

Pharmacological Profiling of Dihydroergotomine and its Relevance to Systemic Delivery via the Respiratory Tract

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OBJECTIVE

This study researched potential for pharmacologically-mediated adverse effect (AE) differences between intravenous and MAP0004, orally inhaled dihydroergotamine mesylate (DHE), and also explored a pharmacological rationale for the absence of local pulmonary effects from this inhaled systemic therapy.

This work first screened for DHE activity at a wide range of targets, and then investigated active receptor pharmacology (serotonin (5-HT), adrenergic, dopaminergic) of dihydroergotamine mesylate in vitro, based on the C_{max} levels reported following MAP0004 and intravenous (IV) dosing in a Phase 1 clinical study.

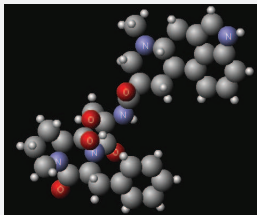
PHARMACOLOGY

DHE (Figure 1) is a semi-synthetic ergot alkaloid used in the treatment of migraine since 1946. DHE is typically administered by intravenous injection in clinical settings or by intranasal delivery or intramuscular/subcutaneous injection in non-clinical environments.

DHE has wide ranging pharmacology mediated by effects on biogenic amine receptors - due to structural similarities with physiological mediators - specifically serotonin (5-HT) subtypes, adrenergic (α and β) subtypes and dopamine (D) subtypes.

In acute migraine therapy, DHE is considered to mediate its effects through 5-HT_{1B} receptors (constriction of intracranial extracerebral blood vessels) and 5-HT_{1D} receptors (inhibition of trigeminal neurotransmission).

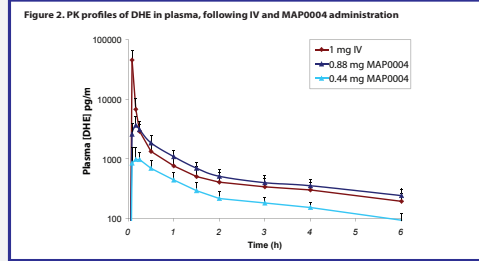
Figure 1. Chemical Structure of DHE



EXPERIMENTAL RATIONALE

A large plasma spike was observed in Phase 1 PK data (Figure 2) following IV administration, but not for MAP0004. This large spike was associated with a greater incidence of adverse effects - nausea (63% IV vs 0% MAP0004), dizziness (44% IV vs 11% MAP0004) and paresthesia (31% IV vs 0% MAP0004).

This plasma spike difference (12 - 15 fold difference) was hypothesized to be associated with the reported IV side effect profile, despite much smaller differences in AUC (of only 20%) between 1 mg IV and 0.88 mg MAP0004. As a result, investigations of receptor activity at the C_{max} concentrations were expected to provide rationale for the differences observed in the AE profile.



METHODS

A comprehensive radioligand pharmacology screen was performed with a high concentration DHE control (5 μ M, 65 times higher concentration than IV C_{max}) across 67 receptors, ion channels and enzymes (NovaScreen Biosciences Corp., Hanover, MD). Activity was defined as percent receptor binding/response where % binding >50% was considered to be an active response and <20% was deemed inactive. For active signals, subsequent receptor binding was completed for DHE at plasma concentrations reflective of C_{max} for IV administration and for 0.44 mg systemic equivalent (2 inhalations) and 0.88 mg systemic equivalent (4 inhalations) (53, 1.4 and 4.3 ng/mL respectively).

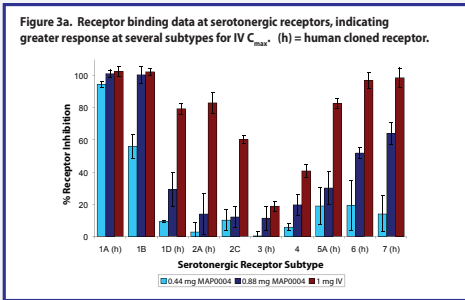
Functional activity at receptors was determined using two G-protein coupled receptor (GPCR) methods. First, calcium flux screening (Millipore Corp, St. Charles, MO) measured the response of DHE at 5-HT_{1B}, 5-HT_{2B}, D₂ and D₂ receptors. Second, Tango β -lactamase assays were used to confirm response at 5-HT_{1B} receptors and probe response at the 5-HT_{1D} target (Invitrogen, Madison, WI).

RESULTS & DISCUSSION

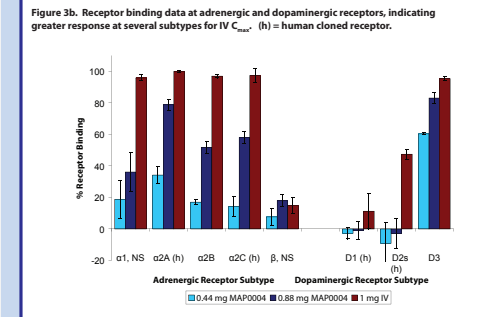
The comprehensive screen at high DHE concentration showed activity (>50% binding) at the following receptors: serotonin (5-HT_{1A}, 1B, 1D, 2A, 2C, 4, 5A, 6, 7), adrenergic (α 1A, 1B, 2A, 2B, 2C), and dopaminergic (D₁, 2, 3, 4) subtypes.

Receptor sites where DHE had been shown to have activity were then screened at the concentrations reflective of C_{max} from clinical trials. Markedly different binding responses between C_{max} for IV and MAP0004 dosing were reported, which might explain the elevated adverse effect frequency for IV DHE (Figures 3a, 3b).

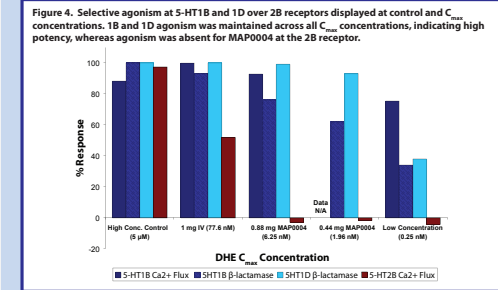
As concentration-dependent binding differences had been established, subsequent functional receptor screens were employed to further probe pharmacological differences between IV and MAP0004 delivery. This approach ensured that binding in fact translated into function at the receptor.



RESULTS & DISCUSSION continued



Selectivity for desired antimigraine action over CVS effects: A comparison between 5-HT_{1B} and 5-HT_{2B} functional responses is presented in Figure 4. Across all C_{max} concentrations, DHE retained its anti-migraine 5-HT_{1B} and 5-HT_{1D} activity, whereas 5-HT_{2B} agonism was only maximal at the high concentration (5 μ M) control, then reduced to 50% agonism at IV C_{max} (i.e. at 12-15 fold higher concentrations than after MAP0004 clinical administration). For C_{max} equivalent to 0.88 mg MAP0004 and lower, the greatly reduced C_{max} elicited no response at this receptor. Chronic 5-HT_{2B} agonism has been associated with adverse long term effects, a fibroproliferative cardiac valvulopathy, for drugs acting at this receptor subtype (e.g. pergolide), raising the possibility that more selective receptor activation, seen with the C_{max} following MAP0004 administration presents an opportunity to minimize the potential for side effects over IV administration. Also, no β blockade (Figure 5) or activation was observed at all concentrations, also supportive of less CVS effects of DHE.



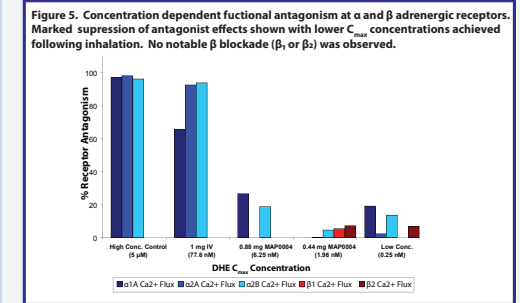
Nausea: Classic nausea targets are 5-HT₃, D₂ and muscarinic receptors.

- Functional screens at the 5-HT₃ receptor showed no activity, in agreement with binding data (Figure 3a), ruling out a role in nausea for DHE at this target (5-HT₃ activation elicits nausea and vomiting).
- Agonist effects at the dopamine D₂ receptor have been implicated as a possible mechanism for DHE side effects (1). However, functional screens confirmed anti-nauseant D₂ antagonism, thus opposing this theory.
- No activation of M₁, M₂, M₃, M₄ and M₅ muscarinic receptors was observed.

Ongoing investigations are targeting the identification of the mechanisms responsible for the high incidence of nausea following IV DHE administration. This may involve a complex interplay between other serotonergic receptors involved in reduced GI tract motility (6,7), known to cause nausea at the peripheral level.

Dizziness: Profiling at adrenergic receptors (α 1A, α 2A and α 2B) showed marked antagonism at C_{max} for IV DHE in accordance with published literature (66%, 92% and 93% antagonism at α 1A, α 2A and α 2B respectively). In contrast, minimal antagonism was recorded at C_{max} for 0.88 mg MAP0004 and lower (Figure 5). Acute alpha adrenoceptor blockade is associated with dizziness, and as such, the increased dizziness reported following IV administration is expected to be associated with these receptors.

Absence of Local Pulmonary Pharmacology: Radioligand binding data showed no activity at the high concentration control (5 μ M) at multiple adenosine, muscarinic and leukotriene receptor subtypes. An absence of functional agonism of the M₃ receptor was confirmed with Ca²⁺ flux assay. Marginal binding of 45% was reported at the β 2-adrenoceptor at 5 μ M, however, subsequent functional agonist and antagonist screens at high concentration control (both <0%) and C_{max} concentrations confirmed a total absence of pharmacological activity at this receptor (Figure 5). From this data, it is expected that DHE is not likely to interfere with asthma pharmacology, which is important as asthma and migraine are co-morbid (8).



CONCLUSION

Pharmacological differences, particularly those that can elicit side effects, have been demonstrated between C_{max} concentrations that model IV DHE and MAP0004. The lower C_{max} associated reduction in receptor activity for MAP0004 correlates with a more favorable side effect profile, while essential activity at anti-migraine receptors is retained. In addition, DHE has not been shown to interact with receptors involved in airways regulation and function, and is therefore less likely to have direct pulmonary effects, in agreement with clinical findings (9).

REFERENCES

- Silberstein S, McCrory D. (2003), "Ergotamine and dihydroergotamine: history, pharmacology, and efficacy," *Headache*, Vol 43, pp 144-166.
- Aelling W. (1984), "Investigation of the vasoconstrictor effect of 8-hydroxy-dihydroergotamine, the main metabolite of dihydroergotamine, in man," *Eur J Clin Pharmacol*, Vol 26, pp 239-242.
- Hannon N, Saunim F, Lanfumey L, Hamon M, Bourgoignie S. (2003), "Dihydroergotamine and its metabolite, 8-hydroxy-dihydroergotamine, as 5-HT_{1A} receptor agonists in the rat brain," *Br J Pharmacol*, Vol 139, pp 424-434.
- Schaerlinger B, Hickel P, Etienne N, Guesnier L, Maroteaux L. (2003), "Agonist actions of dihydroergotamine at 5-HT_{2B} and 5-HT_{2C} receptors and their possible relevance to antimigraine efficacy," *Br J Pharmacol*, Vol 140, pp 277-284.
- Shrewsbury S, Cook R, Taylor G, Edwards C, Ramadan N. (2007), "Comparative clinical pharmacokinetics of parent drug and metabolites following inhaled dosing with dihydroergotamine mesylate via a novel system (TempoTM Inhaler)," *Abstract*, *Headache*, Vol 47, pp 754.
- Taladhar B, Ge L, Naylor RJ. (2003), "5-HT₇ receptors mediate the inhibitory effect of 5-HT on peristalsis in the isolated guinea-pig ileum," *Br J Pharmacol*, Vol 138, No 7, pp 1210-14.
- De Ponti F, Tonini M. (2001), "Irritable bowel syndrome: new agents targeting serotonin receptor subtypes," *Drugs*, Vol 61, No 3, pp 317-32.
- Scher A, Bigal M, Lipton R. (2005), "Comorbidity of migraine," *Curr Opin Neurol*, Vol 18, pp 305-310.
- Shrewsbury S, Kori S, Miller D, Pedinoff A, Weinstein S. (2008), "Randomized, double blind, placebo controlled study of the safety, tolerability and pharmacokinetics of MAP0004 (orally-inhaled DHE) in adult asthmatics," *Curr. Med. Res. Opin.*, Vol 24, pp 1977-1985.