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Advanced renal cell carcinoma - The role of orellanine and associated therapeutic challenges

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i hörsal Arvid Carlsson, Academicum, Medicinaregatan 3, fredagen den 25 januari 2019, klockan 09:00

av Deman Hadi Najar

Fakultetsopponent: Professor Mårten Segelmark.
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Avhandlingen baseras på följande delarbeten

I. Deman Najar, Börje Haraldsson, Magnus Braide, Kerstin Ebefors, and Jenny Nyström. Chronic peritoneal dialysis in uremic, anuric rats. *Manuscript*

II. Deman Najar, Börje Haraldsson, Annika Thorsell, Carina Sihlbom, Jenny Nyström, and Kerstin Ebefors. Pharmacokinetic properties of the nephrotoxin orellanine in rats. *Toxins.* 2018 Aug 17;10(8). doi: 10.3390/toxins10080333

III. Lisa Buvall, Heidi Hedman, Alina Khramova, Deman Najar, Lovisa Bergwall, Kerstin Ebefors, Carina Sihlbom, Sven Lundstam, Anders Herrmann, Hanna Wallentin, Emelie Roos, Ulf A. Nilsson, Martin Johansson, Jan Törnell, Börje Haraldsson, and Jenny Nyström. Orellanine specifically targets renal clear cell carcinoma. *Oncotarget.* 2017; Jul 25;8(53):91085-91098 doi:10.18632/oncotarget.19555

SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR NEUROVETENSKAP OCH FYSIOLOGI

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Advanced renal cell carcinoma - The role of orellanine and associated therapeutic challenges

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Abstract

Orellanine is a fungal nephrotoxin selectively toxic to the human tubular epithelial cells (HTEC) of the kidney nephrons leading to kidney failure. Patients treated with renal replacement therapy after orellanine poisoning show no signs of damage to other organs in the body.

Aims: Our main aim in this thesis is to develop chronic peritoneal dialysis (PD) in anuric rodents, to better understand the pharmacokinetic properties of orellanine and to evaluate orellanine as an experimental treatment against metastasized clear cell renal cell carcinoma (ccRCC).

Methods: The first paper is an *in vivo* study of chronic automated PD in anuric rats. Orellanine was used to induce uremia. Blood, dialysis fluid, and tissue samples were examined for electrolyte profiles, inflammatory status, and morphology. The second paper is an *in vivo* study in which rats were given intravenous injections of labeled/unlabeled orellanine. The distribution of orellanine was imaged, and orellanine plasma concentrations were measured over different time points. The third paper had two parts: an *in vitro* part examining the effect of orellanine on HTEC, epithelial cells, ccRCC cells, and other cancer cell lines, and an *in vivo* part with a xenograft rat model testing the effect of orellanine on metastasized ccRCC tumors.

Results: The levels of urea and creatinine in orellanine-treated rats indicated severe uremia. The automated PD system developed in our lab provided adequate dialysis. The rats gained weight and had normal homeostasis. Orellanine was cleared renally and was mainly distributed to the renal cortex and the urinary bladder. Orellanine induced necrosis, apoptosis, and disruption of cellular functions and growth on HTEC and ccRCC cells while having no significant effect on other tested cell lines at the same doses. Finally, orellanine induced significant apoptosis and necrosis in the xenografted tumors *in vivo*.

Conclusions: Orellanine selectively causes renal failure, which is irreversible at high doses. We describe the first successful treatment of rats with severe uremia that, despite anuria, were kept healthy over a period of at least 21 days. The system can be used to improve PD and to study various aspects of uremia. The pharmacokinetic properties of orellanine were investigated and it was shown that orellanine is distributed mainly to the urinary system. Orellanine induced significant apoptosis and necrosis in metastasized xenografted tumors *in vivo* and showed no signs of affecting other organs. Therefore, we suggest that its therapeutic effects should be further examined as a treatment option for late stage ccRCC patients.

Keywords: orellanine, fungal toxin, nephrotoxin, peritoneal dialysis, ESRD, renal carcinoma, ccRCC, cancer therapy, radioiluminography, ^{3}H-labeled orellanine

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