

NON-INVASIVE MONITORING OF PROMETHAZINE

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INTRODUCTION

Approximately 70% of the crewmembers on the US Space Shuttle experience symptoms of space motion sickness (SMS). SMS results in increased sensitivity to motion and head movement, as well as other symptoms including headache, malaise, lethargy, stomach awareness, loss of appetite, nausea, and episodic vomiting. The frequency and severity of these symptoms has restricted extravehicular activities such that they can begin no sooner than 72 hours after launch.

Since March 1989, Intramuscular (IM) Promethazine (PMZ) has been successfully used to treat the symptoms of SMS. IM administration is preferable to oral administration since SMS itself often leads to inhibition of gastric mobility; further, IM administration minimizes drug loss due to intestinal absorption and liver metabolism. Additionally, IM PMZ remains active at high levels for a relatively long duration (up to about 12 hours). In one study, IM PMZ was more than 90% effective at alleviating SMS symptoms. In another study, the same dosage of IM PMZ was initially reported to be 90% effective at alleviating SMS, but the efficacy dropped to 75% after several hours.

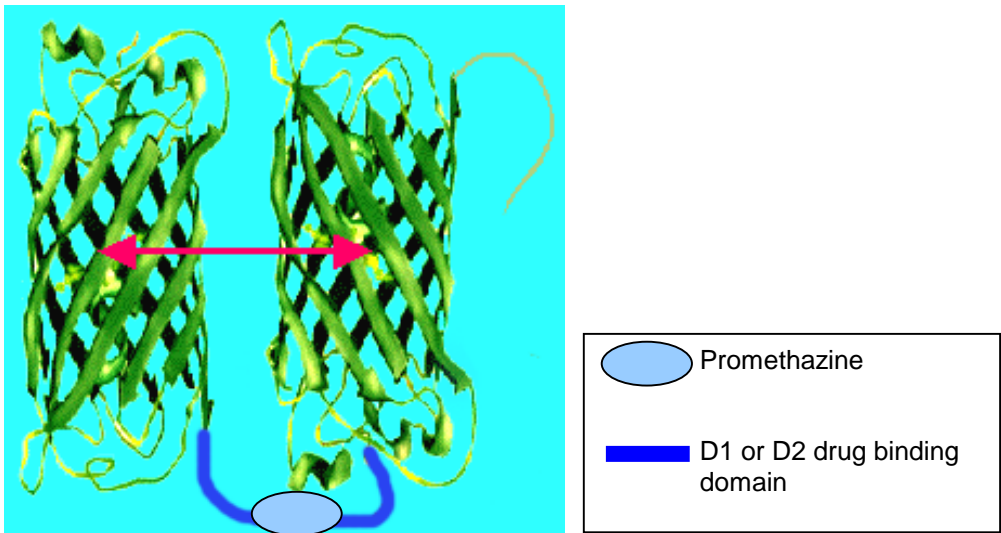
While effective at alleviating SMS, PMZ unfortunately has several side effects including sedation, nausea, vomiting, loss of appetite, and enhanced stomach awareness. On earth, there is a high incidence of sedation associated with PMZ administration and, such an effect propagated to space flight, would undoubtedly impair astronaut ability to operate machinery and carry out assigned mission tasks safely. Interestingly, data from 14 Space Shuttle flights has shown that, of the 21 crew members who received IM promethazine (25-50 mg), only one experienced any associated sedation, reportedly with no operational impact. These limited data show a mere 4.8% of incidence of side effects in space flight as compared to a 73% incidence on earth. [Bagian et al. J Clin Pharmacol 34: 649-651 (1994)]

In all, the pharmacokinetics and bioavailability of IM PMZ administered in space seems to be different than on earth. Because IM PMZ appears to be an invaluable pharmaceutical for space flight, and since there are apparent differences in the drug behavior during space flight, we are developing biosensor to non-invasively monitor the drug metabolism via interstitial fluid.

METHODS

It has been reported that changes in the fluorescence resonance energy transfer (FRET) between two variants of green fluorescent protein (GFP) can be used to monitor cleavage of a protease site localized on a linker between the two GFP variants. Persechini et al. have also demonstrated that a red-shifted and blue-shifted variant of GFP, separated by a calmodulin binding domain linker, exhibit FRET while the two GFP fluorophores are in proximity. If calmodulin, bound to calcium, binds to the calmodulin binding domain between the GFPs, FRET is disrupted. In either of these examples the FRET, or disruption thereof is readily detectable by standard optical techniques.

We are designing a biomolecular sensor that can monitor PMZ levels non-invasively. Our detector molecule is based on the previously successful molecule used for intracellular detection of calcium designed by Persechini et al. We are not choosing to utilize the Histamine H1 receptor for two reasons. First, the clone is not readily available (it was cloned by a Japanese group). Secondly, the dopamine receptor may be more versatile in that we may be able to use this molecule to monitor other drugs that bind to the dopamine receptor, including drugs such as methamphetamines. Thus, in our new biomolecular sensor, the DA receptor extracellular drug binding domains from D1 and D2 will be synthesized by polymerase chain reaction (PCR) and engineered such that they can be readily cloned as a linker between two GFP variants. The resulting molecule will resemble that illustrated in the figure. The thickened line in the picture indicates the DA receptor linker, while each of the green beta-barrels signifies two different variants of the GFP. When PMZ (or dopamine or another control drug) binds to the DA domain, it is thought that the protein will change conformation and the two barrels will separate, disrupting FRET.



Ultimately, we will use this molecule to monitor Ca^{2+} , PMZ, dopamine in interstitial fluid. Interstitial fluid is acquired using a laser porator supplied by SpectRX. This instrument ablates four 80 micron diameter small holes into the skin. This procedure is virtually painless because the holes penetrate only the stratum corneum above the pain receptors. Approximately 1 microliter of interstitial fluid is collected per minute by using this technique. When light suction is applied to the poration holes, patency can be maintained for over 72 hours. Thus, the dynamics of drug metabolism during microgravity exposure can be investigated.

RESULTS

Steady-state and lifetime data of the GFP variants taken with a tau-3 ISA fluoremeter will be presented. Fluorescence lifetime data will be used calculate changes in the distance between red-shifted and blue-shifted GFP variants with and without the target analytes (Ca^{2+} , PMZ, or dopamine). Fluorescence ratio measurements will be presented and compared with the actual concentration of the analyte of interest (Ca^{2+} , PMZ, or dopamine).

CONCLUSION

If GFP-FRET linkers can be adapted to bind analytes of interest with a high degree of selectivity and specificity, this type of biosensor could represent an exciting technology to perform both intercellular and intracellular monitoring.

INDEX TERMS: FRET, biosensor, GFP, promethazine, calcium, dopamine, fluorescence, assay, interstitial fluid