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Original Article

Pharmacokinetics and pharmacodynamics of intravesical and intravenous TMX-101 and TMX-202 in a F344 rat model

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Abstract

Objectives: To evaluate and compare the pharmacokinetic and pharmacodynamic properties of 2 investigational Toll-like receptor 7 agonists, TMX-101, and TMX-202 after intravenous and intravesical administration in a rat model. TLR-7 agonists are successfully used as topical treatment for various (pre)malignant skin lesions and are now under investigation as intravesical therapy for non–muscle-invasive bladder cancer.

Methods: Rats received an intravesical instillation with TMX-101, TMX-202, or vehicle. Additionally 2 groups of rats received an intravenous injection with TMX-101 or TMX-202. Blood sampling was performed at different time points, including pre-exposure and postexposure to determine the plasma concentrations of study drugs for pharmacokinetic and pharmacodynamic analyses and to determine the plasma concentrations of cytokines (IL-2, IL-6, and TNF-α).

Results: We observed no signs of toxicity after intravesical or intravenous administration. There was a limited dose dependent systemic uptake of TMX-101 and TMX-202 after intravesical administration. The systemic uptake of TMX-202 after intravesical instillation was 25 times lower compared to TMX-101.

Conclusions: This in vivo study confirms the safety of intravesical TMX-101 and TMX-202 administration, with TMX-202 showing lower systemic uptake. TMX-202 has a larger molecule-mass compared to TMX-101, and it may therefore have a favorable safety profile when treating patients with non–muscle-invasive bladder cancer intravesically.

Keywords: Immune modulator; Imiquimod; Non–muscle-invasive bladder cancer; Pharmacodynamics; Pharmacokinetics; Toll-like receptor

1. Introduction

The current treatment of non–muscle-invasive bladder cancer (NMIBC) is suboptimal as demonstrated by recurrence rates of up to 78% after 5 years [1]. European and American Guidelines recommend transurethral resection of the bladder tumor followed by adjuvant intravesical treatment for intermediate and high-risk patients [1,2]. Nevertheless, even after adjuvant treatment with intravesical bacillus Calmette Guérin, the risk of recurrence after 5 years is as high as 41.3% [3]. Because of these modest results, new treatment options are needed.

The Toll-like receptor (TLR) family (TLR-1–TLR-10) is a conserved group of transmembrane proteins playing a pivotal role in innate- and adaptive immunity [4,5]. TLR activation leads to a coordinated immune response culminating in antigen presentation by mature dendritic cells and enhanced production of antigen specific T-cells [4,6–8]. On top of activation of innate immunity against pathogens, the involvement of TLR in antitumor immunity has been shown [9]. Indeed, the success of bacillus Calmette Guérin therapy for urothelial carcinoma (UC) is (partly) based on TLR activation [6,7,10,11].

Imiquimod, a synthetic TLR-7 agonist, is used as first line topical treatment for benign and malignant skin lesions [12,13] including genital condylomas and penile carcinoma in situ [14].

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Imidazoquinolines like imiquimod also have a direct effect on UC [6] and may therefore represent an alternative intravesical treatment moiety against UC.

TMX-101 is an imiquimod formulation, suited for intravesical instillation [15,16]. Preclinical [15] and early-phase clinical trials [17–19] have convincingly demonstrated the safety of TMX-101. TMX-202 consists of a TLR-7 ligand conjugated to a phospholipid. It is a highly specific agonist of TLR-7 and in in vitro cellular essays, proinflammatory cytokine levels are approximately 2-fold higher compared to imiquimod [20,21]. Our aim was to evaluate and compare the pharmacokinetic and pharmacodynamic behavior of the investigational drugs TMX-101 and TMX-202 in the Fischer F344-rat after intravesical and intravenous administration.

2. Material and methods

2.1. TLR-7 expression

To determine TLR-7 expression in F344-rat tissue we isolated RNA from F344 rat bladder samples ($n = 3$), bladder without urothelial layer ($n = 1$), prostate ($n = 1$), and spleen ($n = 2$) for reverse transcription-polymerase chain reaction (PCR) analysis using a rat TLR-7 specific primers: Tlr7-rat-foreward: TCTCAAGCTCTGTTCTCC TCC (intron spanning), Tlr7-rat-reverse TACCATC-GAAACCCAAGGAC, as control gene GAPDH was used. Primers GAPDH forward ATGGGAAGCTGGTCAT-CAAC, GAPDH reverse GGATGCAGGGATGATGTTCT.

2.2. Animals

Animal protocols were approved by the Institutional Animal Care and Use Committee, Committee for Animal Experiments (Radboud University Nijmegen Medical Centre, The Netherlands) and were in compliance with Dutch and European regulations (EU-Directive 2010/63/EU). Forty-two female Fischer F344-rats (Charles River, L′Arbresle Cedex, France) were housed for 2 weeks before the experiment. The rats, age 9 to 11 weeks, weighing 170 ± 10 g, were housed in cages (Techniplast, Milan, Italy) with goldflake bedding (SPPS, Frasne, France) in a temperature controlled environment with a 12-hour light/dark cycle with free access to chow and water. Daily, the rats were weighed and monitored for wellbeing. The sample size of 6 rats per group was chosen to collect adequate volumes of blood samples and minimize the risk of confounding variability between the rats. F344-rats were chosen because for future efficacy studies we may use the syngeneic F344-AY27 orthotopic bladder cancer rat model [22].

2.3. Agents

The following agents were used for intravesical instillation: TMX-101, 2.08 mM (0.05%), and 4.16 mM (0.1%), a formulation of imiquimod (1-(2-methylpropyl)-1*H*-imidazo [4,5-c]quinolin-4-amine). TMX-202 (2-(4-((6-amino-2-(2-methoxyethoxy)-8-oxo-7H-purin-9(8 H)-yl) methyl)benzamido)ethyl 2,3-bis (dodecanoyloxy) propyl phosphate), in 2 concentrations: 2.08 mM (0.19%) and 4.16 mM (0.38%). The solutions were sterile and dissolved in 0.1 M lactic acid, 16% poloxamer-407 and 5% hydroxypropylbetacyclodextrin as vehicle. For intravenous administration sterile solutions of TMX-101 6.24 mM (0.15%) in 5% hydroxypropylbetacyclodextrin and TMX-202 6.24 mM (0.56%) in 5% hydroxypropylbetacyclodextrin were used. Agents were provided by Telormedix, S.A. Intravesical TMX-101 was subject of multiple clinical trials [17–19] and further developed under the brand name Vesimune by UroGen Pharma.

Drug concentrations for intravesical administration were within the expected clinical significant range. As safety margin, 1.5 times the highest concentration of intravesical administration was used as concentration for intravenous administration.

2.4. Experimental design

Experiments were performed under inhalation anesthesia (isoflurane 2%, nitric oxide 0.5 l/min, and oxygen 1 l/min). Rats were randomly assigned to 1 of 7 experimental groups. Five groups of 6 rats received an intravesical instillation with 0.5 ml TMX-101 and TMX-202 at 2 concentrations, or vehicle (Table 1). The bladder was transurethrally catheterized with a 16-gauge intravenous cannula (BD Biosystems, Erembodegem-Aalst, Belgium) and drained before instillation with 0.5 ml drug or vehicle. To resemble the clinical situation, an indwelling time of 1 hour was used. Blood samples were collected before and 15, 30, 60, 120, and 480 minutes after drug instillation. Furthermore, 2 groups of 6 rats received an intravenous injection of 0.2 ml 6.24 mM TMX-101 or TMX-202 (0.3 mg [1.76 mg/kg] and 1.12 mg [6.59 mg/kg], respectively) and blood samples were taken before administration and after 5, 15, 30, 60, 120, and 240 minutes. At the first 2 time points, blood was withdrawn from all rats. For other time points an alternating scheme was used to diminish the risk of animal loss, leading to 4 blood samples per rat. Per time point blood samples were taken from at least 3 rats within a treatment group.

Table 1
Experimental groups and corresponding treatments

Group	Treatment	Dosage	Route	*N*
1	TMX-101	2.08 mM (0.05%), 0.5 ml	Intravesical	6
2	TMX-101	4.16 mM (0.1%), 0.5 ml	Intravesical	6
3	TMX-202	2.08 mM (0.19%), 0.5 ml	Intravesical	6
4	TMX-202	4.16 mM (0.38%), 0.5 ml	Intravesical	6
5	Vehicle control	0.5 ml	Intravesical	6
6	TMX-101	6.24 mM (0.15%), 0.2 ml	Intravenous	6
7	TMX-202	6.24 mM (0.56%), 0.2 ml	Intravenous	6