2024 Summer Research Fellowship Program Application

Project Description (1-2 pages)

Title: Remimazolam Pharmacogenetics

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Location: The study will be mainly conducted in Dr. Wang's lab on the RGE4th floor. The data analysis could be completed remotely.

Abstract:
Annually in the United States, over 100,000 deaths and 2 million hospitalizations occur due to drug adverse reactions. With nearly 40 million anesthetic and sedative administrations yearly, and over 2 million reported complications, optimizing these medications is crucial for patient safety, comfort, and procedural success. Remimazolam (Byfavo®), a recently approved ester-linked benzodiazepine for sedation by the US FDA, offers distinct advantages in sedation with its rapid action and predictable duration, highly suitable for widespread anesthesia use. However, its notable interindividual variability in patient responses, accompanied by an adverse effect rate surpassing 50%, poses a significant challenge, leading to patient distress and procedure failure. Consequently, there is an urgent clinical need for optimizing remimazolam use to improve the sedative/anesthetic outcomes.

The favorably short and predictable action of remimazolam is largely attributed to its fast metabolism, primarily involving the hydrolysis of its ester group. Thus, the hydrolysis of remimazolam plays a crucial role in its deactivation, which in turn significantly influences its action duration, overall efficacy, toxicity, and contributes to the variability in its response. Nevertheless, the enzyme(s) responsible for the hydrolysis of remimazolam remain controversial. Carboxylesterase1 (CES1) serves as the primary hepatic hydrolase in humans, participating in the metabolism of numerous therapeutic agents. Significant interindividual variability in expression and activity of CES1 has been consistently reported. Our previous publications have demonstrated that the *CES1* genetic polymorphisms and inhibitors are associated with significantly altered metabolism and/or efficacy of several selective CES1 substrate drugs. Given the ester structure of remimazolam, characterized by a small alcohol group and large acyl group, which aligns with the substrate preference of CES1, however, the experimental evidence identifying the enzymes responsible for remimazolam hydrolysis remains lacking.

The **overarching objective** of this proposal is to identify the enzyme responsible for remimazolam deactivation and to determine the impact of genetic polymorphisms on remimazolam deactivation. Our **central hypothesis** is that the remimazolam is deactivated by CES1 and functional *CES1* genetic polymorphisms can significantly affect the remimazolam deactivation in human liver. The central hypothesis will be examined in two specific aims:

Aim1: To identify the enzyme responsible for CES1 deactivation.
Human liver and intestine s9 fractions, human plasma, CES1 and CES2 recombinant enzymes will be used to test the hypothesis that remimazolam is deactivated by hepatic CES1 specifically.

Aim2: To examine the impact of CES1 genetic polymorphism on the deactivation of remimazolam *in vitro*.
We will assess the impact of CES1 G143E on remimazolam deactivation using wild type CES1, CES1 G143E, and vector transfected cell lines.

Significance:
There is an urgent need for researchers to probe the factors contributing to the interindividual variabilities in drug responses[1]. Every day, millions of individuals are prescribed medications that may not provide effective treatment. Shockingly, among the top ten highest-grossing drugs in the United States, their efficacy rate ranges from benefiting only 1 in 25 to 1 in 4 of those who take them. For some commonly used medications, such as statins, the benefit rate may decrease to as low as 1 in 50[2]. Undesired drug responses contribute significantly to the overall costs of health care, and the associated adverse drug reactions even lead to significant patient morbidity and mortality. Understanding the factors that shape a person's response to a particular treatment is crucial for developing more tailored and effective medical interventions[3].

Approximately 40 million short diagnostic and therapeutic procedures requiring sedation are performed in the US annually (FDA 2012)[4]. However, over 50% of the patients receiving anesthetics or sedatives experienced associated side effect or potential complications. These complications can lead to pain, discomfort,

procedural failure or even additional health issues [5] Thus, the importance of precision anesthesia and sedation cannot be overstated, which ensures not only patient comfort and safety during interventions but also swift recovery, reducing monitoring needs and room occupancy time, consequently saving cost for healthcare system. Moreover, the ability to tailor anesthetic and sedative use, significantly enhances the quality of health care during millions of procedures, ultimately contributing to increased procedural efficiency and optimal patient outcomes[6].

The development of remimazolam represents a remarkable advancement in sedative and anesthetics, which is expected to be widely used in anesthesia and sedation procedures. In January 2020, remimazolam (Byfavo®), a novel benzodiazepine ester derivative, was approved as a general anesthetic in Japan firstly in the world. It received US FDA approval for induction and maintenance of procedural sedation in adults in July 2020. In comparison to current corner stone anesthetics and sedatives, like propofol and midazolam, remimazolam minimizes risk of respiratory depression and offers a smoother patient recovery. Due to its promising profile, remimazolam is increasingly being considered for a wide range of surgical and medical procedures. The extensive range of potential applications underscores the critical need for the precise use of remimazolam, given its capacity to significantly enhance patient care across various medical settings[7-9].

Significant interindividual variability in responses to remimazolam has been observed, which can lead to severe and potentially life-threatening adverse effect[10]. According to an analysis of 36 case reports and 73 trials involving 6740 patients who received remimazolam, common adverse reactions following remimazolam administration include hypotension (33-58%), hypertension (20-42%), respiratory acidosis (19%), bradycardia (4-11%), hypoxia (22%), respiratory rate increased (14%), tachycardia (8%), pyrexia (4%), nausea (4%), and headache (3%). Notably, 68 cases reported delayed emergence, ten cases reported anaphylaxis, and eight cases reported re-sedation[10]. However, the factors contributing to the interindividual variability in remimazolam response remain unclear. Therefore, there is a **critical clinical need** to identify the factors contributing to the interindividual variability in remimazolam response, so that anesthesiologists and medical professionals can tailor anesthetic use to minimize these unwanted consequences for patients.

Research methods:

Aim1: To identify the enzyme responsible for CES1 deactivation.

We will conduct *in vitro* incubation study to determine the enzyme and tissue responsible for the deactivation of remimazolam using human liver and intestine S9 fractions and human serum. Both CES1 and CES2 are expressed in human liver, while CES1 greatly exceeds CES2. In the human small intestine, only CES2 is present. Hydrolytic activity in human serum is attributed to several esterases, including butyrylcholinesterase (BChE), Paraoxonases (PONs), acetylcholinesterase (AChE) and albumin, but neither CES1 nor CES2 is expressed in human serum. We will then validate the results using recombinant CES1 and CES2 enzymes

Aim2: To examine the impact of CES1 genetic polymorphism on the deactivation of remimazolam in vitro.

We will assess prepare s9 fractions from WT CES, CES1 G143E and vector transfected cell lines. Then we will compare the remimazolam deactivation rates among these three groups. The remimazolam inactive metabolite formation will be measured by LC-MS/MS after incubation using the method established in our lab.

Expected Results

We expected 1) remimazolam is only deactivated in the s9 fractions from human liver, but no appreciable hydrolysis in human intestine or serum. Remimazolam is only deactivated in CES1 but not CES2 recombinant enzyme. 2) Remimazolam deactivation rates are significantly lower in the G143E transfected cells compared to the WT CES1.

Contribution to the success of the overall research

At the completion of this study, it is our expectation that we will have established the critical role of CES1 genetic variants on remimazolam metabolism. The findings from this translational project will form a solid foundation for future clinical studies that will validate the impact of CES1 genetic polymorphisms and the drug-drug interactions on the outcomes of remimazolam use in human. This line of research holds promise for improving the effectiveness of anesthesia by revealing an important gap in our understanding of interindividual variability in remimazolam therapy, which will ultimately optimize anesthetic options and dosing precision. Therefore, the findings will be invaluable for advancing tailored treatment strategies for patients receiving anesthetics, promoting more effective and individualized care.

Student Fellow Training/Mentoring Plan (Limit of one half page)

Training Goal

the ORSP summer research fellowship program will provide the student fellow a great opportunity to broaden research horizons, gain deep knowledge of translational research, and develop skills in 1) CES1 pharmacogenetics, 2) *in vitro* drug metabolism assay 3) LC-MS/MS, 4) Scientific writing, 5) Scientific communication and oral presentation.

Mentoring Plan

With regarding to the science training for the proposed project, the key areas I would like to help the student improve are listed as below:

a) Pharmacogenomics

- We have Journal Club to share research trend/development and critically evaluate published articles in the field. In the Journal Club, the student will be participating in the discussion of current development in our fields. We will critically discuss the cons & pros of the research, and how the study may be designed differently to better answer the research questions.
- We have weekly lab meeting to discuss the research progress of the summer project and guide the students for trouble shooting.

b) *In vitro* drug metabolism assay

- One-on-one mentoring will be offered to help student learn how to conduct *in vitro* drug metabolism assay.

Presentation skills development

- The student will present the research updates weekly in our lab meeting and present articles in our Journal Club. He will also have opportunity to present the whole project in our department seminar after summer.

Scientific Writing

a) Read publications on scientific Journal

- Reading well-written scientific articles is the key to learn and improve scientific writing. At the beginning, I will select some publications relevant to the summer project for the student to read. In the meantime, the student will also learn to search for references of interest using Google Scholar and PubMed. We will update the reading progress in Journal Club.

b) Practice and Revise

- Practice is necessary to improve writing skills. I find the writing-revision cycles between trainee and faculty is a very efficient and individualized way to polish writing skills. I will find opportunities for the student to improve writing skill through the personalized writing-revision cycles.