Species-Specific Urothelial Toxicity with an Anti-HIV Non-Catalytic Site Integrase Inhibitor (NCINI) is Related to Unusual pH-Dependent Physicochemical Changes.

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Abstract 3180/ P163

1. Objective

The development of GS-9695 and GS-9822 was suspended following urothelial toxicity. Here we describe these findings and the investigations conducted to understand their origin.

2. Background & Introduction

Significant advances in the treatment of HIV1 have reduced the number of new infections over the past decade. However, resistance to existing antiviral agents remains a challenge. GS-9695 (Figure 1B) and GS-9822 (1C) are next generation non-catalytic site integrase inhibitors (NCINIs) with improved potency against HIV compared with previous drugs such as BI-224436 (1A). GS-9822 is structurally distinct from GS-9695, possesses a much-improved resistance profile and has a significantly higher resistance barrier than the approved, second-generation, reverse transcriptase inhibitor rilpivirine (Mitchell et al., 2017). Based on these favorable parameters and the high selectivity index based on cellular toxicity (data not shown), GS-9695 entered early toxicology testing, followed by GS-9822. Development of both stopped due to vacuolation of the bladder urothelium seen in cynomolgus monkey but not in rat; this lesion was absent in equivalent preclinical studies with BI-224436 (tested in dog and rat). Here we investigate the lesion and its significance for development of NCINIs.

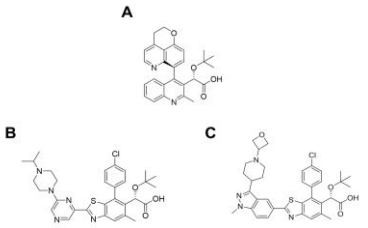

Figure 1. *GS-9695 and GS-9822 are zwitterions with pKa values of 4 and 7.8 (GS-9695) and 4.2 and 5.8 (GS-9822), respectively. Note the piperidine group attached to an oxetane group has a pKa of ~6–7.*

3a. Results

GS-9695 and GS-9822 caused vacuolation of the bladder urothelium in cynomolgus monkey (Figure 2; Table 1) but not in rat (data not shown); this lesion was absent in equivalent preclinical studies with BI-224436 (tested in dog and rat). Lesions were unlikely to be attributable to target since NCINIs specifically target viral integrase protein and no mammalian homologue is known. Secondary pharmacology studies, mitochondrial toxicity studies, immunophenotyping and analysis of proteins implicated in cell-cell interactions and/or bladder integrity (e-cadherin, pan-cytokeratin, uroplakins) failed to offer any plausible explanation for the species-specificity of the lesion (data not shown).

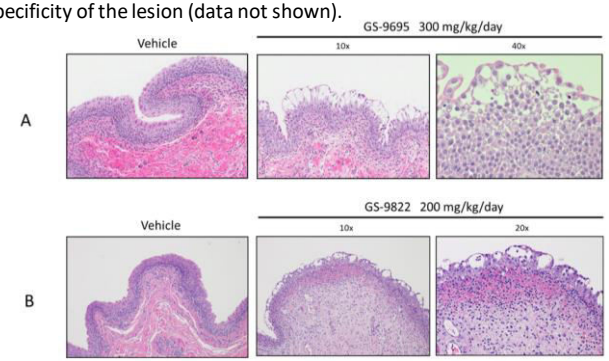

Figure 2. *Cynomolgus monkey bladder urothelium after 7 days oral gavage treatment with (A) GS-9695 or (B) GS-9822. Transitional cell vacuolation, inflammation and hemorrhage are evident in the treated sections compared with vehicle controls.*

Tissue/finding	Sex	Male				Female			
GS-9695 Dose (mg/kg/day)		0	20	60	300	0	20	60	300
Urinary Bladder	No. examined:	3	3	3	3	3	3	3	3
Vacuolation, urothelium									
Total number affected		0	1	3	3	0	1	2	3
Minimal		0	1	1	0	0	1	0	0
Slight		0	0	1	2	0	0	1	1
Moderate		0	0	1	0	0	0	1	2
Marked		0	0	0	1	0	0	0	0
Hemorrhage									
Total number affected		0	0	1	1	0	0	0	2
Minimal		0	0	1	1	0	0	0	1
Slight		0	0	0	0	0	0	0	1
Inflammation									
Total number affected		0	0	0	3	0	0	1	3
Minimal		0	0	0	1	0	0	0	0
Slight		0	0	0	0	0	0	0	1
Moderate		0	0	0	2	0	0	1	2
Kidney	No. examined:	3	3	3	3	3	3	3	3
Vacuolation, urothelium									
Total number affected		0	0	1	3	0	0	2	2
Minimal		0	0	0	1	0	0	1	1
Slight		0	0	1	0	0	0	1	1
Moderate		0	0	0	2	0	0	0	0
Inflammation, pelvis									
Total number affected		0	0	1	2	0	0	0	1
Minimal		0	0	0	0	0	0	0	0
Slight		0	0	1	1	0	0	0	1
Moderate		0	0	0	1	0	0	0	0
Ureter	No. examined:	3	3	3	3	3	3	3	3
Vacuolation, urothelium									
Total number affected		0	0	0	2	0	0	0	1
Minimal		0	0	0	0	0	0	0	1
Slight		0	0	0	2	0	0	0	0
Inflammation									
Total number affected		0	0	0	2	0	0	0	1
Minimal		0	0	0	1	0	0	0	1
Slight		0	0	0	1	0	0	0	0

Table 1. *Incidence and severity of histological observations in the renal tract of cynomolgus monkeys treated for 7 days with GS-9695. Similar results were seen with GS-9822 (data not shown)*

3b. Results

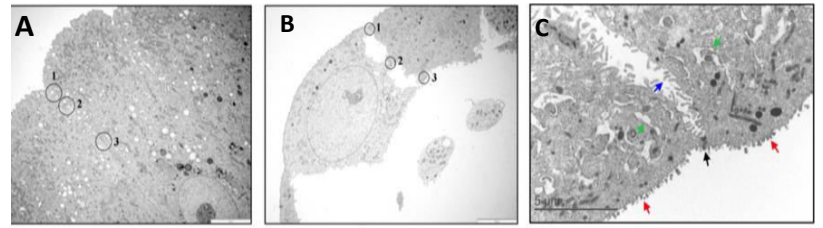

Figure 3. *TEM of cynomolgus monkey urothelium after 7 days oral gavage treatment with GS-9695 (B) or GS-9822 (C) compared with control (A). In B, there is increased clear space between adhesion molecules along lateral surfaces and desmosomes along the basal border of the superficial cells are dissociated. In C, green arrows indicate moderately injured superficial cells with increased cytoplasmic vacuolation. Except for the region of the adluminal junctional complex (black arrow), there is marked separation of the cells, with the presence of prominent microvilli (blue arrows). Projections from the luminal surface are shorter and plumper than in controls (red arrows). Left and middle images are at 2650x. Right image is at 12000x.*

Urinalysis in cynomolgus monkeys indicated a mean concentration of GS-9695 in the urine of 5.06 µM at Day 1 rising to 16.97 µM at Day 7 (data not shown). Urinalysis in rats indicated a lower concentration of GS-9695 (data not shown) but data points overlapped between species at Day 1. The solubility of GS-9695 in urine was similar between rat and cynomolgus monkey (data not shown). No unexplained solid material, crystalline or otherwise, was detected in any urine samples.

Since the lesion was characterized by inflammation and disruption of urothelial morphology (Figure 4), we investigated physicochemical changes in the bladder of cynomolgus monkey (urinary pH 5.5-7.4) that might not occur in the bladder of rats (urinary pH 7.3-8.5). In measurements of surface activity (Figure 3), GS-9822 showed an unusual transition from a monolayer to a bilayer at the air/water interface with decreasing pH, attributed to the strong association between drug molecules in adjacent bi-layer leaflets and expected to be highly disruptive to the urothelium. Structural analysis of GS-9822 and GS-9695 showed zwitterionic characteristics over the range of pH expected in cynomolgus monkey but not rat urine.

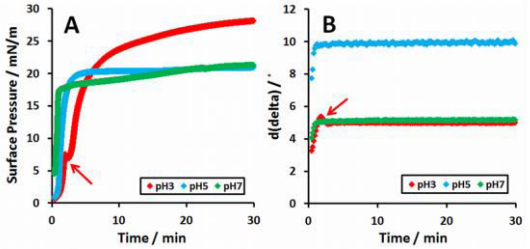

Figure 4 *Time-resolved measurements of (A) the surface pressure and (B) ellipsometric phase shift at the air/water interface for solutions of 20 µM GS-9822 in 0.1 M NaCl/0.1 M KCl with respect to the solution pH; aspiration was used to set the zero-time point. The former (A) is related to the ability of the drug to lower the surface tension of the air/water interface and the latter (B) is a measure of the amount of adsorbed drug*

4. Conclusions

Although there are some gaps in the data, they provide useful insights to guide current or future clinical development of NCINIs, related compounds, and those with zwitterionic characteristics. Specifically, structural analysis of GS-9822 and GS-9695 showed zwitterionic characteristics over the range of pH expected in cynomolgus monkey but not rat urine. This exotic surface behaviour is unlikely with BI-224436 since it would transition from neutral to cationic (never zwitterionic) with decreasing pH. These data provide useful insights to guide discovery and development of NCINIs, related compounds and zwitterions.

COI: RR, CS and PS are employees of ApconiX, an integrated toxicology and ion channel company that provides expert advice on non-clinical aspects of drug discovery and drug development to academia, industry, and not-for-profit organizations. Clients of ApconiX include Gilead. RC was contracted to ApconiX to conduct the work reported in this paper. MO, AC, LABN, YX, JYF, RS, MM, EP, and JW were all employees of Gilead at the time this work was carried out.

Mitchell et al 2017: Novel Non-Catalytic Site Integrase Inhibitor with Improved Resistance Profile. Conference on Retroviruses and Opportunistic Infections (CROI), February 13-16, 2017, Seattle, Washington. Abstract 343, available at http://www.croiwebcasts.org/p/2017croi/croi33637.