Vascular effects of the vascular targeting agent NGR-hTNF in patients with advanced solid cancer: a dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) study

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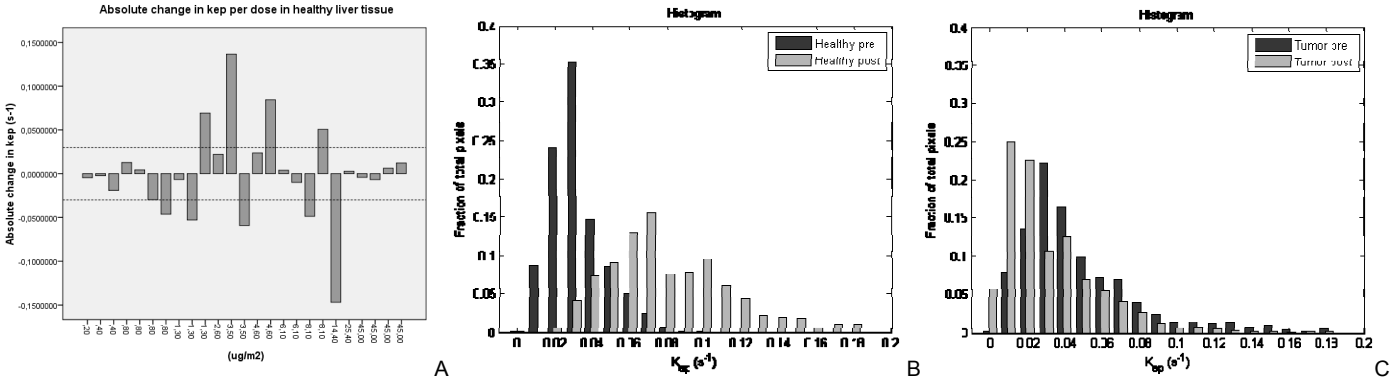
Introduction: Vascular targeted TNF, NGR-hTNF, has antivascular properties. In a recent phase I study, it was not possible to select an optimal biological dose of NGR-hTNF from DCE-MRI measurements.(1) This study aims to examine the reasons for this. We hypothesized that the following factors could play a role: 1) insufficient reproducibility of the DCE-MRI method; 2) lack of specific targeting of tumor vasculature by NGR-hTNF; 3) lack of sufficient tumor neovasculature to enable NGR-hTNF efficacy; 4) non-vascular effects of NGR-hTNF interfering with the anti-vascular effects.

Patients and Methods: DCE-MRI,CT, clinical and laboratory data of patients treated in the phase I dose escalation study of NGR-hTNF in patients with advanced solid cancer were analyzed. The utility of DCE-MRI imaging as a predictive biomarker for clinical response and determination of optimal biological dose was an objective for this study. The results of the phase I study were published separately.(1) NGR-hTNF was administered intravenously once every 3 weeks in 20-60 minutes in cohorts of 3-6 patients. DCE-MRI was performed baseline and two hours after start of the first administration of NGR-hTNF in all patients with either primary or metastatic tumors in the liver (n=26) or the head and neck region (n=5). A 1.5 T Siemens MR system was used. After conventional T1- and T2-weighted imaging, 15 ml 0.5M Gadolinium-DTPA was administered intravenously in 6 seconds by a Spectris^{TM} MR injection system. Using a T1-weighted fast low-angle shot (FLASH) sequence with a time resolution of 2 seconds Gd-DTPA uptake in the tissue was monitored. An arterial input factor (AIF) was determined in a carotid artery (for the head and neck region) or in the spleen (for the liver). Sequence parameters were: TR 50 ms, TE 4.4 ms, α 45º, slice thickness 7mm, 4-6 slices, FoV 512x416. DCE-MRI data were acquired for 90 seconds. For follow-up scans slice positions were matched with the first session using anatomical hallmarks as a reference. We obtained maps for k_{ep} and K^{trans} as described previously.(2) From each map, the mean k_{ep} and K^{trans} of the whole tumor/metastasis. To assess tumor heterogeneity, histogram analyses were performed. For the first hypothesis, reproducibility measurements were performed in five additional patients with liver metastases without systemic treatment. The method of Bland Altman was used to determine repeatability coefficients.(3) The second hypothesis, the effect of NRG-hTNF on healthy liver tissue, was analyzed by measuring mean k_{ep} and K^{trans} values as well as histogram shift in 3 ROIs containing healthy liver tissue in the patients with liver metastases. These data were correlated to liver function. For the third hypothesis, we considered that sufficient tumor neovasculature is essential for the intratumoral delivery of NGR-hTNF. We expected that in larger tumors the neovasculature is more mature, the effect of diffusion from surrounding healthy liver tissue is less, and there may be more necrotic parts. We therefore correlated DCE-MRI parameters and histogram results with largest diameters as measured on the baseline CT according to the RECIST criteria. At last, we evaluated the effect of the shedding of soluble TNF receptors (sTNF-RI and sTNF-RII) and development of anti-TNF antibodies.

Results and discussion: Reproducibility was tested in the 5 additional patients with liver metastases. A mean k_{ep} of 0.059 s^{-1} and mean K^{trans} of 0.046 s^{-1} was found in metastases with a reproducibility coefficient of 0.013 s^{-1} for k_{ep} and 0.055 s^{-1} for K^{trans}. For healthy liver tissue a mean k_{ep} value of 0.088 s^{-1} was found with a reproducibility coefficient of 0.030 s^{-1}, and mean K^{trans} was 0.058 s^{-1} with a repeatability coefficient of 0.024 s^{-1}. In both metastases (n=31) and healthy liver tissue (n=26) of patients treated with NGR-TNF, no significant changes were found in mean absolute values of k_{ep} ($p>0.1$) and K^{trans} ($p>0.1$) Despite this, the changes in mean k_{ep} values exceeded the repeatability coefficient in metastases in 6 patients and in healthy liver tissue in 9 patients (fig. A). The fraction of pixels with k_{ep} values below the lower threshold (TV_{low}) significantly increased (k_{ep} $p=0.002$) for metastases. This is in contrast with an increase in fraction of pixels above TV_{high} in healthy liver tissue ($p=0.03$) (fig. B, C). Therefore, NGR-hTNF seems less tumor specific than expected, although this did not result in a correlation between DCE-MRI parameters of healthy liver tissue and liver function. Mean values of delta k_{ep} and K^{trans} were not correlated with longest tumor diameters of the liver metastases and metastases in the head and neck region. The change in percentage of pixels with K^{trans} values below TV_{low} was inversely associated with the longest diameters of the tumors ($r2= -0.171$, $p=0.021$) but not significantly correlated to the change in pixels with k_{ep} values below the TV_{low} ($p=0.067$). Therefore, the effect of NGR-hTNF seems higher in smaller tumors with less mature vessels. At low dose (<1.3 mg/m2) the levels of sTNF-RI and sTNF-RII were scattered around zero and increased in a dose proportional manner ($r^2=0.53$, $p=.0014$ and $r^2=0.61$, $p=.0003$, respectively), with an apparent plateau observed at ≥25 mg/m^2. There was no relation between sTNF-RI and sTNF-RII and DCE-MRI parameters. No increase of anti-hNGR-TNF antibodies was observed.



A B C

A: Absolute change in k_{ep} in healthy liver tissue per patient, 9 patients exceed the reproducibilty limits.B and C: Histogram analyses of a patient before (pre) and after (post) NGR-hTNF administration in a healthy ROI (panel B) and a metastatic ROI (panel BC), indicating a shift of number of pixels with higher k_{ep} values in healthy liver tissue (B), in contrast to an increase in the number of pixels with lower k_{ep} values in metastases (C).

Conclusions: The failure of the applied DCE-MRI approach to determine an optimal biological dose of NGR-hTNF was not due to inadequate reproducibility in the metastases. Our results suggests that this was caused by a combination of the following factors: (*i*) less adequate reproducibility in healthy liver tissue due to more than expected heterogeneity in vascular response, (*ii*) more than expected changes in healthy liver tissue which influences the amount of contrast between metastases and healthyliver tissue (*iii*) difference in the effect of NGR-hTNF between tumors related to tumor size and (*iv*) the development of soluble TNFα receptors.

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