Isolated Regional Perfusion for Metastases of Malignant Melanoma
Clinical and Experimental studies

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs universitet kommer att offentligen försvaras i Hörsal Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg, fredagen den 15:e November 2013, kl 13.00

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Avhandlingen baseras på följande delarbeten:

I. **Olofsson R**, Mattsson J, Lindnér P. *Long-term follow-up of 163 consecutive patients treated with isolated limb perfusion for in-transit metastases of malignant melanoma.* Int J Hyperthermia. 2013 Sep;29(6):551-7

II. **Olofsson R**, Cahlin C, All-Ericsson C, Hashimi F, Mattsson J, Rizell M, Lindnér P. *Isolated Hepatic Perfusion for Ocular Melanoma Metastasis - Registry Data Suggests a Survival Benefit.* Ann Surg Onc. In press. DOI:10.1245/s10434-013-3304-z.

III. **Olofsson R**, Lindberg E, Karlsson-Parra A, Lindnér P, Mattsson J, Andersson B. *Melan-A specific CD8+ T lymphocytes after hyperthermic isolated limb perfusion: A pilot study in patients with in-transit metastases of malignant melanoma.* Int J Hyperthermia. 2013 May;29(3):234-8

IV. Eldh M[*], **Olofsson R**[*], Lässer C, Svanvik J, Sjöstrand M, Mattsson J, Lindnér P, Choi DS, Gho YS, Lötvall J. *MicroRNA in exosomes isolated from the liver circulation in patients with metastatic uveal melanoma. In manuscript*.
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ABSTRACT

Isolated Regional Perfusion for Metastases of Malignant Melanoma - Clinical and Experimental studies

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Background Isolated regional perfusion is a treatment option mainly for localized metastatic disease. The principle idea is to surgically isolate a region of the body and connect the circulation to a heart-lung machine. A high concentration of a chemotherapeutic agent is then delivered to the tumour, while systemic toxicity is avoided. The aim of this thesis was to evaluate clinical outcome of isolated regional perfusion for extremity and liver metastases of malignant melanoma, investigate associated immunological mechanisms, and to explore the potential use of tumour-derived exosomes as future biomarkers.

Methods Clinical outcome was analysed by retrospective studies of patient medical records and by data from the national patient registers. Tumour specific T-cells were studied by flow cytometry analyses before and after perfusion. Exosomes were isolated from liver perfusate by ultra-centrifugation and thereafter characterized by electron microscopy, flow cytometry and real-time PCR of the RNA content.

Results Between 1984 and 2008, 163 patients with melanoma in-transit metastases underwent isolated limb perfusion (ILP). The overall response rate was 85%, with 65% of the patients having a complete response. Local progression occurred in 63% of the patients after a median time of 16 months. Predictive factors for response were mainly attributed to tumour burden. Thirty-four patients, with uveal melanoma liver metastases, underwent isolated hepatic perfusion (IHP). The overall response rate was 68%. There was a significant median overall survival advantage of 14 months ($p=0.029$) compared with the longest surviving patients in Sweden during the same time period. Immunological factors were studied in twelve patients after ILP, and the results showed a significant elevation of Melan-A+ CD8+ T-cells after four weeks in 30% of the patients. Exosomes were isolated from the liver perfusate and were shown to be Melan-A positive with a miRNA profile associated with melanoma.

Conclusion ILP is a surgical method with a high response rate for the palliative treatment of patients with in-transit metastases of melanoma. IHP is a treatment option with a high response rate, and with a potential survival benefit of more than one year. A small increase in Melan-A specific T-cells is induced after ILP, however the clinical significance needs to be further assessed. Tumour-derived exosomes can be isolated from liver perfusate during IHP. The miRNA characteristics of these exosomes could be a potential source for future biomarkers.

Keywords: Malignant Melanoma; Uveal Melanoma; Isolated Limb Perfusion; Isolated Hepatic Perfusion; Immunology; Exosomes

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