials. We concluded that tumour size and tumour location are the most important factors determining the risk of local failure following RFA.

In **Chapter 9** all aforementioned results and future perspectives of local ablative therapies and their possible interactions with immunotherapy are discussed. We conclude that local ablative therapies are a valuable addition to the therapeutic treatment possibilities for colorectal liver metastases, whose value may be further increased in the near future by enhancing their stimulating effects on the immune system.