Human pharmacology of current and novel gaba(a)-ergic treatments for anxiety

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DISCUSSION AND CONCLUSION

For more than two decades, no mechanistically novel anxiolytic agents have been approved and launched into the market for the treatment of anxiety disorders. Such situation may be attributed to the lack of a solid understanding on the underlying pathophysiology of anxiety disorders, as well as the insufficiency in the development and application of valid animal models and their inability to reliably predict clinical anxiolytic effects in humans [1]. In addition, the term 'anxiety disorders' actually represents a heterogeneous group of illnesses that share a core phenomenology of both excessive fear and anxiety in terms of apprehension and worry about future events. Psychiatrists are still struggling to define the appropriate nosological classification of these disorders and current diagnostic classifications lack a robust neurobiological basis for clinical anxiety-related phenomena. The changing diagnostic landscape and uncertain boundaries between both the various anxiety disorders and mood disorders introduce further challenges for drug development [1]. Meanwhile, the search of novel pharmacotherapies for various anxiety disorders is driven by the growing medical need derived from clinically available drugs for the improvement of their effectiveness and/or for the reduction of their side-effect profiles [2].

The pharmacotherapeutic pipeline of anxiolytic treatments in development can be outlined into three major trends: 1) exploration of compounds acting on novel targets that address the underlying neural circuits of anxiety disorders, in which the glutamate, various neuropeptides and the endocannabinoid systems show particular promise as the targets of future drug development [4-6]; 2) design of compounds with established mechanism of action for anxiety but have modified or additional pharmacological properties than the traditional drugs: the development of subtype-selective GABA(A)-ergic partial agonists is an example of this approach; likewise, the recently marketed multi-target serotoninergic compounds, such as vortioxetine, vilazodone, and agomelatine [1], have been proved effective as antidepressant agents, and their efficacy on anxiety disorders has been shown in small population of patients; 3) repositioning of registered drugs for other indications in the treatment of anxiety disorders, such as clinical trials investigating the effects of antipsychotic drugs on anxiety disorders, and the approval of pregabalin by the European Medicines Agency for treatment of GAD in 2006 is a successful example of this approach [1,3].

Benzodiazepines were discovered by serendipity in the 1950s. Thereafter, due to the widespread therapeutic use of GABAergic agents on anxiolysis, sedation, seizure suppression, muscle relaxation, etc., as well as the cumulating understanding about GABA(A) receptor subunit neurophysiology and subtype-specific pharmacology, GABA(A) receptors have become a highly appreciated target in preclinical-to-clinical translational strategies. In the area of anxiety disorders, increasing evidence

from neuroscience indicates that anxiety disorders result from a functional imbalance in the modulation of brain circuits that regulate the emotional response to potentially threatening stimuli. In this context, the inhibitory network of GABAergic neurotransmission system is proposed to contribute to the pathogenesis of anxiety and hence serves as a promising therapeutic target for the treatment of human anxiety disorders [7].

In addition to anxiolytic effects, benzodiazepines also display potent sedative-hypnotic properties. For anxiety-related symptomatology like insomnia, these properties are useful. However, for the management of daytime anxiety, such effects are undesirable. The sedative effects and their ensuing cognition impairment and the potential for tolerance development and abuse liability are the major obstacles against wide and long-term use of benzodiazepines in the treatment of anxiety disorders. Previous research suggested these untoward effects are associated with the off-target pharmacological activities of benzodiazepines on the GABA(A) receptors containing α_1 and α_5 subunits [8-11]. As a result, novel GABA(A)-ergic α_1- and α_5-subtype sparing partial agonists, with either disproportional binding affinity or disproportional *in vitro* efficacy at the benzodiazepine-targeted GABA(A)-ergic receptor subtypes, are expected to separate anxiolytic effects from the BZDs-induced sedative and cognition-impairing effects.

Across the industry, the most common reason for developmental failure in Phase 2 in was lack of efficacy [12]. There are many areas of uncertainty regarding the translation of preclinical pharmacology data to human. These questions cannot be readily answered unless we know whether the drug actually expressed the intended pharmacology by modulating its target(s). In the entire process of clinical drug development, the demonstration of pharmacological effects with clinically tolerable doses is termed as proof-of-mechanism (POM) study. Generally speaking, these types of studies should comprise three goals: 1) observing drug exposure at the target site of action; 2) detecting drug interaction with the intended drug target; and 3) exploring effect of the drug on human biology using biomarker(s). Such investigational approach may be especially useful for anxiety disorders, in which therapeutic exploratory studies in patients can be difficult to achieve a clinically meaningful end-point due to the nature of subjective assessments, the relatively large sample size, the high probability of placebo effect, and other ethical or practical issues [13,14].

This thesis presents the early-phase proof-of-mechanism studies evaluating the pharmacokinetics and pharmacodynamics of three $\alpha_{2,3}$-subunit-selective GABA(A) agonists (i.e., AZD7325, AZD6280 and NS11821), in at least two dose levels, compared with active control (lorazepam), in its therapeutic dose, and placebo control. Most

of the studies were single-dose, double-blind, randomized, cross-over trials in healthy volunteers. A number of validated pharmacodynamic measurements were taken to address the effects of these novel drugs on psychomotor, neurophysiological, and neuroendocrine functions.

The results of each study provided a comprehensive picture about the pharmacological 'fingerprint' of the investigated compounds on a variety of CNS domains [15-17]. The concept of pharmacological selectivity was demonstrated by the relatively dominant effects of these novel compounds on saccadic eye movements, which measure the $\alpha_{2,3}$-subtype GABA(A) receptor related pharmacodynamic responses, in comparison with their minimal or none effects on postural stability, subjective alertness (i.e., measurements reflecting GABA(A) receptor α_1-subtype modulation) and cognition (i.e., GABA(A) α_5-subtype specific effects) [18]. In contrast, lorazepam-induced SPV reduction was generally consistent with its effect size on the other non-SPV neurophysiologic biomarkers. Considering the potential relation between SPV decline and clinical anxiolysis [19], the similarity in the effect size of these GABA(A) subtype-selective agonists on SPV implied the possibility of comparable anxiolytic effect between the novel compounds at certain dose levels and the active control, and was therefore taken as supportive evidence for future dose selection and the decision of further clinical development. Meanwhile, the flat concentration-effect curves of the novel GABA(A)-ergic compounds on subjective alertness, visuo-motor coordination, postural balance, and cognition indicate relatively favorable clinical side-effect profiles of these drugs versus the traditional non-subtype-selective full GABA(A) agonists, such as lorazepam. However, since the dose potency of the novel GABA(A)-ergic drugs might not be equivalent to that of lorazepam 2 mg, the lack of effects on the abovementioned CNS domains cannot be directly interpreted as improvement of adverse effects. In order to resolve this problem, we incorporate these pharmacodynamic (PD) measurements into an SPV-normalized regression model.

As is indicated in the previous chapters, the abovementioned repeat pharmacodynamic measurements all presented a clear dose/exposure-response relationship in healthy volunteers administered with benzodiazepines and subtype-selective GABAergic compounds. The PD-SPV regression models established on simultaneously measured pharmacodynamic endpoints actually reflect the relative effect profiles of the investigated drug across a wide range of plasma drug concentrations. The effect size on SPV was used as the normalizer because SPV has been shown associated with $\alpha_{2,3}$ GABA(A) receptor subtype modulation [20]. Interestingly, recent studies [21] reported quantitative correlation between disturbed performance in saccadic eye movement paradigm and the severity of various anxiety disorders. These results suggested measurements of saccadic eye movements might also

serve as neuropathophysiological biomarkers for the status or severity of anxiety. Moreover, two additional findings suggested performance of saccadic eye movement may be a predictive biomarker for clinical anxiolytic effect: 1) TPA023, a previously developed GABA(A) receptor $\alpha_{2,3}$ subtype-selective agonist, induced significant SPV reduction and minimal sway impairment and no memory change in single-dose study performed in healthy volunteers [22], has demonstrated a better-than-placebo anxiolytic effect in its phase 2 proof-of-efficacy studies [19]; 2) our study with both GABA(A)-ergic and non-GABA anxiolytic compounds showed similar SPV-depressive effects by alprazolam and pregabalin with single doses of these drugs at their clinically anxiolytic doses [23].

The pooled data analysis on the studies of this GABA(A) modulator family was performed to not only summarize the common pharmacological characteristics of these compounds but also evaluate the sensitivity and specificity of the selected CNS-pharmacodynamic measures. Three $\alpha_{2,3}$-selective GABA(A) agonists (i.e., TPA023, TPACMP2, SL65.1498), one α_1-selective GABA(A) agonists (zolpidem), and another full GABA(A) agonist (alprazolam) were examined through this approach. Pharmacological selectivity was assessed by determination of regression lines for the change of a pharmacodynamic endpoint (ΔPD) versus the change from baseline of SPV (ΔSPV). The absolute slopes of the ΔPD-ΔSPV relations were consistently lower with the $\alpha_{2,3}$ selective GABA(A) agonists than with lorazepam, indicating that their effects on non-SPV pharmacodynamic measurements are less than their effects on SPV. The ΔSPV-ΔPD relations of lorazepam were comparable to those of alprazolam. In contrast, zolpidem, an α_1 selective GABA(A) agonist, showed relatively higher impairments in the α_1-relevant PD parameters relative to the effect on SPV, although its ΔPD-ΔSPV profiles did not statistically significantly differ from those of lorazepam. These ΔPD-ΔSPV findings support the pharmacological selectivity of the $\alpha_{2,3}$-selective GABA(A) agonists, implying that the clinical anxiolytic effect of these drugs might be accompanied with fewer untoward side effects on psychomotor and cognitive function.

In summary, the development of novel GABAergic compounds can be structured, step-by-step, as the preclinical-to-clinical translation process depicted in Figure 1. First of all, the neurobiological investigation about anxiety and the clinical experience with benzodiazepines both cast light on the GABAergic neurotransmission system as a potential pathway for new drug development. Further knock-in animal studies suggest that the pharmacological selectivity of a ligand for a certain GABA(A) receptor subtype can be achieved either by affinity differentiation (i.e., forming or not forming a receptor-ligand complex) or by efficacy differentiation (i.e., eliciting or not eliciting a biological response after binding to the receptor) [24]. Using ^{18}F-flumazenil as the tracer, a positron emission computed tomography

(PET) study provides information on the dose-dependency or exposure-dependency of the drug's *in vivo* GABA(A) receptor occupancy, and thereby helps to determine the dose range to be administered in future clinical development. In a clinical pharmacology study, the compound is assessed for its pharmacological effects and pharmacokinetic exposures within the tolerated dose range, at which considerable receptor occupancy can be reached based on the findings of previous neuroimaging study. The observed effects indicate biological interactions between the ligand and the targeted receptors. More specifically, in the case of GABA(A)-ergic novel compounds, the subtype-specific pharmacodynamic biomarkers, in conjunction with the simultaneously measured plasma drug concentrations, allows addressing the effect amplitude and effect potency of GABA(A) receptor subtype modulation elicited by the investigated drug and the active control, and demonstrates the PK/PD profile distinctions that one would expect between full agonist and partial agonist [25]. Also, the relationship of these effects builds up a bridge that connects the *in vitro* pharmacological activity to the *in vivo* physiological responses and supports the concept of pharmacological selectivity for $\alpha_{2,3}$-subtype selective GABA-A agonists in general and, in the case of this thesis, AZD7325, AZD6280 and NS11821.

The results of our research were informative and affected the decision of further clinical development of each specific novel compound: 1) since 10 mg AZD7235 was associated with 80-90% receptor occupancy, the small effect size of 2 mg and 10 mg AZD7325 indicated insufficient receptor modulation of the compound at the investigated dose [26]; 2) for NS11821, the pharmacodynamic effects observed at the moderate-to-high dose levels in the first-in-human study helped to identify and select the pharmacologically active doses for future clinical trials [27]; 3) for AZD6280, the pharmacodynamic effect size on SPV was similar to that of lorazepam, suggesting potentially comparative clinical anxiolytic effect, while the ignorable effects of this compound on body sway and $VAS_{alertness}$ were thought to predict a reduced profile of CNS side-effects [25]. Such clinical pharmacological profile was considered promising for further development and future clinical doses were probably limited to the range of 10 to 40 mg.

In order to link the neurophysiological and neuropsychological biomarkers to the pathophysiological alteration of anxiety patients in fear extinction, we integrated a fear-potentiated-startle (FPS) paradigm with the Neurocart pharmacodynamic set, and, in particular, with the repeated assessments of subjective calmness ($VAS_{calmness}$). The FPS paradigm is a procedure that mimics the fear extinction experiment in rodents [28] and aims to induce stressfulness in human. To evaluate the feasibility and utility of this approach, a validation study was conducted in healthy vopunteers. The PD effects of three marketed comparator drugs (i.e. two anxiolytic drugs and one hypnotic drug) were characterized by applying them as

pharmacological probes in the FPS study. The findings of this study corroborated the sensitivity and specificity of the CNS-PD measures to single therapeutic dose of GABAergic (alprazolam) and non-GABAergic (pregabalin) anxiolytic compounds, and reinforced the clinical relevance of saccadic eye movement measurements to clinical anxiolysis. In conjunction with the FPS paradigm, significant increase of subjective calmness was observed with the two anxiolytic drugs, which warrants the use of stress-challenged subjective measurements and neurophysiological tests for the prediction of clinical anxiolytic drug effect [24].

Last but not least, the exploration of potential endocrine biomarkers regarding the differential effects of selective and nonselective GABA receptor modulators suggested a compensatory approach for the pharmacodynamic evaluation of novel anxiolytic agents. The overall effects of the nonselective benzodiazepine lorazepam, as well as two novel $\alpha_{2,3}$ subunit-selective GABA(A) receptor modulators AZD7325 and AZD6280, on prolactin levels were measured within 8 hours post-dose in healthy male volunteers. Following administration of lorazepam at 2 mg and AZD6280 at 10 mg and 40 mg, prolactin levels increased significantly compared with placebo (difference 42.0%, 19.8%, and 32.8%, respectively), suggesting that the α_2 and/or α_3 receptor subtypes are involved in GABAergic modulation of prolactin secretion, although possible roles of the α_1 and α_5 receptor subtypes cannot be excluded. The increases in prolactin levels after administration of AZD7325 at 2 mg and 10 mg doses (difference 7.6% and 10.5%, respectively) did not reach statistical significance. Such results were consistent with the non-significant responses observed on the other neurophysiological and neuropsychological measurements with AZD7325 [15], reinforcing the conclusion that the investigated doses of AZD7325 or the intrinsic efficacy of AZD7325 at the α_2 and α_3 receptor subtypes may have been too low [29].

CONCLUSION

The GABAergic system has been implicated in the pathogenesis of various anxiety disorders. Pharmacological treatments, like benzodiazepines, have been proven to target the GABA(A) receptors and exert quick-onset anxiolytic effect in anxiety patients. However, the side effects of these non-selective GABA(A)ergic compounds, such as sedation, postural imbalance, or potential abuse, limit their use for clinical anxiolysis. Based on the understanding of benzodiazepines' mechanism of action, the emergence of $\alpha_{2,3}$ subtype-selective GABA(A) modulator is expected to provide a novel pharmacological approach that alleviates anxiety symptoms but spares the common undesired side effects. Most of these compounds are still in early clinical development, in which stage proof-of-mechanism studies are usually performed

in healthy volunteers. The findings from our studies consistently present a similar pattern in the pharmacodynamic effect profiles of the $\alpha_{2,3}$ subtype-selective GABA(A) modulators versus those of the non-selective full GABA(A) agonist, lorazepam. Future application of anxiogenic symptom provocation models that combine subjective measurements and/or neuroendocrine biomarker assays may provide further construct validity for clinical anxiolytic effects of $\alpha_{2,3}$ subtype-selective GABA(A) modulators. Also, such findings are expected to provide insights into the translation of preclinical pharmacological properties of $\alpha_{2,3}$ subtype-selective GABA(A)-ergic compounds to clinical effects in patients with anxiety disorders through human pharmacology studies.

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Figure 1 · Schematic graph about the developmental steps of GABAergic novel compounds from pathway/target identification to clinical research