Effect of AQX-1125 on Urinary Bladder Inflammation and Pain Induced by Cyclophosphamide in Rats, by Targeting the SHIP1 Pathway

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ABSTRACT

Rationale: Cyclophosphamide (CP)-induced cystitis is a well-characterized model of sub-acute, inflammatory visceral pain in rodents. In addition to increases in urinary frequency, an increase in behavior indicative of pain, that peaks shortly after CP injection and persists for up to 4 hours, has been described.[1-3] *The* CP-induced bladder hyperactivity model is commonly used as an experimental model for bladder pain syndrome/interstitial cystitis (BPS/IC). It was previously demonstrated that a single intraperitoneal (i.p.) injection of CP induces urinary bladder pain and inflammation 4 hours post-administration.[4-5]

Aim: The aim of this study was to evaluate the effect of AQX-1125 (3 and 30 mg/kg doses) on urinary bladder pain and inflammation in acute CP-induced cystitis in female Sprague Dawley rats.

Conclusion: Oral AQX-1125 inhibited the nociceptive pain scores induced by CP at both the 3 and 30 mg/kg doses. The effects were not dose related as maximum effects were observed at both doses. In addition, AQX-1125 significantly reduced hemorrhage scores at both doses.

STUDY DESIGN

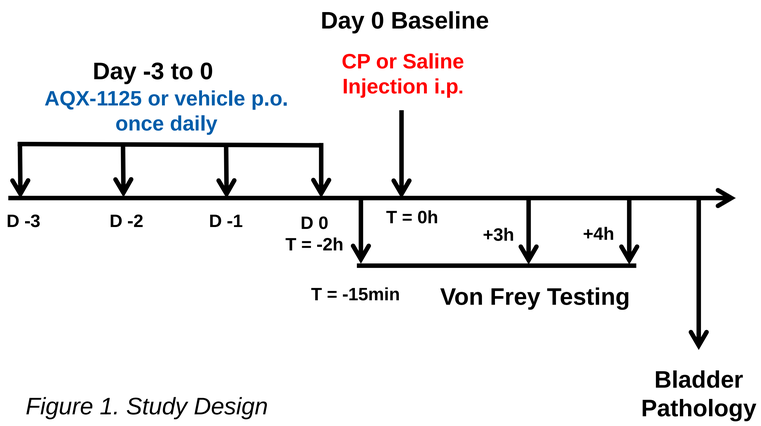

Figure 1. Study Design

Oral AQX-1125 was administered once-daily at 3 and 30 mg/kg/day for 4 days, prior to a single injection of CP. Pain was assessed by von Frey testing on three occasions (prior to, 3 and 4 h post CP injection). On each occasion, Von Frey testing was completed 3 times for each rat at forces between 1 and 60 g. Nociceptive pain score at each force is expressed as the % of the maximum score. At the end of the study urinary bladders were assessed for cytokine levels and bladder pathology.

Ibuprofen (300 mg/kg) was used as the positive control to ensure the sensitivity of the test system and was administered orally 5 minutes before the time of the CP injection (not shown in *Figure 1*).

RESULTS

A single i.p. injection of CP (150 mg/kg) induced pain characterized by decreased mechanical threshold (allodynia) and increased nociceptive pain scores (hyperalgesia) in response to von Frey forces (*Figure 2:* -●- *vs.* -○-). This pain was associated with an acute inflammation of the urinary bladder, characterized by an increase in urinary bladder weight and wall thickness, associated with strong edema and hemorrhage (*Figure 3:* CP/ Vehicle *vs.* Saline/ Vehicle).

The presence of local inflammation was also confirmed by high levels of the pro-inflammatory cytokines IL-1β and IL-6 and increases in chemokine (MCP-1) and adhesion molecules (VCAM) *(Data now shown).*

The observed effect of AQX-1125 (3 and 30 mg/kg, p.o.) on CP-induced pain scores is shown in *Figure 2*. Results are expressed as mean ± S.E.M. (n=10/group). AQX-1125 decreased the mechanical pain threshold at 3 mg/kg and 30 mg/kg ($p<0.01$) when compared to vehicle.

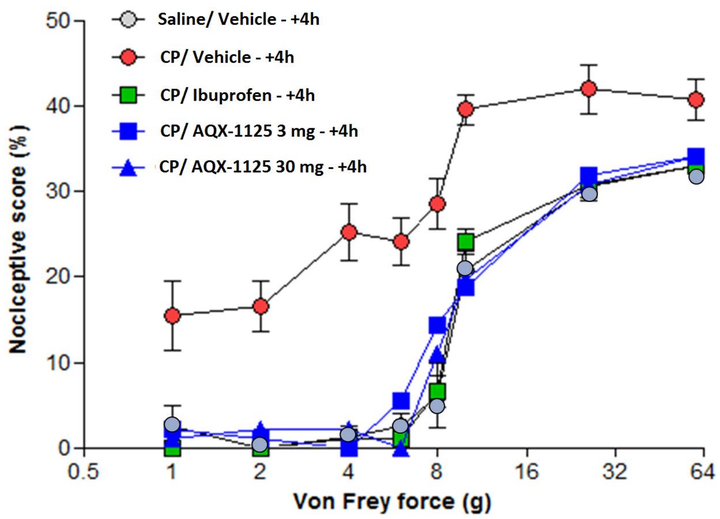

Figure 2. Effect of AQX-1125 on Nociceptive Score

AQX-1125 significantly inhibited the CP-induced nociceptive pain score at both 3 mg/kg and 30 mg/kg ($p<0.0001$) when compared to vehicle. Tissue levels of the four acute inflammatory mediators were not significantly affected by administration of AQX-1125.

The observed effect of AQX-1125 (3 and 30 mg/kg, p.o.), its vehicle or ibuprofen on hemorrhage scores 4 hours after saline or CP treatment in rats, can be seen in *Figure 3*. Results are expressed as mean ± S.E.M. (n=9-10/group).

RESULTS

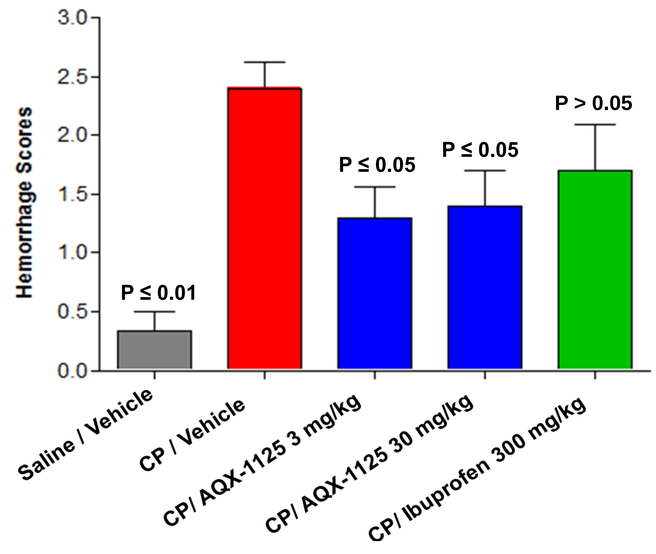

Figure 3. Effect of AQX-1125 on Hemorrhage Scores

Compared to saline-treated rats, acute CP induced a significant increase in hemorrhage scores at 4 hours post-administration (*Figure 3*). Compared to CP/vehicle, a significant decrease in hemorrhage scores was observed in animals administered AQX-1125 at both doses (*Figure 3*).

SUMMARY

In summary, AQX-1125 significantly inhibited the nociceptive pain response in CP induced rats. AQX-1125 was also shown to prevent bladder hemorrhage.

Aquinox is exploring other pre-clinical BPS/IC models in inflammatory urology, as well as currently having an ongoing Phase II trial investigating AQX-1125 in BPS/IC patients (LEADERSHIP study) with pain as the primary endpoint.

REFERENCES

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