Photodynamic therapy with TOOKAD® in an orthotopic human prostate cancer xenografted in nude rat

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D. Blanc[1], H. Ficheux[1], N. Just [2], O. Duchamp[2], F. Bichat[2]

(1) NEGMA-LERADS – STEBA NV, Magny-les-Hameaux (78), France ; (2) ONCODESIGN Biotechnology, Dijon (21), France

Introduction

- Photodynamic therapy (PDT) is a cancer therapy in which drug action is locally activated by light: the photosensitization of a non-toxic circulating sensitizer generates cytotoxicity and causes cell death and necrosis of the tumor tissue,
- Although, PDT was used in superficial human lesions readily accessible to laser illumination, few studies have been reported in human prostate cancer, one main limitation of the technique being the limited depth of the treatment due to the properties of the sensitizer,
- Tookad® is a bacteriopheophorbides photosensitizer for PDT which targets the tumor vasculature upon illumination with light in the near-infrared wavelengths,
- Tookad® has been successfully evaluated in several preclinical models,
- The objective of this study was to evaluate the antitumor effects of PDT with Tookad® in the PC3-M human prostate cancer model orthotopically xenografted in nude rats.
- OT tumor induction

Experimental Methodology

Ten millions human PC3-M prostate cells were injected orthotopically (OT) in the prostate of *Rowett Nude* rats (Harlan SD Inc., Indianapolis) at D0. The treatments started at D10 when the mean OT tumor diameters reached about 5-10 mm. The treatments were performed as described in table below :

- Treatments

No rats	Photosensitizer			Laser conditions			
	Treatments	Doses (mg/kg)	Infusion rate (µl/min/kg)	Energy (J/cm²)	Power (mW/cm²)	Duration (sec)	Delay (min)
11	Not Treated	-	-	-	-	-	-
11	Vehicle Tookad®	-	267	125	200	625	5
11	Tookad®	2	267	50	200	250	5
12	Tookad®	2	267	75	200	375	5
12	Tookad®	2	267	100	200	500	5
12	Tookad®	2	267	125	200	625	5
7	Tookad®	2	267	175	200	875	5
12	Tookad®	2	267	0	0	0	0
12	Navelbine®	2	IV *bolus*	-	-	-	-

Concomitant illumination of OT prostate tumor by the laser with infusion of Nude rat with Tookad® by the tail vein

Firstly, an optic fiber of 5 mm length was inserted in the centre of OT tumor and the adjacent healthy tissues were protected from light using aluminium paper slided over the animal abdomen just before start of treatment. Then, the rats received a 3-min intravenous infusion of Tookad® (WST09) at 2 mg/kg via a catheter coupled to a syringe-pump (267µl/kg/min) followed by a laser illumination (λ 763 nm) of OT tumor 5 min after the start of Tookad® infusion. The laser power was maintained at 200 mW/cm² while the laser energy varied from 50 to 175 J/cm². One group of rats received a single IV bolus injection of Navelbine® at 2 mg/kg at D10 and D17.

- Histological study of vascularity and hypoxia of tumors

The rats were sacrificed at D13 to collect and weigh the tumor. Previously, the rats received a single IV *bolus* injection of Pimonidazole at 60 mg/kg 60 min before sacrifice. The histology was analyzed in OT tumor sections. The immunohistochemical detection of vessels and hypoxia was performed with anti-CD-31 and anti-pimonidazole antibodies (hydroxyprobe assay kit), respectively.

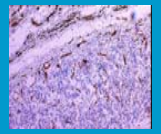
- MRI protocol

MRI was performed with a 4.7 T BRUKER Pharmascan spectrometer using a 6 cm diameter cylindrical coil on rats at D10 (before start of treatment), D11 and D17. Prior to MRI, each rat bearing a PC3-M human prostate tumor was anaesthetized using isoflurane. A catheter was inserted in the tail vein for the IV bolus injection of Gd-DOTA contrast agent (Dotarem®, Guerbet, France) at a dose of 0.3 mmol/kg. A T2-weigthed TurboRare sequence was used for morphological image acquisitions and determination of the tumor volume (in both sagittal and axial planes). A diffusion- weighted spin-echo (SE) imaging sequence was applied on axial slices of rats before and after treatment. Diffusion gradients were linearly increased using 4 b values. A T2*-weighted multi-gradient echo sequence was used for T2* acquisitions. Prior to dynamic contrast-enhanced MRI (DCE-MRI), the tumor precontrast T1 was determined using a FISP T1-weighted imaging sequence. A reference tube with a known T1 value was placed in the FOV and imaged at the same time as the rat to correct for slice profile imperfections. Finally, DCE-MRI images were acquired using FLASH 2D sequence. Precontrast T1-weighted images were acquired during 1 minute prior to a short (5 seconds approximately) bolus intravenous injection of Gd-DOTA. A series of T1-weighted FLASH 2D images were acquired during the remaining 20 minutes of acquisition.

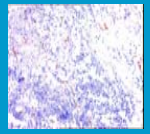
All images were transferred to a Linux workstation to be analysed under OPASIS software. T2*, ADC, and Ktrans maps were generated yielding a value for each pixel. All the images were evaluated by means of regions of interest (ROIs). The Tofts and Kermode model was used for the quantification of Ktrans on a pixel by pixel basis.

Results

Vessels staining via immunohistochemistry detection of CD-31

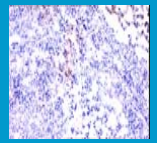


Control OT tumor with viable rim of tumor and presence of vessels

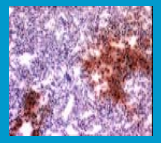


Tookad® + 125J/cm² treated OT tumor with large necrosis areas

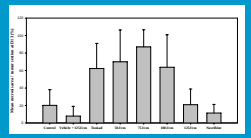
Hypoxia staining via immunohistochemistry detection of pimonidazole



Control OT tumor with low level of hypoxia



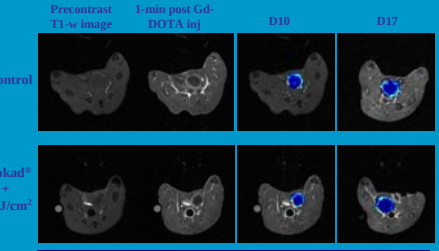
Tookad® + 125J/cm² treated OT tumor with high level of hypoxia



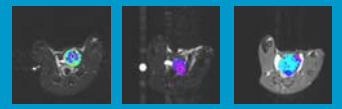
Necrosis level (%) in OT prostate tumors at D13

- The histological analyses of tumors at D13 showed a significant increase of necrosis areas for rats treated with Tookad® with or without laser illumination (62 to 87% of necrosis vs 8-20% in vehicle and Navelbine® treated groups).

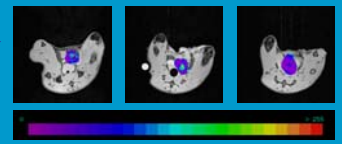
DCE-MRI images and Ktrans maps



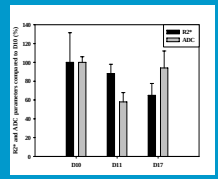
ADC false color map overlaid over a diffusion-weighted image



R2* false color map overlaid over a T2*-weighted image

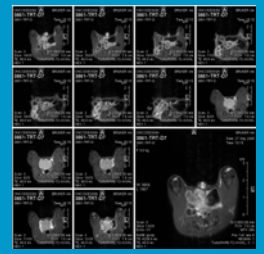


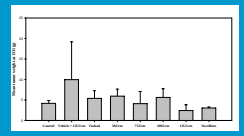
Mean ADC and R2* MRI parameters measured in OT prostate tumors, at D11 and D17 compared to D10.



- No change of Ktrans parameter in treated OT tumors compared to control tumors,
- Significant decrease of ADC (42% at D11) and R2* (35% at D17) MRI parameters in tumors from rats treated with Tookad® + 175 J/cm compared to D10.

T2-weighted morphological images of OT tumor bearing rats





Mean OT prostate tumor weight (g) at D31

- Significant tumor effect was seen at D31 on the tumor weight of groups of rats treated with PDT at 125 J/cm² and Navelbine® compared to control (2.42 ± 1.42 and 3.04 ± 0.23 vs 4.18 ± 0.41, respectively).

Conclusions

- The PC3-M prostate carcinoma orthotopically xenografted in Nude rats appears suitable to evaluate antitumor activity of PDT,
- In this model, Tookad® injected at 2 mg/kg just before the laser illumination of OT tumors at 125 J/cm inhibits PC3-M OT tumor development and confirms its interest for the treatment of locally prostate cancer,
- ADC and R2* MRI markers confirmed early response and onset of necrosis upon PDT treatment.