Deucrictibant Inhibits Carrageenan-Induced Edema in Bradykinin B2 Receptor Transgenic Rat

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Introduction

- Deucrictibant is an orally administered, specific antagonist of the bradykinin B2 receptor under development for prophylactic and on-demand treatment of hereditary angioedema (HAE) attacks
- Deucrictibant is a potent antagonist at the human bradykinin B2 receptor but is a weak antagonist at the rat ortholog (>100-fold lower potency), which is indicative of species selectivity[1]
- To address the challenge of deucrictibant's species selectivity in experimental models (eg, the paw edema model in rats), a humanized bradykinin B2 receptor transgenic (Tg) rat line was developed and validated
- Following the validation, the Tg rat was used in the carrageenan-induced paw edema model to investigate the *in vivo* primary pharmacodynamic (PD) effects of deucrictibant

Materials and Methods

- A Tg rat line, expressing a humanized bradykinin B2 receptor, was generated on a Sprague-Dawley background using CRISPR/Cas9- mediated gene editing. This rat line showed no adverse phenotypes and appeared a healthy strain
- The potency of deucrictibant and icatibant, an established bradykinin B2 receptor antagonist, to inhibit the bradykinin-induced intracellular Ca2+ mobilization was evaluated in HEK293 cells stably expressing the recombinant wild type (WT) or Tg rat bradykinin B2 receptor using a fluorimetric method. The half maximal inhibitory concentration (IC_{50}) and the equilibrium dissociation constant (Kb) values were calculated
- Membrane preparations from WT and Tg rat uterus were used in radioligand binding inhibition experiments to determine the affinity (Ki value) of deucrictibant for the endogenously expressed B2 receptor. The assay was validated with icatibant
- The *in vivo* effects of deucrictibant and icatibant were examined on paw edema induced by unilateral intraplantar injection of carrageenan (0.75 mg in 0.05 mL/paw) in the hind paw of female Tg rats.
- The carrageenan-induced paw edema model is widely used to assess the activity of anti-inflammatory agents and marketed HAE medicines
- Given that multiple inflammatory pathways are active in this model, compounds in general show a partial inhibition of carrageenan-mediated paw swelling
- Deucrictibant was administered orally at 0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg in female Tg rats, 30 minutes before carrageenan injection
- Icatibant (1 mg/kg) was administered intravenously 2 minutes before carrageenan injection.
- The positive control acetylsalicylic acid (512 mg/kg) was administered orally 60 minutes before carrageenan injection
- The volume of the paw was measured by hydroplethysmometry prior to carrageenan injection and at 2, 4, and 8 hours after carrageenan injection

Results

Potency of deucrictibant at recombinant WT and Tg rat bradykinin B2 receptors

- The potency of the agonist bradykinin measured as the half maximal effective concentration (EC_{50}) at the recombinant WT and Tg rat bradykinin B2 receptors was 105 and 113 pM, respectively.

Table 1: Antagonist potency of icatibant and deucrictibant at recombinant WT and Tg rat bradykinin B2 receptors in HEK293 cells

	WT B2		Tg B2		Ratio K_b WT B2 vs Tg B2
	IC_{50} (nM)	K_b (nM)	IC_{50} (nM)	K_b (nM)	
Icatibant	2.45 ± 0.12	0.59 ± 0.02	2.04 ± 0.48	0.53 ± 0.10	1.10
Deucrictibant	251.00 ± 76.00	61.00 ± 23.00	1.72 ± 0.45	0.45 ± 0.10	136.00

Values are mean ± SD; n=3 to 4 for icatibant and deucrictibant. IC_{50}, half maximal inhibitory concentration; K_b, equilibrium dissociation constant; WT, wild type

- Icatibant was equally potent at the WT and Tg rat bradykinin B2 receptors (**Table 1**)
- The antagonist potency of deucrictibant increased 136-fold at the heterologously expressed Tg receptor as compared to the WT rat receptor
- The potency of deucrictibant 0.45 nM for the Tg rat B2 receptor is similar to the potency for the human bradykinin B2 receptor (0.15 nM)
- Based on these data it was decided to create a Tg rat line expressing this humanized bradykinin B2 receptor

Affinity of deucrictibant for the bradykinin B2 receptor in uterus tissue of WT and Tg rats

- Saturation binding experiments with [^{3}H]BK showed a mean binding capacity (Bmax) of 0.027 and 0.010 pmol/mg protein, and a mean dissociation constant (KD) of 0.72 and 0.39 nM for WT and Tg rat uterus membranes, respectively (n=3)

Table 2: Affinity of icatibant and deucrictibant for bradykinin B2 receptors in uterus from WT and Tg rats

	WT B2		Tg B2		Ratio K_i values WT vs Tg rat
	IC_{50} (nM)	K_i (nM)	IC_{50} (nM)	K_i (nM)	
Icatibant	0.58 ± 0.18	0.34 ± 0.10	0.32 ± 0.10	0.14 ± 0.05	2.40
Deucrictibant	25.20 ± 6.90	14.90 ± 4.10	1.24 ± 0.33	0.55 ± 0.14	27.00

Values are mean ± SD; n=5 for icatibant and n=3 for deucrictibant. IC_{50}, half maximal inhibitory concentration; K_i, inhibitory constant; Tg, transgenic; WT, wild type.

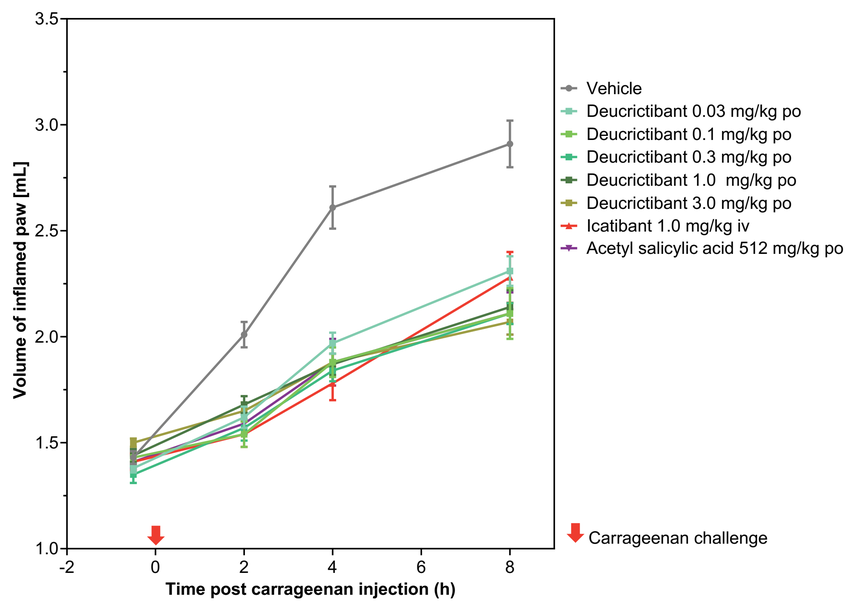
- The affinity of icatibant for the bradykinin B2 receptor in Tg rat uterus membranes was in the same order of magnitude as the affinity for the bradykinin B2 receptors in WT rat uterus membranes (**Table 2**)
- Humanization of the bradykinin B2 receptor resulted in a 27-fold increase in affinity of deucrictibant for the bradykinin B2 receptor in membrane preparations from Tg rat uterus compared to WT rat uterus
- The Ki at the bradykinin B2 receptor in uterus membranes from Tg rats is in the same range as the reported Ki for the recombinant human bradykinin B2 receptor (0.55 nM versus 0.47 nM)[1]

Results

Effect of deucrictibant on carrageenan-induced paw edema in Tg rats

- Intraplantar injection of carrageenan in the hind paw of Tg female rats induced a marked increase in paw volume at 2, 4, and 8 hours pos-tdose, indicative of gradual development of edema (**Figure 1**)

Figure 1: Inhibition of carrageenan-induced paw edema



- Deucrictibant, icatibant, and the control acetylsalicylic acid partially prevented carrageenan-induced development of paw edema at 2, 4, and 8 hours after carrageenan injection (**Figure 1, Table 3**)

Table 3: Inhibition of carrageenan-induced paw edema

	% Inhibition of carrageenan-induced paw edema		
Time post-carrageenan injection	**2 hours**	**4 hours**	**8 hours**
Deucrictibant 0.03 mg/kg po	58.7 ± 6.2	49.5 ± 4.3	37.4 ± 4.1
Deucrictibant 0.1mg/kg po	81.7 ± 12.1	61.6 ± 6.8	53.9 ± 9.1
Deucrictibant 0.3 mg/kg po	61.5 ± 9.2	58.1 ± 5.0	48.2 ± 2.7
Deucrictibant 1.0 mg/kg po	57.6± 6.4	63.2 ± 4.3	52.7 ± 6.1
Deucrictibant 3.0 mg/kg po	74.8 ± 9.3	68.3 ± 4.8	61.7 ± 5.0
Icatibant 1 mg/kg iv	78.1 ± 10.5	68.2 ± 5.7	40.9 ± 7.9
Acetyl salicylic acid 512 mg/kg po	68.3 ± 6.0	60.1 ± 8.0	52.6 ± 6.2

Values are mean ± SEM for n=11. iv, intravenous; po, administered orally

- All doses of oral deucrictibant inhibited paw edema in Tg rat.
- Doses of 0.3, 1, and 3 mg/kg retained their efficacy up to the 8-hour time point (**Table 3**)

Conclusions

- **The genetically engineered humanized bradykinin B2 receptor Tg rat model is a viable tool to address the challenge of human species selectivity of deucrictibant**
- **Deucrictibant showed a 136-fold increased antagonist potency for the recombinantly expressed humanized bradykinin B2 receptor vs WT**
- **Deucrictibant showed a near 30-fold increased affinity for the endogenous bradykinin B2 receptor in uterus tissue from the Tg rat vs WT rat**
- **The rat line is pharmacologically responsive to bradykinin B2 receptor antagonists and can be used to study the pharmacodynamic properties of deucrictibant *in vivo***
- **Oral deucrictibant was effective at inhibiting carrageenan-induced paw edema in humanized bradykinin B2 receptor Tg rats**

References

1. Lesage A., Marceau F., Gibson C., et al. Int Immunopharmacol. 2022:105:108523.

COI: A.L.: employee of GrayMatters Consulting and consultant to Pharvaris, holds stocks/stock options in Pharvaris; advisor to Kosa Pharma; **B.L:** consultant to Pharvaris, holds stocks/stock options in Pharvaris; **E.P.:** employee of Pharvaris, holds stock in Pharvaris; **C.G.:** employee of AnalytiCon Discovery, holds stocks/stock options in Pharvaris; **J.K.:** employee of JCK Consult and consultant to Pharvaris, holds stocks/stock options in Pharvaris.

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