Rationale for MRGPRX4 Antagonism as a Treatment for Cholestatic and Uremic Pruritus

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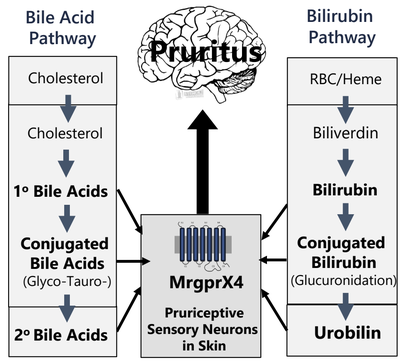
INTRODUCTION

Cholestatic and uremic pruritus represent a large unmet medical need:

- Patients with cholestatic liver disease, such as primary biliary cholangitis and primary sclerosing cholangitis, often suffer from chronic itch (cholestatic pruritus, CP), that is experienced by up to 70-80% of these patients over the course of their disease.[1]
- Similarly, chronic itch (uremic pruritus, UP) is commonly observed in chronic kidney disease, with a prevalence of up to 55% among patients with end-stage renal disease (ESRD) receiving hemodialysis.[2]
- The pathophysiology of CP and UP is not fully understood as the itch-causing pruritogen(s) and their cognate receptor(s) have remained largely elusive. There is compelling clinical evidence that bile acids are involved in CP, but similar evidence is absent for UP.
- Recent publications have identified the novel multi-ligand chemosensory receptor MRGPRX4 to be activated by both bile acids and heme (bilirubin) metabolites *in vitro,* and it has been demonstrated that MRGPRX4 agonists can cause itch in humans.[3-5]
- MRGPRX4, located in a subset of pruriceptive sensory neurons of the dorsal root ganglia (DRG) that innervate the skin, is activated by human bile extract and specifically by numerous bile acids and bilirubin which are often elevated in patients with cholestatic liver disease.



- We have identified additional novel MRGPRX4 agonists, including urobilin, a bilirubin metabolite that is renally excreted and capable of inducing a strong, dose-dependent itch response in mice. These findings further strengthening the involvement of MRGPRX4 as a novel target for treatment of both CP and UP.

AIM

To further define the pharmacology of MRGPRX4 agonism and identify endogenous agonists as potential mediators of pruritus in cholestatic liver disease and chronic kidney disease patients.

METHODS AND MATERIALS

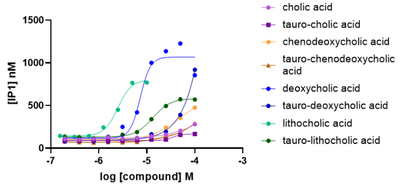
In Vitro studies: HEK293 cells were stably transfected to express human MRGPRX4 with an N-terminal HA tag (Beacon Discovery, San Diego, CA). IP-1 was measured using the IP-One – G_q Kit purchased from Cisbio.

In Vivo studies: Mice were equilibrated in chambers for 30 minutes prior to subcutaneous drug (MRGPRX4 agonist or vehicle) administration at the nape, between the scapulae. Individual scratch bouts were defined as any event where the hind paw was clearly lifted from the platform and a high frequency scratching motion towards the body or head was observed.

RESULTS

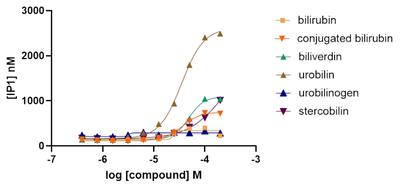
Identification of novel MRGPRX4 agonists: most components of hepatic bile activate MRGPRX4.

Figure 1a. Conjugated and unconjugated primary and secondary bile acids activate MRGPRX4 with varying potencies and efficacies



Results for glyco-conjugates (not shown) were similar to those for the tauro-conjugates

Figure 1b. Among heme metabolites, urobilin was the most effective activator of MRGPRX4 with efficacy ~10-fold higher than bilirubin



MRGPRX4 is activated by several approved medications associated with itch.

- Nateglinide, a meglitinide approved for the treatment of type 2 diabetes that has been shown to cause itch when injected intradermally in humans[6], activates MRGPRX4.
- Obeticholic acid, a synthetic bile acid derivative approved for the treatment of PBC and associated with dose-dependent itch[7], and its glyco- and tauro-conjugates also activate MRGPRX4.
- Ursodeoxycholic acid, and to a much lesser extent its glyco- and tauro-conjugates, also activate MRGPRX4.

Figure 2. MRGPRX4 agonist properties of nateglinide, obeticholic acid, ursodeoxycholic acid and their respective conjugates

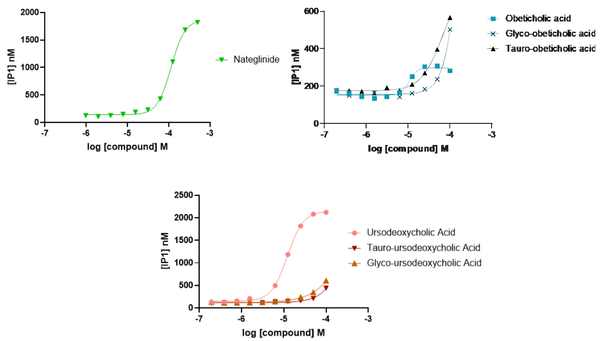
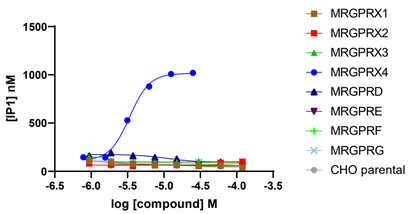


Table 1. MRGPRX4 agonist potencies and efficacies of bile acids, heme metabolites, and medications

	EC_{50} (μM)	% Efficacy
Primary bile acids		
Cholic acid	>84	26%
Tauro-cholic acid	>100	10%
Chenodeoxycholic acid	29	52%
Tauro-chenodeoxycholic acid	>100	15%
Secondary bile acids		
Deoxycholic acid	9	108%
Tauro-deoxycholic acid	>70	172%
Lithocholic acid	2	88%
Tauro-lithocholic acid	20	45%
Heme metabolites		
Biliverdin	68	21%
Bilirubin	10	14%
Conjugated Bilirubin	78	52%
Urobilinogen	>53	16%
Stercobilinogen	>40	18%
Urobilin	18	76%
Medications		
Nateglinide	160	109%
Obeticholic acid	10	42%
Glyco-obeticholic acid	>100	35%
Tauro-obeticholic acid	70	74%
Ursodeoxycholic acid	60	150%
Glyco-ursodeoxycholic acid	>60	8%
Tauro-ursodeoxycholic acid	>60	4%

Specificity: The agonist properties of bile acids are selective for MRGPRX4, with no activity detected at any of the other 7 human MRGPRs (shown below for deoxycholic acid)

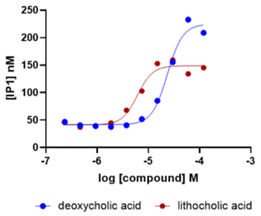
Figure 3. Deoxycholic acid is a selective agonist for MRGPRX4



Bile acids activate MRGPRX4 in primary SH4 cells.

- We established that SH4 melanoma cells endogenously express MRGPRX4 at sufficient levels to measure agonist induced IP-1 levels and demonstrated activation by bile acids and urobilin.

Figure 4. Bile acids activate SH4 cells that natively express MRGPRX4

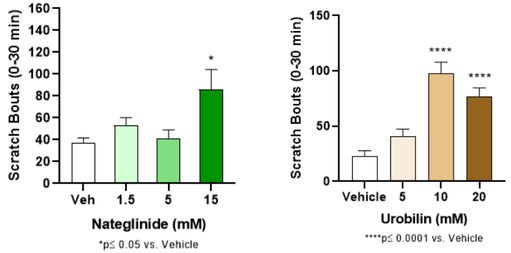


	SH4 EC_{50} (μM)	CHO EC_{50} (μM)
Deoxycholic acid	24	9
Lithocholic acid	6	2

***In vivo*, both nateglinide and urobilin induce itch in C57BL/6J male wild type mice.**

- This effect is likely mediated by a yet to be fully identified rodent ortholog(s) of human MRGPRX4.

Figure 3. Urobilin and nateglinide induce itch in mice



CONCLUSION

- **Most components of hepatic bile, including a broad range of primary and secondary bile acids as well as heme metabolites, were found to be agonists at MRGPRX4.**
- **These findings confirm and expand upon prior reports that MRGPRX4 is activated by bile acid and heme metabolites, including those that may be elevated in cholestatic and/or uremic states.**
- **Variability in the accumulation of different bile acids and heme metabolites, both in blood[7] and skin[8], coupled with differences in their affinity, potency, and efficacy at MRGPRX4, may in part explain inter-individual differences in the occurrence and intensity of CP in patients with cholestatic liver disease.**
- **Our findings support the role of MRGPRX4 as a central and specific mechanism of pruritus in CP and UP.**
- **MRGPRX4 antagonism, aimed at non-competitively blocking the activation of the receptor by the full range of agonists, is thus a potentially attractive and elegant therapeutic approach to meaningful relief of the itch associated with both cholestatic and uremic diseases.**

REFERENCES

1) Mittal, 2016: Curr Probl Dermatol, 50:142-8
2) Hu et al., 2018: Medicine, 21:e10633
3) Meixiong et al., 2019: PNAS, 116:10525-30
4) Meixiong et al., 2019: eLife, 8:e44116
5) Yu et al., 2019: eLife, 8: e48431
6) Nevens et al., 2016: N Engl J Med,18:375
7) Hegade et al., 2019: Liver International, 39:967-75
8) Schoenfield et al., 1967: Nature, 213:93-4
9) Yeager et al., AASLD 2021, Poster Publication Number 1262
10) Taylor et al., AASLD 2021, Poster Publication Number 1272

DISCLOSURES

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