

# PRECISE PULMONARY DELIVERY OF DIHYDROERGOTAMINE AND MECHANISM FOR REDUCED ADVERSE EFFECT PROFILE

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## OBJECTIVE

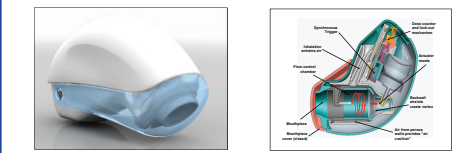
This study investigated potential mechanisms to explain how MAP0004, orally inhaled dihydroergotamine (DHE), retains efficacy but minimizes pharmacologically-mediated adverse effects (AE) compared to intravenous (IV) DHE as observed in clinical trials. The pharmacological rationale for the absence of local pulmonary effects from this inhaled systemic therapy was also explored.

DHE activity at a wide range of targets, and active receptor pharmacology (serotonin (5-HT), adrenergic, dopaminergic) of dihydroergotamine mesylate were investigated in vitro. These evaluations were conducted at DHE concentrations equivalent to the C<sub>max</sub> levels observed following MAP0004 and intravenous administration in human pharmacokinetic (PK) studies.

## MAP0004 BACKGROUND

MAP0004 is orally inhaled and self-administered at home using MAP Pharmaceuticals' proprietary Tempo<sup>®</sup> inhaler (Figure 1). The Tempo inhaler conveniently and consistently dispenses drug automatically when the patient inhales.

Figure 1: The Tempo inhaler (left) and detailed cross section (right) outlining the main functional elements of the inhaler, with the mouthpiece in the closed position.



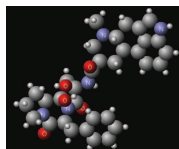
## PHARMACOLOGY

DHE (Figure 2) 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<sub>1B</sub> receptors (constriction of intracranial extracerebral blood vessels) and 5-HT<sub>1D</sub> receptors (inhibition of trigeminal neurotransmission).

Figure 2: Chemical structure of DHE



## EXPERIMENTAL RATIONALE

High plasma concentration peaks (C<sub>max</sub>) were observed in PK data (Figure 3a) for IV administration, but not for MAP0004. Subjects with high C<sub>max</sub> reported correspondingly higher incidence of adverse effects (Figure 3b).

This 12 - 15 fold difference in C<sub>max</sub> was hypothesized to be associated with the increased IV side effect profile, despite much smaller differences in AUC (of only 20%) between 1 mg IV and 0.88 mg MAP0004. Receptor activity at these C<sub>max</sub> concentrations were evaluated to explore the rationale for the differences observed in the AE profile of the two delivery routes.

Figure 3a: PK profiles of DHE in plasma, following IV and MAP0004 admin

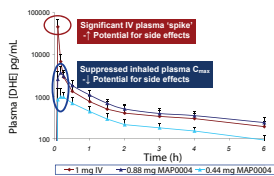
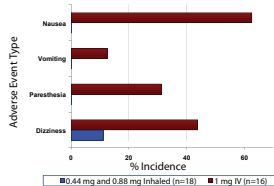


Figure 3b: Frequency of adverse effects (%)



## METHODS

A comprehensive radioligand pharmacology screen was performed with a high concentration DHE control (5 μM, 65 times higher concentration than IV C<sub>max</sub>) 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<sub>max</sub> 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. Calcium flux screening (Millipore Corp., St. Charles, MO) measured the response of DHE at 5-HT<sub>2B</sub>, D<sub>2</sub> and β<sub>2</sub> receptors. Also, Tango β-lactamase assays were used to confirm response at 5-HT<sub>1B</sub> and at the 5-HT<sub>1D</sub> target (Invitrogen, Madison, WI).

## RESULTS & DISCUSSION

### A Receptor Binding Data

A comprehensive screen of receptors, ion channels and enzymes at high DHE concentration showed activity (>50% binding) at the following receptors: serotonergic (5-HT<sub>1A</sub>, 1B, 1D, 2A, 2C, 4, 5A, 6, 7), adrenergic (α<sub>1A</sub>, 1B, 2A, 2B, 2C), and dopaminergic (D<sub>1</sub>, 2, 3, 4) subtypes.

Receptor sites where DHE had been shown to have activity were then screened at the concentrations reflective of C<sub>max</sub> from clinical trials. Markedly different binding occupancy between C<sub>max</sub> for IV and MAP0004 dosing were reported, which might explain the elevated adverse effect frequency for IV DHE (Figures 4a, 4b).

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.

Figure 4a: Receptor binding data at serotonergic receptors, indicating greater occupancy at several subtypes for IV C<sub>max</sub>. (h) = human cloned receptor.

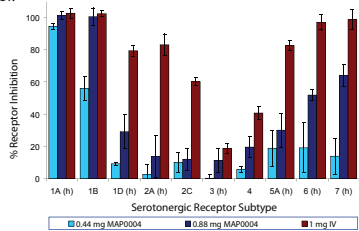
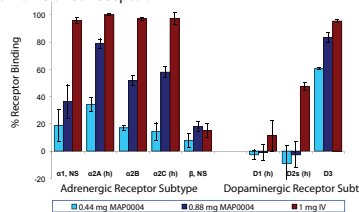


Figure 4b: Receptor binding data at adrenergic and dopaminergic receptors, indicating greater occupancy at several subtypes for IV C<sub>max</sub>. (h) = human cloned receptor.

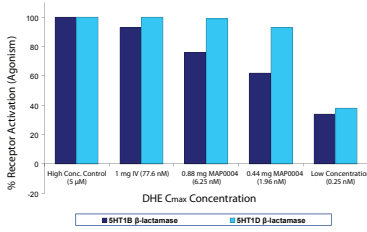


### B Receptor Functional Data

MAP0004 maintains desirable anti-migraine activity at key receptors.

Across all C<sub>max</sub> concentrations (IV and MAP0004), DHE retained its anti-migraine 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> activity (Figure 5). Sub-nanomolar potency was confirmed using functional binding curves to determine EC<sub>50</sub> at the receptor targets (the concentration at which 50% agonism is achieved). EC<sub>50</sub> was 0.81 nM and 0.35 nM for 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptors, respectively.

Figure 5: Agonism at antimigraine 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptors was maintained across all C<sub>max</sub> concentrations, indicating high potency.



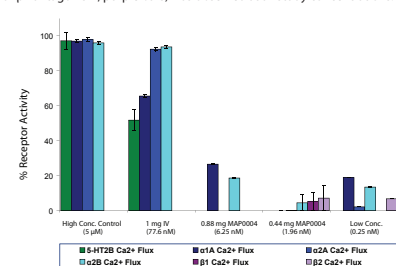
## RESULTS & DISCUSSION

### C Cardiovascular and Other Side Effect Profile

No β<sub>1</sub> adrenoceptor blockade (Figure 6) or activation was observed at all concentrations investigated. This is supportive of a reduced tendency for DHE to exert cardiovascular effects, particularly on blood pressure.

In addition, chronic 5-HT<sub>2B</sub> agonism has been associated with adverse long term effects that may present as a fibroproliferative cardiac valvulopathy, for drugs acting at this receptor subtype (e.g. pergolide). Results confirmed that 5-HT<sub>2B</sub> agonism was only maximal (Figure 6) at the high concentration (5 mM) control, then reduced to 50% agonism at IV C<sub>max</sub> (i.e. at 12-15 fold higher concentrations than after MAP0004 clinical administration). Importantly, at C<sub>max</sub> equivalent to 0.88 mg MAP0004 and lower, the greatly reduced C<sub>max</sub> elicited no functional response at this receptor. This concentration dependent effect shows greater receptor selectivity for MAP0004, as the reduced C<sub>max</sub> following MAP0004 administration presents an opportunity to minimize the potential for side effects over IV administration.

Figure 6: Agonism at 5-HT<sub>2B</sub> clearly absent for MAP0004 equivalent concentrations (green bars - agonism present only for IV C<sub>max</sub> concentration and high concentration control). Concentration dependent functional antagonism at adrenergic receptors was observed (blue bars), as marked suppression of antagonist effects were shown with lower C<sub>max</sub> concentrations achieved following MAP0004 administration. No notable β blockade (β<sub>1</sub> or β<sub>2</sub> antagonism, purple bars) was observed at all study concentrations.



**Nausea:** Classic nausea targets are 5-HT<sub>3</sub>, D<sub>2</sub> and muscarinic receptors. Functional screens at the 5-HT<sub>3</sub> receptor showed no activity, in agreement with binding data (Figure 4a), ruling out a role in nausea for DHE at this target (5-HT<sub>3</sub> activation elicits nausea and vomiting). Agonist effects at the dopamine D<sub>2</sub> receptor have been implicated as a possible mechanism for DHE side effects (1). However, functional screens confirmed anti-nausea D<sub>2</sub> antagonism, thus opposing this theory. No activation of M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub>, M<sub>4</sub> and M<sub>5</sub> muscarinic receptors was observed.

Ongoing investigations are targeting the identification of the mechanisms responsible for the high incidence of nausea following IV DHE administration, but not seen with lower C<sub>max</sub> orally inhaled DHE. 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<sub>1A</sub>, α<sub>2A</sub> and α<sub>2B</sub>) showed marked antagonism at C<sub>max</sub> for IV DHE in accordance with published literature (66%, 92% and 93% antagonism at α<sub>1A</sub>, α<sub>2A</sub> and α<sub>2B</sub> respectively). 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. In contrast, minimal antagonism was recorded at C<sub>max</sub> for 0.88 mg MAP0004 and lower (Figure 6), providing a mechanism for minimal potential for dizziness by this route at these concentrations.

**Absence of Local Pulmonary Pharmacology:** Radioligand binding data showed no activity at the high concentration control (5 mM) at multiple adenosine, muscarinic and leukotriene receptor subtypes. An absence of functional agonism of the M<sub>3</sub> receptor was confirmed with Ca<sub>2+</sub> flux assay. Marginal binding of 45% was reported at the β<sub>2</sub>-adrenoceptor at 5 mM, however, subsequent functional agonist and antagonist screens at high concentration control (both <0%) and C<sub>max</sub> concentrations confirmed a total absence of pharmacological activity at this receptor (Figure 6). From these data, it is expected that DHE is not likely to interfere with asthma pharmacology, which is important as asthma and migraine have been reported as co-morbid conditions (8) and in agreement with previously reported clinical findings (9).

## CONCLUSION

Markedly different binding occupancy and pharmacological differences, particularly those that can elicit side effects, have been demonstrated between C<sub>max</sub> concentrations simulating IV DHE and orally inhaled MAP0004. The C<sub>max</sub> associated reduction in receptor activity for MAP0004 correlates with a more favorable side effect profile including cardiovascular effects, dizziness and nausea, while activity at anti-migraine receptors is retained at these lower C<sub>max</sub> doses. In addition, DHE has not been shown to interact with receptors involved in airway regulation and function, and is therefore less likely to have direct pulmonary effects, in agreement with previously reported clinical findings (9).

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