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Effect of sunitinib treatment on skin sodium accumulation in patients with renal cancer: a pilot study

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Recent evidence suggests that skin could play a crucial role in sodium homeostasis and therefore in blood pressure regulation. This buffering mechanism for salt in the skin seems to be driven by immune cell-derived lymphatic growth factor VEGF-C (vascular endothelial growth factor-C); here VEGF-C activates the VEGF type 3 receptors (VEGFR3) on the lymphatic capillary network leading to hyperplasia and hypertrophy of these capillaries and ultimately to the clearance of interstitial sodium.[1] In line with the idea that VEGF-C improves skin sodium clearance, increased skin sodium content in healthy elderly people was found to be associated with lower VEGF-C levels.[2] Although animal studies further support a causality between VEGF-C, VEGFR3, skin sodium and blood pressure,[3] human data are limited to aforementioned observation of associations.

In the last decade of cancer research different treatment strategies have been developed to inhibit VEGF signaling in order to inhibit neoangiogenesis. RTKI (receptor tyrosine kinase inhibitors) - such as sunitinib - are among the most commonly used. These agents are featured by adverse events; hypertension being one of the most common and well-documented.[4]

Based on experimental studies with rodents[3] and observations in humans[2] here we aimed to test the hypothesis that inhibition of VEGF signaling leads to a skin sodium and VEGF-C associated blood pressure elevation. To test our hypothesis, we performed an open-label clinical pilot study to investigate the effect of sunitinib treatment on tissue sodium accumulation in otherwise healthy men with metastatic renal cancer who were candidates for starting treatment with sunitinib (https://clinicaltrials.gov/ct2/show/NCT04368546). The institutional review board of the Charité – Universitätsmedizin Berlin approved the study

(EA1/044/15) and written informed consent was obtained from all participants before study entry. According to the standard treatment protocol, sunitinib, 50 mg once daily, was taken for 4 weeks followed by 2 weeks off treatment period. Patients were all normotensive and had similar systolic blood pressure to the age-matched healthy controls at all measured time points (Figure [A]). Nevertheless, sunitinib-induced systolic blood pressure changes could be observed (Figure [A]). Skin sodium content was similar in renal cell carcinoma patients before initiation of a sunitinib treatment in comparison to age-matched healthy subjects, but elevated after sunitinib treatment and stayed elevated independently from medication (Figure [B]). Skin water content was similar in patients during the whole observation time in comparison to age-matched healthy subjects (Figure [C]). Similar observation could be made for muscle (triceps surae) sodium content, however in this case sunitinib-induced sodium accumulation was accompanied with water content elevation (data not shown). Treatment with sunitinib led to an elevation in plasma endothelin-1 concentrations, which returned to levels similar to those at baseline in the treatment off phase, but were not significantly elevated again after the second treatment period with sunitinib (Figure [D]). Similar changes could be observed for plasma VEGF-A concentrations (Figure [E]). In contrast to VEGF-A, high baseline VEGF-C concentrations dropped after sunitinib treatment and stayed low independently from further treatment phase of sunitinib (Figure [F]).

Although the here presented data are derived from a small number of subjects we could verify earlier findings of sunitinib treatment on blood pressure and on circulating endothelin-1 and VEGF levels[5, 6]. More importantly, it was enough powered to detect clear changes on skin sodium content and in plasma VEGF-C concentrations; sunitinib treatment decreased VEGF-C

levels and led to an increase of skin sodium content. This resembles the findings in animal models,[3] and suggests a VEGF-C-dependent tissue sodium clearance. Interestingly, we found that a single sunitinib treatment cycle suppressed circulating VEGF-C sustainably, and skin sodium content remained on a higher level. This observation has to the best of our knowledge not been reported before and needs further investigation. Although we did not measure sodium consumption of the patients, based on self-reporting, eating habits of patients did not change during the study. Moreover, we could not detect skin sodium change by MRI in another study of our group (https://clinicaltrials.gov/ct2/show/NCT02509962) after an increase of habitual salt intake by 6 g per day (unpublished data), reassuring us that this is not a dietary bias.

In summary, we found that treatment with a drug targeting VEGF-receptors in humans leads to skin sodium content changes associated altered circulating VEGF-C levels. Our findings also suggest that whereas a side-effect such as blood pressure elevation is a reversible phenomenon of a sunitinib treatment, it could have a long-lasting effect on plasma VEGF-C concentrations and thereby on skin sodium homeostasis. Further studies are warranted to validate this concept.

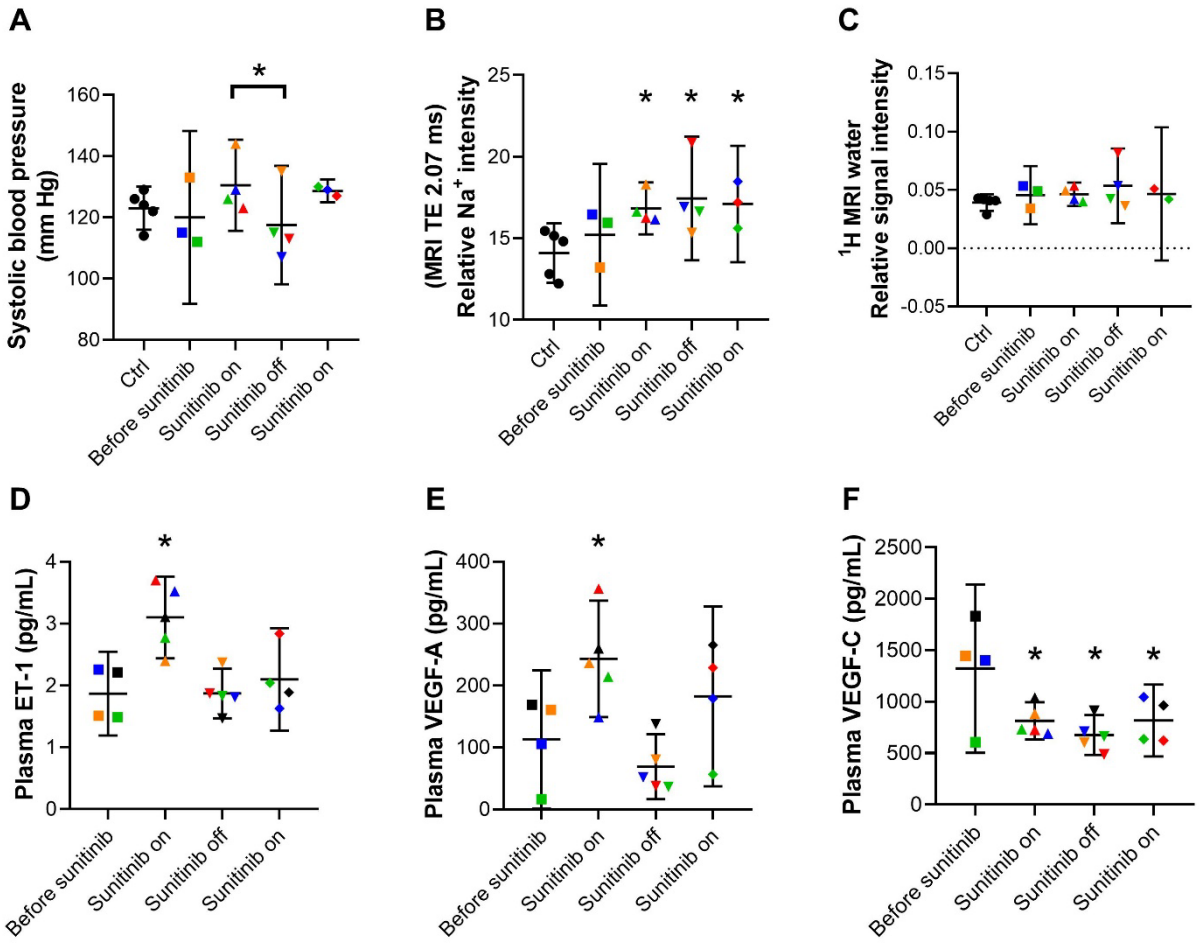

Figure. Intermitting treatment with receptor tyrosine kinase inhibitor sunitinib induces circulating ET-1 (endothelin-1)- and VEGF-A (vascular endothelial growth factor A)-associated blood pressure changes, and VEGF-C - but not blood pressure-associated - skin sodium changes. All measurements were performed in the week before starting sunitinib (Before sunitinib), after 4 weeks of sunitinib administration (Sunitinib on) and at the end of the 2 weeks-off treatment period (Sunitinib off) and again after 4 weeks of sunitinib administration (Sunitinib on). Systolic blood pressure, skin sodium and water content were measured and compared to values of cancer patients additionally in a healthy, age-matched control group (EA1/325/13).

Figure panels include 5 control individuals (except for panels D-E) and 4 sunitinib patients. Of note, one patient could not be measured before sunitinib treatment and another one could not attend his last visit. Figure scatter plots indicate mean±95% confidence intervals. Patients are color coded and each color represents data of the same patient during the course of their treatment with sunitinib. **A,** Systolic blood pressure in healthy subjects and in patients. Office blood pressure was measured after at least 15 minutes of sitting for 20 minutes at 5 minutes intervals using an automatic ambulatory blood pressure monitor (Mobil-O-Graph, I.E.M. GmbH, Aachen, Germany) in both patients at all visits and in healthy subjects. P=0.27, one-way ANOVA; a separate paired analysis on patients only between first sunitinib on and sunitinib off phase; half tick-down line, *P<0.05, two-sided, paired t-test. **B,** Skin sodium content in the skin of healthy subjects and patients. Skin sodium measurements were performed on the left lower leg using a transmit/receive birdcage ^{23}Na knee-coil (Rapid Biomedical, Rimpar, Germany) as described earlier by Dahlmann et al.[2] with the exception that here a Philips Ingenia 3.0 Tesla magnetic resonance imaging (Amsterdam, Netherlands) scanner was used instead of a Siemens scanner with device-specific adjustment of the scanning sequence. *FDR-adjusted P values (q value) <0.01, one-way ANOVA, two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli test. **C,** Relative water signal intensity in the skin of healthy subjects and patients. Using ^{1}H-MRI, measurements of the lower leg water content were performed in the same area as sodium measurements in each individual. **D**, Plasma concentration of ET-1 (endothelin-1), **E**, VEGF-A (vascular endothelial growth factor A) and **F**, VEGF-C. Blood was collected in a standardized manner after blood pressure measurement (i.e., after ca. 45 minutes of sitting) and always at the same daytime. All measurements were performed using Quantikine enzyme-linked immunosorbent assays (ELISA; R&D systems, Minneapolis, USA). Of note, Figures **D-E**

include measurements from an additional patient (black symbols) too, which could participate in blood parameter measurements only. *FDR-adjusted P values (q value) <0.01, one-way ANOVA, two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli test.

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