TOOKAD Photodynamic Therapy of orthotopic PC3-MM2 prostate tumors in nude rats : investigation using BOLD-MRI, Diffusion-weighted-MRI and Dynamic-Contrast Enhanced MRI

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Introduction : The local activation of a non-toxic sensitizer such as TOOKAD by near-infrared wavelengths was used as an effective cancer therapy by generating cytotoxicity that causes cell death and necrosis. Although TOOKAD was used successfully in several pre-clinical models of cancer, few studies were reported in orthotopic prostate cancers. In this work, the treatment schedules and doses of either the TOOKAD photosensitizer or the laser illumination were validated on orthotopic PC3-M prostate tumors. The TOOKAD anti-tumor activity at various laser energies was studied by tumor growth inhibition measurements, MRI and immunohistochemistry.

Methods : We conducted MR measurements on PC3-MM2 tumors orthotopically xenografted in the prostate of five nude rats. Tumor growth measurements and immunohistochemical measurements were performed on 59 nude rats bearing PC3-MM2 prostate tumors orthotopically xenografted. As described in table 1, 6 groups of rats underwent Tookad + PDT by a 3-minute Tookad infusion at a dose of 2 mg/kg in the tail vein and insertion of an optical fiber in the the center of the tumors 5 minutes after Tookad infusion at D10 after cell inoculation. Sacrifice of rats occurred at D21 post-treatment after FITC-DEXTRAN and pimonidazole injection. Groups 9 and 10 were imaged in a 4.7T magnet (Bruker, Wissembourg) prior to treatment (D0) and 1 and 7 days post treatment. Multi-slice T2-weighted MR images covering the entire tumor were followed by multi-gradient echo T2* MR acquisitions (TR/TE/α=500ms/ 4, 10, 16, 22, 28ms/45°; FOV=70x70mm; Matrix=256x256, slice thickness=2mm). Spin echo diffusion weighted MRI was also performed (Linearly increased gradients in the read direction 0-150mT/m, Δ=50ms; δ=3ms, 4 b values=2.970, 104.92, 64.66, 782.19 s/mm^2, FOV = 70x70 mm, 128x64, 2mm slice thickness). DCE-MRI was performed using a FLASH-2D sequence with a temporal resolution of 12.8s per image (TR/TE/α=100ms/3.3ms/70°; FOV=70x70mm, Matrix=128x128, slice thickness=2mm). T1-weighted pre-contrast images were acquired 1 minute prior to an IV bolus injection of 0.3 mmol/kg Gd-DOTA (GUERBET, France). 115 post-contrast images were acquired during 20 minutes. Images were processed under IDL 6.1 using in-house written software. Region of interest (ROI) analysis and pixel by pixel analysis were performed to determine ADC, R2* values and K^{trans} and ve values using the Tofts and Kermode pharmacokinetic model.

Results: Results showed significant tumor weight reduction in 100J/cm and 125J/cm PDT treated rats compared to dark control, Navelbine treated rats and untreated rats at D21 post-treatment (t-test $p < 0.03$). Histology showed that 60 to 80% PDT treated tumors were necrotic compared to only 10-30% in untreated, vehicle, dark control and Vinorelbine groups. ADC and R2* MRI markers demonstrated onset of necrosis upon PDT treatment as soon as D1 post-treatment. In PDT treated rats ADC dropped by 42% at D1 and re-increased at D7. R2*had dropped by more than 50 % at D7. The perfusion study showed that tumor rims remained viable after PDT treatment with no significant changes in Ktrans and ve post-treatment.

Conclusions: Reduced ADC was detected at D1 after Tookad + PDT treatment followed by a re-increase in ADC values at D7. These results confirm results found by another team in subcutaneously xenografted prostate tumors (1) and emphasize the ability of MRI techniques to detect early response to Tookad + PDT treatment with reproducible and reliable markers such as ADC values easily transferable to the clinics.

Table1: Groups and Tookad+PDT conditions used for investigations of PC3-M response to treatment

Animals			Photosensitizer			Laser conditions			
groups	**Tumor**	**No rats**	**treatments**	**Doses (mg/kg)**	**Infusion Rate (µl/min/kg)**	**Energy (J/cm)**	**Power (mW/cm)**	**Duration (s)**	**Delay (min)**
1	**PC3-M**	**11**	**Not Treated**	**-**	**-**	**-**	**-**	**-**	**-**
2	**PC3-M**	**11**	**Vehicle WST09**	**-**	**267.0**	**125**	**200**	**625**	**5**
3	**PC3-M**	**11**	**Tookad**	**2**	**267.0**	**50**	**200**	**250**	**5**
4	**PC3-M**	**12**	Tookad	**2**	**267.0**	**75**	**200**	**375**	**5**
5	**PC3-M**	**12**	Tookad	**2**	**267.0**	**100**	**200**	**500**	**5**
6	**PC3-M**	**12**	Tookad	**2**	**267.0**	**125**	**200**	**625**	**5**
7	**PC3-M**	**12**	Tookad	**2**	**267.0**	**0**	**0**	**0**	**0**
8	**PC3-M**	**12**	**Vinorelbine**	**2**	**IV -bolus**	**-**	**-**	**-**	**-**
9	**PC3**	**2**	**Not**	**0**	**0**	**0**	**0**	**0**	**0**
10	**PC3**	**3**	**Tookad**	**2**	**267**	**175**	**200**	**875**	**5**

(1) Plaks V et al (2004) Neoplasia 6, 224-233