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

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ENGLISH SUMMARY

Anxiety disorders are highly prevalent psychiatric disorders that are associated with significant personal and societal costs. The transition from adaptive negative affect such as fear and anxiety to an anxiety disorder in humans is mediated by an interplay between psychosocial factors and a wide array of neurobiological alterations.

The introduction of this thesis (**chapter 1**) provides a detailed overview of the definition, classification, neurobiology and current psychopharmacological treatment of anxiety disorders. On a conceptual level, anxiety disorders result from disruptions of highly interconnected neuronal circuits that normally serve to process the stream of potentially threatening stimuli detected by the human brain from the outside world. Perturbations in any of these circuits cause imbalance in the entire system, resulting in a fundamental misinterpretation of sensory information as threatening and leading to inappropriate emotional hyperarousal, physical symptoms and behavioral responses that are characteristic of anxiety disorders. Although monoamine modulating drugs such as the selective serotonin reuptake inhibitors (SSRI's) and gamma-aminobutyric acid (GABA) agonists are widely applied to modulate central emotional processing centers in patients with anxiety disorders, their effectiveness is limited in a large proportion of patients due to either inefficacy or untoward effects. This obviously unmet clinical need in the treatment of anxiety disorders opens an opportunity for novel pharmacological approaches.

As the predominant inhibitory neurotransmitter system in the human brain, the GABAergic system has been implicated in the pathophysiology of anxiety disorders. Evidence from preclinical studies suggests distinct physiological effects of the benzodiazepines-targeted α1, α2, α3, and α5 GABA(A) receptor subunits: α2/α3-subunits predominantly mediate analgesia and anxiolysis, while α1- and α5-subunits are associated with sedation and cognition, respectively. The relatively high affinity or *in vitro* efficacy of novel α2,3 subtype-selective GABAergic receptor modulators therefore represents a potentially useful innovative pharmacological approach for the treatment of anxiety disorders. This thesis is largely devoted to the early development of these innovative compounds, and to methods to show their effects in humans.

In the subsequent chapters, we report three first-in-human (FIH) clinical pharmacology studies which evaluated the pharmacokinetics and pharmacodynamics of the α2,3-subunit-selective GABA(A) agonists AZD7325 (**chapter 2**), AZD6280 (**chapter 3**) and NS11821 (**chapter 4**), respectively. Because of their pharmacological selectivity at the α2,3 GABA(A) receptor subtypes, these compounds are expected to elicit clinical anxiolysis without inducing unwanted sedative effects in humans. Therefore, these studies aimed to characterize the pharmacodynamic effects and evaluate the pharmacologically active doses/exposure levels of these compounds by applying Neurocart, a battery of previously validated pharmacodynamic measurements

that assess different functional central nervous system (CNS) domains. In all studies, at least two dose levels were explored and were compared with placebo and the non-selective GABA-A receptor agonist lorazepam as active control. The results of these studies demonstrate compound-specific effect profiles on the neurophysiological functions postural balance, visuo-motor coordination, cognition and subjective feelings for most compounds. Moreover, the concept of pharmacological selectivity is demonstrated by the relatively dominant effects of these novel compounds on saccadic eye movements, which reflects the GABA(A) α2,3-subtype receptor related pharmacodynamic responses, in comparison with their minimal or absent effects on postural stability and subjective alertness (i.e., α1-subtype receptor modulation) and cognition (i.e., α5-subtype-specific effects). In contrast, lorazepam-induced SPV reduction is generally similar to its effect size on the other non-SPV neurophysiologic biomarkers, indicating a comparable interaction with different GABA(A) receptor subtypes. These findings are corroborated by the α2,3-subtype-selective GABA(A) partial agonist TPA023, which previously demonstrated SPV reduction in healthy volunteers that translated to a clinical anxiolytic effect in patients with generalized anxiety disorder. Therefore, similar effect sizes of the evaluated α2,3 GABA(A) subtype-selective agonists on SPV suggest potentially efficacious anxiolytic effects comparable to the clinically effective dose of non-subtype-selective GABA(A) modulator, lorazepam. On the other hand, the flat concentration-effect curves of the α2,3-selective GABA(A)-ergic compounds on subjective alertness, visuo-motor coordination, postural balance and cognition, indicate a relatively favorable clinical side-effect profile of these drugs versus the traditional non-subtype-selective full GABA(A) agonists, such as lorazepam. Taken together, the demonstration of an equipotent α2,3 GABA(A) effect in the absence of either α1 or α5 effects provides support to further pursue clinical development and can potentially guide future dose selection for studies in both healthy volunteers and patients with anxiety disorders.

In **chapter 5**, we present a pooled data analysis based on studies with the α2,3 subtype-selective GABA(A) modulator family that were previously published by our group. The pharmacological selectivity of three α2,3-selective GABA(A) agonists (i.e., TPA023, TPACMP2, SL65.1498), one α1-selective GABA(A) agonist (zolpidem), and another non-selective GABA(A) agonist (alprazolam) were examined by modeling their regression lines for the effect on one of the (unwanted) pharmacodynamic endpoints (ΔPD) versus the simultaneous (desired) effect on SPV (ΔSPV). The absolute slope of the relation between the unwanted and desired pharmacodynamics effect (ΔPD-ΔSPV) was consistently lower with the α2,3 selective GABA(A) agonists than with lorazepam. Moreover, the ΔSPV-ΔPD relations of lorazepam were comparable to those of alprazolam, but slightly lower than zolpidem. Together, these ΔPD-ΔSPV findings further support the pharmacological selectivity of the α2,3-selective GABA(A) agonists, and as a consequence, imply

that the clinical anxiolytic effect of these drugs might be accompanied with fewer untoward side effects on psychomotor and cognitive function compared to the non-selective benzodiazepines.

Next to the neurophysiological, emotional and cognitive effects that were investigated in previous chapters, anxiety responses are also characterized by neuroendocrine reactions. This was explored further in **chapter 6**, which focused on potential potential peripheral neuroendocrine biomarkers for the effects of selective and non-selective GABA receptor modulators. The effects of two novel α2,3 subunit-selective GABA(A) receptor modulators, AZD7325 and AZD6280, on serum prolactin levels were evaluated in healthy male volunteers, compared with the non-selective GABA(A) modulator lorazepam. Prolactin levels increased significantly after administration of AZD6280 and lorazepam, whereas increases in prolactin levels after administration of AZD7325 did not reach statistical significance, probably because the dosages were too low. These findings suggest that the α2 and/or α3 receptor subtypes are involved in GABAergic modulation of prolactin secretion, although possible roles of the α1 and α5 receptor subtypes cannot be excluded. The observed drug effects on serum prolactin levels support the use of serum prolactin level as a neuroendocrine biomarker complementary to the validated pharmacodynamic measurements in clinical pharmacology study of novel anxiolytic agents.

The previous chapters mainly describe the pharmacological effects of GABA-ergic compounds on the CNS. To get an impression of their potential anxiolytic effects, anxiety and fear can be examined in healthy volunteers. Finally in **chapter 7**, the fear-potentiated-startle (FPS) paradigm is used to experimentally simulate conditioned and unconditioned threat in healthy volunteers. Conceptually the former scenario represents fear whereas the latter relates to anxiety. In this study, FPS is combined with saccadic and smooth pursuit eye-movement tests, visual analogue scales measuring subjective alertness, visuo-motor coordination and postural balance to evaluate anxiolytic drug effect on the FPS-stimulated neurophysiological and neuropsychological responses. The PD effects of two anxiolytic drugs (alprazolam and pregabalin) and one hypnotic drug (diphenhydramine) were characterized in the presented study. None of the treatments reliably reduced either fear or anxiety-potentiated startle responses, probably due to methodological complexity and the variability of startle responses between and within study participants. However, decrease of subjective calmness from baseline was evident after the stressful FPS procedure during the placebo treatment, while alprazolam and pregabalin maintained subjective calmness to its baseline level following FPS. Such findings corroborate the sensitivity and specificity of the CNS-PD measures to a single therapeutic dose of GABAergic (alprazolam) and non-GABAergic (pregabalin) anxiolytic compounds. In fact, clinically available anxiolytic drugs, such as benzodiazepines or SSRIs also do not consistently induce

significant increases of subjective calmness in healthy volunteers under stress-free experimental conditions. Therefore, the measurable effects on subjective calmness, as well as the test procedure modification with FPS integration, may warrant the use of stress-challenged subjective measurements and neurophysiological tests for the simulation of clinical anxiolytic drug effect.

CONCLUSION

The GABAergic system has been implicated in the pathogenesis of various anxiety disorders. Clinically effective pharmacological treatments like benzodiazepines have been demonstrated to target the GABA(A) receptors, by which they exert acute anxiolytic effects in patients with anxiety disorders. However, the side effects of these non-selective GABA(A)-ergic compounds, such as sedation, postural imbalance, cognitive effects and potential abuse limit their use in clinical practice. Based on the understanding of benzodiazepines' mechanism of action, the emergence of α2,3 subtype-selective GABA(A) modulators 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 α2,3 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 α2,3 subtype-selective GABA(A) modulators. Also, such findings are expected to provide insights into the translation of preclinical pharmacological properties of α2,3 subtype-selective GABA(A)-ergic compounds to clinical effects in patients with anxiety disorders through human pharmacology studies.

Overall, the work in this thesis illustrates an important step in a structured translational process of novel subtype-selective GABAergic compounds from preclinical development to early phase clinical trials : 1) **pathway identification** of the gabaergic neurotransmission system informed by the neurobiology of anxiety and clinical efficacy of benzodiazepines; 2) **novel drug design and discovery** based on knock-in animal studies that suggest the distinct pharmacological activities of various GABA(A) receptor subtypes; 3) **proof-of-target study** using neuroimaging tools to demonstrate the drug's *in vivo* GABA(A) receptor occupancy; 4) **proof-of-mechanism study** assessing the drug's pharmacodynamic effects and pharmacokinetic exposures within the tolerated dose range; 5) **proof-of-efficacy study** exploring the drug's clinical efficacy in the target patient population; 6) **proof-of-therapy study** confirming the drug's clinical utility and effectiveness in in clinical practice.