

Improved SPECT-Imaging of Folate Receptor-Positive Tumors in Mice Using ¹¹¹In-DTPA-Folate in Combination with the Antifolate Pemetrexed

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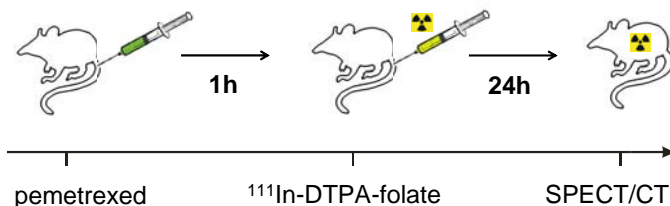
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Introduction. The high affinity folate receptor (FR) is overexpressed in various tumor types (e.g. ovarian, endometrial carcinomas). Thus, using the vitamin folic acid as a molecular “Trojan Horse” is a promising strategy for targeted delivery of diagnostic or therapeutic probes into FR-positive tumor cells [1,2]. ¹¹¹In-DTPA-folate (Endocyte Inc, IN) was the first radiofolate that has been tested in patients [3]. In spite of promising results, kidney accumulation of activity was high due to the expression of FRs in the renal proximal tubule cells. Recently, we developed a protocol for the combined application of a radiofolate and an antifolate that significantly decreased kidney accumulation of radioactivity in mice [4]. In this study we were able to demonstrate the beneficial effect of the antifolate pemetrexed (PMX) applied in combination with ¹¹¹In-DTPA-folate.

Preparation of the ¹¹¹In-DTPA-Folate

The DTPA-folate (a kind gift from Prof. P. S. Low, Purdue University, IN) was incubated with ¹¹¹InCl₃ for 1 h at RT in a buffered solution of pH 6.5. Quality control performed via HPLC showed high radiochemical purity (> 98%).

Pemetrexed Protocol

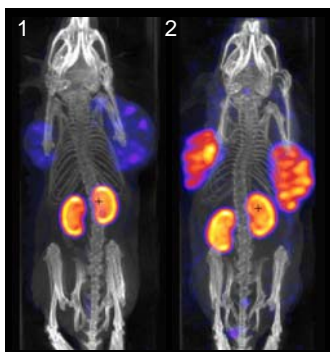


Tumor-to-Background Ratios of ¹¹¹In-DTPA-Folate

The values represent the ratios as mean ± SD of 3 mice with s.c. IGROV-1 tumors (ovarian carcinoma) determined at different times post injection (p.i.).

| IGROV-1 | control 4 h p.i. | PMX 4 h p.i. | control 24 h p.i. | PMX 24 h p.i. |
|-----------------|---------------------|-----------------|----------------------|------------------|
| tumor-to-blood | 47.0 ± 12.6 | 61.6 ± 25.3 | 50.0 ± 20.8 | 98.4 ± 9.6 |
| tumor-to-kidney | 0.11 ± 0.04 | 0.49 ± 0.09 | 0.09 ± 0.03 | 0.45 ± 0.13 |

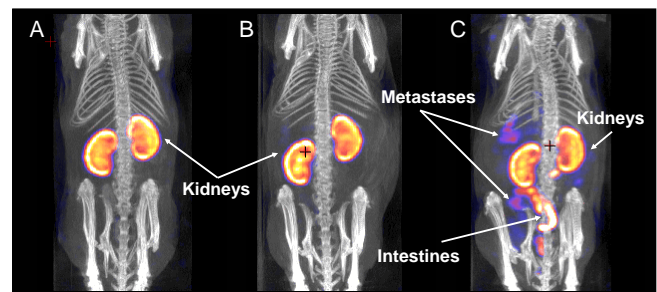
SPECT/CT of Female Mice with s.c. IGROV-1 Tumors



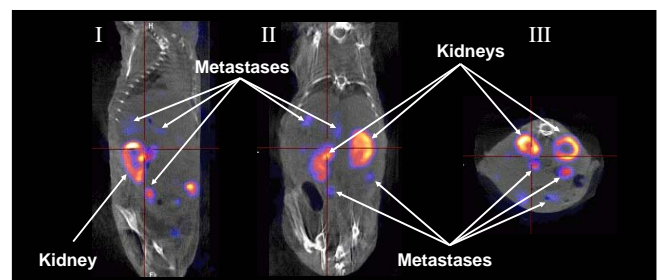
Scans were performed with a four-headed SPECT/CT camera (NanoSPECT/CT™). Each head was outfitted with apertures of nine 1.4 mm-diameter pinholes. Whole-body scans were acquired in mice bearing IGROV-1 xenografts (human ovarian carcinoma), 24 h after injection of the ¹¹¹In-DTPA-folate (50 MBq) only (1) or in combination with 400 µg PMX (2).

SPECT/CT of Female Mice with SKOV-3 Metastases

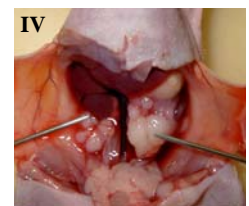
Female mice were i.p. injected with SKOV-3 cells (human ovarian carcinoma). Four weeks later, the mice were injected with ¹¹¹In-DTPA-folate (90 MBq) with/without administration of 400 µg PMX 1 h prior to the radioactivity. Whole-body SPECT/CT scans were acquired 24 h p.i..



Control mouse without metastases (A), mice with SKOV-3 metastases after injection of ¹¹¹In-DTPA-folate alone (B) or ¹¹¹In-DTPA-folate in combination with PMX (C) in a 3D view. While A and B could not be distinguished, C allowed the detection of even smallest metastases after adjustment of the scale to the same kidney uptake as shown in control scans.



Sagittal (I), coronal (II) and transaxial sections (III), of a female nude mouse injected with ¹¹¹In-DTPA-folate and PMX (C). Visualization of metastases (IV) was achievable since the tumor-to-kidney ratio of radioactivity could be significantly increased after PMX-injection



Conclusion. The application of the antifolate PMX prior to the injection of ¹¹¹In-DTPA-folate resulted in a significantly improved tumor-to-kidney ratio of radioactivity in IGROV-1 tumor bearing mice due to reduction of radioactivity retention in the renal tissue. The importance of this effect became in particular obvious in mice bearing SKOV-3 i.p. metastases that could only be visualized in mice that received PMX. For the development of a folate-based radiotherapy this protocol will be crucial in order to prevent renal damage by particle-emitting radiation.