TC-5214 (S-(+)-Mecamylamine): A Neuronal Nicotinic Receptor Modulator with Antidepressant Activity

Patrick M. Lippiello, Jessica S. Beaver, Gregory J. Gatto, John W. James, Kristen G. Jordan, Vincent M. Traina, Jianxun Xie & Merouane Bencherif

Targacept, Inc., Winston-Salem, North Carolina, USA

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Correspondence

Patrick M. Lippiello, Ph.D.
Targacept, Inc.
200 East 1st Street, Suite 300,
Winston-Salem, NC 27101, USA.
Tel.: 336-480-2100;
E-mail: Lippiello@Targacept.com

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Both clinical and preclinical data support a potential therapeutic benefit of modulating the activity of CNS neuronal nicotinic receptors (NNRs) to treat depression and anxiety disorders. Based on the notion that the depressive states involve hypercholinergic tone, we have examined the potential palliative role of NNR antagonism in these disorders, using TC-5214 (S-(+) enantiomer of mecamylamine), a noncompetitive NNR antagonist. TC-5214 demonstrated positive effects in a number of animal models of depression and anxiety. TC-5214 was active in the forced swim test in rats (minimum effective dose (MED) = 3 mg/kg i.p.), a classical depression model. It was also active in the behavioral despair test in mice (0.1–3.0 mg/kg i.p.), another model of depression. In the social interaction paradigm in rats, a model of generalized anxiety disorder (GAD), TC-5214 was active at a dose of 0.05 mg/kg s.c. In the light/dark chamber paradigm in rats, a model of GAD and phobia, TC-5214 was also active at a dose of 0.05 mg/kg s.c. Although TC-5214 shows modest selectivity among NNR subtypes, the antidepressant and anxiolytic effects seen in these studies are likely attributable to antagonist effects at the $\alpha 4\beta 2$ NNRs. This is supported by the observation of similar effects with $\alpha 4\beta 2$ -selective partial agonists such as cytisine and with $\alpha 4\beta 2$ -selective antagonists such as TC-2216. TC-5214 was well tolerated in acute and chronic toxicity studies in mice, rats, and dogs, showed no mutagenicity and displayed safety pharmacology, pharmacokinetic and metabolic profiles appropriate for therapeutic development. Overall, the results support a novel nicotinic cholinergic antagonist mechanism for antidepressant and anxiolytic effects and highlight the potential of NNR antagonists such as TC-5214 as therapeutics for the treatment of anxiety and depression.

Introduction

Based on the observation that cholinomimetic drugs aggravate depressive symptoms and anticholinergic drugs alleviate them, it was hypothesized over 30 years ago that a cholinergic imbalance (i.e., hypercholinergic tone) was a primary factor in depressive illnesses [1,2]. Since that time a number of key findings from both animal and human studies have supported this hypothesis and focused it specifically on central nicotinic cholinergic rather than muscarinic pathways [3,4]. Most clinically prescribed antidepressants target catecholaminergic and indoleaminergic neurotransmitter systems. However, many of the

drugs that target these systems, such as selective serotonin or norepinephrine (NE) reuptake inhibitors and tricyclic antidepressants, also act as noncompetitive antagonists of neuronal nicotinic receptors (NNRs) at clinically effective doses [5–7]. Studies have shown that nonselective NNR antagonists such as mecamylamine, as well as NNR-selective antagonists and partial agonists, can exhibit antidepressant effects in animal models of depression and anxiety [8]. In addition, chronic nicotine administration (patch) has been shown to substantially reduce HAM-D scores in patients with major depression [9], an effect believed to be due to nicotine's time-averaged antagonistic effects on NNRs. These effects

have been attributed primarily to inhibition of a high affinity NNR subtype in the brain, which is composed of $\alpha 4$ and $\beta 2$ subunits, representing the predominant mammalian brain NNR subtype [10]. This is also supported by studies with cytisine, a partial agonist at the $\alpha 4\beta 2$ subtype, which produced antidepressant effects in several animal models and reduced c-fos levels in the basolateral amygdala, similar to what is seen with classical antidepressants [11]. Cytisine's antidepressant effects were attributed to partial blockade of high affinity $\alpha 4\beta 2$ NNRs. Additional evidence for the involvement of $\alpha 4\beta 2$ NNRs includes the loss of antagonist-mediated antidepressant effects in $\beta 2$ knock-out models [12] and increased anxiety in $\alpha 4$ knock-in models [13]. Emerging evidence now suggests that the $\alpha 4\beta 2$ NNR subtype exists in two distinct subunit configurations with different sensitivity to agonists and antagonists [14–17]. Although the physiological relevance of these differences has not as yet been elucidated, it may necessitate reevaluation of previous findings relative to subtype selectivities and activities of cholinergic ligands.

Mecamylamine is a potent, noncompetitive, relatively nonselective antagonist of NNRs and is a racemic mixture comprised of the optical isomers S-(+)-mecamylamine and R-(–)-mecamylamine. Mecamylamine HCl was originally developed as a ganglion-blocking agent and is approved as Inversine[®] (Siegfried CMS Ltd., Zofingen, Switzerland) for the treatment of moderately severe to severe essential hypertension and in uncomplicated cases of malignant hypertension [18]. Its use for this indication diminished as new generations of antihypertensive products became available. Although mecamylamine was originally investigated as a ganglion-blocking agent with hypotensive action, it became apparent that the compound was capable of exerting a number of central actions. Studies demonstrated that mecamylamine, in common with other ganglion blockers, was capable of preventing nicotine-induced seizures in several species [19]. It was believed that the convulsive effects of nicotine were due to a central action of the drug, and mecamylamine showed anticonvulsive action at doses lower than those necessary to reduce blood pressure or elicit other peripheral signs of postganglionic blocking activity. Subsequently, it was shown that mecamylamine was capable of crossing the blood–brain barrier and distributing in brain tissues. In contrast to its competitive blockade of nicotinic receptors in autonomic ganglia [20], mecamylamine does not interact directly with the receptor-binding site either at neuromuscular nicotinic receptors or at CNS nicotinic receptors. It has been known for some time that a primary mechanism of action at CNS NNRs involves mecamylamine binding to a site in the open ion channel of the receptors [21]. NNRs display

considerable subtype diversity, in common with many neurotransmitter receptors. While there are some differences in receptor subtype sensitivity, mecamylamine interacts with all NNR receptor subtypes examined to date.

More recently mecamylamine has been investigated as a potential therapeutic for additional clinical indications, at doses lower than those which produce ganglionic hypotensive effects. It has been studied in children for use in the treatment of Tourette's syndrome (TS), which is a neuropsychiatric syndrome with childhood onset that is characterized by the repetitive and unwanted expression of both motor and vocal movements (tics and coprolalia) and frequently-associated behavioral and emotional problems such as attention deficit hyperactivity disorder (ADHD) and depression. Somewhat unexpectedly, mecamylamine was found to be particularly effective in reducing the associated dysfunctions of ADHD and depression in children with Tourette's without reducing the incidence of physical manifestations [22–24]. Although physical manifestations were not eliminated in these children, their ability to concentrate on tasks and function in a social context without emotional outbursts was remarkably improved. Of particular relevance, there was a significant beneficial effect on sudden mood changes and depression in a subset of subjects who comprised the most severely affected patients.

Aims

The present report summarizes the results of preclinical pharmacology and toxicology studies that support the further development of TC-5214, the S-(+) enantiomer of mecamylamine, as an antidepressant and discusses the potential of a NNR antagonist mechanism as a new approach to the treatment of major depression and anxiety.

Results

Chemistry

The structure of TC-5214 ((1R,2S,4S)-N,2,3,3-Tetramethylbicyclo[2.2.1]heptan-2-amine hydrochloride) is shown in Figure 1. The molecular weight of the base is 167.3 and that of the salt 203.8. TC-5214 is freely

Figure 1 Chemical structure of TC-5214 (S-(+)-mecamylamine).

Figure 2 Effects of TC-5214 in a forced swim test of depression in mice. TC-5214 (0.1, 0.3, 1, and 3 mg/kg), saline, and fluoxetine (5 and 10 mg/kg) were administered intraperitoneally to male Swiss mice. Thirty minutes after dosing, mice were individually placed in a cylinder containing water (at a depth of 6 or 10 cm) from which they could not escape and the duration of immobility was measured. The results represent the mean $\pm$ standard error. * $P < 0.05$.

soluble in water, soluble in alcohol and glycerol, and sparingly soluble in isopropanol. TC-5214 free base has a pKa value of 11.5. Unless otherwise stated, all doses reported herein are expressed as free base equivalents.

Behavioral Pharmacology

Forced Swim Test in Mice (Depression)

Several studies have evaluated the antidepressant effects of TC-5214 in mice and rats using the forced swim test, in which animals are forced to swim in a situation from which they cannot escape and rapidly become immobile [25]. Classical antidepressants decrease the duration of immobility in this task. The effects of acute administration of TC-5214, saline, and fluoxetine were evaluated in male mice. Thirty minutes after dosing, mice were individually placed in a cylinder containing water from which they could not escape and the duration of immobility was measured. The results are shown in Figure 2. A decrease in the duration of immobility compared to the saline-treated group was seen with statistical significance for TC-5214 at 0.1 (–33%), 1 (–37%), and 3 mg/kg (–46%), and for fluoxetine at 5 (–32%) and 10 mg/kg (–34%). Clearly any compound that increases motor activity could reduce immobility; therefore, a separate study to assess locomotor activity of TC-5214 was conducted. Male Swiss mice ($n = 8/\text{group}$) were injected via the intraperitoneal route with either saline or TC-5214 (0.1, 1 and 3 mg/kg), immediately prior to placement in the locomotor chambers (40 Digiscan activity

Figure 3 Effects of TC-5214 in a forced swim test of depression in rats. Male Sprague–Dawley rats were individually placed in a cylinder containing water (at a depth of 13 or 35 cm) for 15 min on the first day, and were then put back in the water 24 h later for a 5-min test. TC-5214 (0.1, 0.3, 1, and 3 mg/kg), saline, and desipramine (10 mg/kg) were administered intraperitoneally. The duration of immobility during the 5-min test was measured. The results represent the mean $\pm$ standard error. * $P < 0.05$.

chambers). Horizontal activity (interruption of photocell beams) was measured over 40 min and data expressed as mean $\pm$ SD. Horizontal counts for the 0.3, 1, and 3 mg/kg TC-5214-treated groups were 3225 ± 236 , 3400 ± 735 , and 3175 ± 624 , respectively. Horizontal counts for the vehicle control groups were 3400 ± 744 (paired with the 0.3 mg/kg TC-5214 group), 3351 ± 717 (paired with the 1 mg/kg TC-5214 group), and 3450 ± 705 (paired with the 3 mg/kg TC-5214 group), respectively. With the failure to produce effects on locomotor activity, the antidepressant-like activity of TC-5214 demonstrated in the mouse forced swim test was not confounded by nonspecific stimulant effects.

Forced Swim Test in Rats (Depression)

In another study, the effects of TC-5214 were determined in a forced swim test in rats. The effects of TC-5214, saline, and desipramine on immobility were evaluated in male Sprague–Dawley rats (Fig. 3). Swim sessions were conducted by placing rats in a Pyrex cylindrical tank (22 cm $\times$ 46 cm) containing water (at 23–25°C) for 15 min. The water depth was 35 cm from the bottom, a depth at which the rat cannot touch the bottom with its tail. The initial 15-min swim pretest was followed 24 h later by a 5 min test. Rats received three injections between the pretest and the test swim (i.e., 23.5, 5, and 1 h before the 5 min swim test). The duration of immobility during the 5-min test was measured. TC-5214 (3 mg/kg) substantially reduced immobility compared

Figure 4 Effects of TC-5214 in a social interaction test of generalized anxiety in rats. Female Sprague–Dawley rats were initially placed individually in a test arena for a 5-min familiarization trial. For the test trial on the next day, two animals were placed in the arena at the same time. The pairs were randomly allocated among the treatment groups receiving subcutaneous injections of saline, TC-5214 (0.005, 0.05, or 0.17 mg/kg), or (–)-nicotine (0.02 mg/kg). Social interaction behaviors and locomotor activity were compared between each group and the saline control group for 5 min. The results represent the mean $\pm$ standard error. * $P < 0.05$.

with the saline control (–24%), consistent with antidepressant activity. Desipramine, the reference compound in this study, also substantially decreased immobility (–41%).

Social Interaction Test (Generalized Anxiety Disorder)

To assess the ability of TC-5214 to reduce the anxiety of animals exposed to a mildly stressful environment, changes in social interaction were studied in pairs of female Sprague–Dawley rats placed in a test arena using methods similar to those described by File et al. [26]. Pairs of subjects were administered saline, TC-5214, or (–)-nicotine. Social interaction behaviors and locomotor activity (to control for nonspecific drug effects) were observed, quantified, and compared between each active treatment group and the saline control group for 5 min (Fig. 4). The social interaction score was substantially higher in the TC-5214 (0.05 mg/kg) and nicotine groups than in the saline group. There were no significant differences in locomotor activity between any active treatment group and the saline group. These results indicate that TC-5214 reversed social interaction-induced anxiety. The fact that nicotine, typically viewed as a classical NNR agonist, also showed a positive signal in this assay reinforces the often-cited concept that chronic nicotine expo-

Figure 5 Effects of TC-5214 in a light/dark test of generalized anxiety in rats. Sprague–Dawley rats were randomly allocated to treatment with a subcutaneous injection of saline, TC-5214 (0.017, 0.05, or 0.17 mg/kg), or (–)-nicotine (0.09 mg/kg). After treatment, each rat was returned to its home cage for 30 min, after which it was placed in the lit portion of the test arena facing the opening between a light compartment and a dark compartment. The percentage of time spent in the light compartment and locomotor activity were compared between each group and the saline control group. The results represent the mean $\pm$ standard error. * $P < 0.05$.

sure can lead to a time-averaged antagonist effect due to receptor desensitization.

Light/Dark Assay (Generalized Anxiety Disorder/Phobia)

TC-5214 was evaluated for its ability to increase the proportion of time spent in a mildly stressful (brightly lit) environment [26]. Sprague–Dawley rats were administered subcutaneous injections of saline, TC-5214, or (–)-nicotine. After treatment, each rat was returned to its home cage for 30 min, after which it was placed in the lit portion of the test arena facing the opening between a light compartment and a dark compartment. The apparatus was equipped with infrared beams that sensed the number of exploratory rearings in both the light and dark sections, the ambulatory activity in each section, the initial latency to enter the dark from the light compartment, the total time spent in each compartment, and the number of transitions between compartments. The percentage of time spent in the light compartment and locomotor activity (to control for nonspecific drug effects) were compared between each group and the saline control group (Fig. 5). The percentage of time in the light was higher in all groups than in the saline group; the result was statistically significant for TC-5214 at 0.05 mg/kg and nicotine and approached statistical significance for TC-5214 at

Table 1 Comparison of efficacy profiles in animal models of depression and anxiety for racemic mecamylamine and its enantiomers

Indication	<i>In vivo</i> model	Species (strain or line)	Mecamylamine (racemate)	TC-5214 S-(+) enantiomer	R-(−) enantiomer	Reference compound
Depression	Forced swim	Mouse (Swiss)	Inactive (0.1–3 mg/kg)	Active (0.1, 1 & 3 mg/kg)	Active (3 mg/kg)	Fluoxetine (5 & 10 mg/kg)
		Mouse (NMRI)	Active (3 mg/kg)	Active (3 mg/kg)	Inactive (0.1–3 mg/kg)	Imipramine (32 mg/kg)
		Rat (SD)	Active (3 mg/kg)	Active (3 mg/kg)	ND	Desipramine (10 mg/kg)
Generalized anxiety disorder	Social interaction	Rat (SD)	Active (0.2 mg/kg)	Active (0.05 mg/kg)	Active (1 mg/kg)	Nicotine (0.09 mg/kg)
	Light/dark	Rat (SD)	ND	Active (0.05 mg/kg)	Inactive (0.005–0.2 mg/kg)	Nicotine (0.09 mg/kg)
Panic disorder	Elevated plus maze	Rat (Wistar)	Inactive (0.1–1 mg/kg)	Inactive (0.1–1 mg/kg)	Inactive (0.1–1 mg/kg)	Clobazam (16 mg/kg)

ND = not determined.

For "active" and reference compounds, doses shown are those for which a statistically significant effect was seen; for tests where compounds were "inactive," doses shown are dose ranges tested.

0.17 mg/kg ($P = 0.051$). There were no significant differences in locomotor activity between any active treatment group and the saline group. These results indicate that TC-5214 reversed anxiety resulting from placement in an aversive (light) environment. In this paradigm, there appeared to be a biphasic dose response. This observation has often been reported for the behavioral effects of nicotine and is referred to as the "inverted U" dose-response effect. Although the exact mechanism(s) for this effect has not been elucidated for nicotinic compounds, Picciotto [27] have discussed possible bases for the phenomenon. They suggest that systemic administration of nicotine results in a behavioral output that is a vector sum of the different NNR targets and neuronal systems that are stimulated, consistent with the broader NNR selectivity of nicotine and TC-5214. In addition, many nicotinic agonists have been reported to be use-dependent channel blockers, initially activating the receptors and subsequently blocking the ion channel. This may partly explain why nicotine (as mentioned above) is as efficacious as TC-5214 in this assay, consistent with functional antagonist activity.

Comparison of TC-5214 Effects to R-(−) Enantiomer

Table 1 summarizes the results of behavioral tests conducted with TC-5214 (S-(+)-mecamylamine), its enantiomer R-(−)-mecamylamine and racemic mecamylamine. The pattern of enantioselectivity appears to be consistent across all of the tests, indicating that TC-5214 has more robust antidepressant and anxiolytic activity

than the R-(−) enantiomer and potency equal to or better than that of the racemate. Interestingly, racemic mecamylamine did not show a significant effect in the mouse forced swim test although the individual enantiomers were both effective. At present, we have no explanation for this apparently anomalous result. However, it is clear that in studies where both enantiomers were found to be active the R-(−) enantiomer was significantly less potent, by an order of magnitude or more. Thus, it can be concluded that TC-5214 is responsible for most of the antidepressant activity demonstrated by racemic mecamylamine. It is interesting to note that the anxiolytic effects of TC-5214 appear to occur at much lower doses than those required for antidepressant activity. Sensitivity of anxiolytic effects to low doses has also been reported for nicotine [28]. This suggests that different NNR targets may be responsible for anxiolytic and antidepressant effects. It is tempting to speculate that these differences are in some way related to differential effects on the HS and LS isoforms of $\alpha 4\beta 2$ NNRs expressed in the CNS (see Introduction), but additional studies will be needed to confirm the exact targets and/or mechanisms involved. Despite the anxiolytic effects seen for TC-5214 in the light-dark and social interaction paradigms, neither mecamylamine nor its enantiomers were active in the elevated plus maze test of anxiety (Table 1). This is reminiscent of the finding that nicotine's effects in the social interaction test do not generalize to the elevated plus-maze because the two tests apparently generate distinct states of anxiety [29]. Equivocal results have often been reported for nicotine in the elevated plus paradigm,

possibly due to a low sensitivity to nicotinic effects in general or that the test may actually be more sensitive to anxiogenic activity.

Safety Pharmacology

In Vitro hERG Studies

The effects of TC-5214 on the human ether-a-go-go related gene (hERG)-related potassium current were assessed using electrophysiology methods and were quantified by analysis of concentration–response relationships. Tests were conducted at concentrations of 3, 10, and 30 μM . Perfusion of 3–30 μM TC-5214 did not produce any physiologically significant inhibition of hERG-mediated potassium currents with a maximum inhibition of 4.46% at the 30 μM concentration. In contrast, the positive control, cisapride produced approximately 74.8% inhibition.

Gut Motility Function in Rats

The acute effects of TC-5214 on gastrointestinal motility were tested in rats via single oral gavage administration. Three treatment groups of 10 male and 10 female rats were administered TC-5214 at dose levels of 1, 5, and 10 mg/kg. Controls received water. At approximately 2.5 h post dose, the test meal of 5% charcoal in 10% gum arabic (acacia) was administered to all animals for gastrointestinal motility via oral gavage at a dose volume of 10 mL/kg. At study termination animals were euthanized, the small intestines were surgically removed and the distance the charcoal traveled from the pylorus was measured. Gastrointestinal motility was defined as the distance the charcoal traveled from the pylorus, expressed as a percentage of the entire length of the small intestine. TC-5214 had a slowing effect on gut motility in rats administered both 5 and 10 mg/kg treatments. The acute no-observable-effect level (NOEL) for TC-5214 in this study was 1 mg/kg.

Pulmonary Function in Rats

A study was conducted to evaluate the potential effects of TC-5214 on pulmonary function. Three treatment groups of eight male rats were administered TC-5214 by oral gavage at dose levels of 1, 5, and 10 mg/kg. Controls received water. Pulmonary function (respiratory rate, tidal volume, and minute volume) was monitored for at least 1 h prior to dosing to establish baseline and for at least 4 h post dose. There were no physiologically meaningful changes in pulmonary function observed following oral gavage dosing of up to 10 mg/kg TC-5214.

Cardiovascular Telemetry Study in Dogs

The potential cardiovascular effects of an oral gavage dose of TC-5214 in conscious, freely-moving beagle dogs was evaluated. The same four male and four female dogs were administered the vehicle water, the positive control article at dose levels of 1, 5, and 10 mg/kg, and TC-5214 at dose levels of 1, 5, and 10 mg/kg with a 7-day washout period between each treatment until each animal received all treatments in ascending order. There were no systematic inductions of either conduction disturbances or QT prolongation associated with TC-5214 following administration of up to 10 mg/kg.

Pharmacokinetics

Rats

Following intravenous administration of 5 mg/kg to Sprague–Dawley rats, the mean plasma half-life was 2.9 ± 1.7 h. The average clearance was 3.1 ± 0.2 L/h/kg, which is 94% of the typical liver blood flow of a rat (3.3 L/h/kg). The average volume of distribution was 4.8 ± 0.7 L/kg, which is greater than the total body water of a typical rat (0.7 L/kg), suggesting extensive distribution throughout the body tissues. Following oral administration of TC-5214 at 10 mg/kg, maximal plasma concentrations were reached within 1 h post dose; the average half-life was 3.1 ± 0.2 h. The mean oral bioavailability for TC-5214, based on AUC_{last} values, was 56%. There were no appreciable differences in terminal plasma half-life with increasing dose level, gender, and multiple dosing. The extent of systemic exposure of rats to TC-5214, characterized by C_{max} and AUC_{last} , increased with increasing dose level over the dose range of 1–50 mg/kg/day. The peak and extent of systemic exposure of rats to TC-5214 were typically higher in females than in males (ratio of C_{max} for females to C_{max} for males within each group 1.10–1.74).

Dogs

After oral administration of TC-5214 to Beagle dogs, peak plasma concentrations of TC-5214 were attained at 0.5 to 1.0 h post dose. Concentrations of TC-5214 then declined and values for the apparent terminal plasma half-life ranged from 1.0 to 2.9 h. There were no appreciable differences in terminal plasma half-life with increasing dose level, gender, and multiple dosing. The extent of systemic exposure of dogs to TC-5214, characterized by C_{max} and AUC_{last} , increased with increasing dose level over the dose range of 1 to 10 mg/kg/day. The peak and extent of systemic exposure were similar in males and females. The gender C_{max} ratio

(ratio of $C_{\max}$ for females to $C_{\max}$ for males within each group) was 0.99–1.09.

Plasma Protein Binding

Binding of TC-5214 to plasma proteins showed 15–44% binding in mouse, rat, guinea pig, dog, monkey and human plasma at 0.1 to 10 μM , except in guinea pig at 0.1 μM which was 77% bound. Plasma protein binding of TC-5214 was consistent within each species from 0.1 to 10 μM , indicating that the binding is not saturated in this concentration range. Binding to human serum albumin and α -glycoprotein was minor, ranging from 21% to 35% and 13% to 19%, respectively, across the concentration range. Binding of TC-5214 in human red blood cells was 40–47%.

Metabolism

Several *in vitro* studies were performed to assess the metabolism of TC-5214 and its potential to induce or inhibit known drug metabolizing enzymes. In metabolic kinetic determinations, TC-5214 appeared relatively stable in human liver microsomes. Kinetic parameters were determined as $K_m = 78 \mu\text{M}$ and $V_{\max} = 328 \text{ pmol/min/mg protein}$. With respect to cytochrome P450 (CYP) involvement in TC-5214 metabolism, there was no significant disappearance at 2 μM over 30 min in 0.5 mg/mL microsomal protein. The compound appeared stable in human liver microsomes, and no CYP enzymes were found to be involved in metabolism. Disappearance rates of TC-5214 in flavin-containing monooxygenase (FMO) active and inactive microsomal reactions were similar, which suggests that FMO enzymes play little or no role in the metabolism of TC-5214. There was slight or no degradation in the absence or presence of the monoamine oxidase (MAO) inhibitor pargyline, suggesting that MAO enzymes play little or no role in the metabolism of TC-5214. The potential of TC-5214 to induce CYP 1A and CYP 3A4 was assessed in fresh human hepatocytes at 0.1, 1.0, and 10 μM . No positive induction was observed for either CYP 1A or CYP 3A4 in the presence of TC-5214 in any of the donor preparations tested. The inhibition potential of TC-5214 on CYP 1A2, 2A6, 2C19, 2C9, 2D6, and 3A4 was evaluated up to a concentration of 10 μM and no significant inhibition was found.

Preclinical Safety Evaluation

Acute Toxicity (Mouse)

In a rising dose acute study, TC-5214 was administered intravenously (i.v.) via tail vein to male and female mice

at single doses of 4.1, 8.2, 12.3, and 16.4 mg/kg. Animals were observed for 14 days post dose followed by necropsy. Doses of 4.1 and 8.2 mg/kg produced no mortality and minimal clinical signs, which included partially closed eyelids and dilated pupils that were not reactive to light. From the mortality observed in this study, the i.v. LD_{50} was estimated to be $>9.9 \text{ mg/kg}$ but $<16.4 \text{ mg/kg}$ in both male and female mice. The maximum dose that produced no mortality in mice following an i.v. dose was 8.2 mg/kg. In another rising dose acute study, TC-5214 was administered by oral gavage to mice at a single dose of 20.5, 41.0, 82.1, and 123.2 mg/kg. The only dose that produced mortality was 123.2 mg/kg (1 male, 1 female). At 20.5 and 41.0 mg/kg, clinical signs included dilated pupils that were not reactive to light, eyelids that were partially closed and hunched posture. At a dose of 82.1 mg/kg, the mice displayed hunched posture, decreased activity, ataxia, tremors, and labored breathing, as well as partially closed eyelids and dilated pupils. In a second phase, animals received a single oral dose of 4.1 or 106.7 mg/kg. At 106.7 mg/kg, three males and four females died soon after dosing. The LD_{50} was estimated to be between 82.1 and 123.2 mg/kg. Based on these data, 82.1 mg/kg was determined to be the maximum tolerated dose and the NOAEL was approximated at 4.1 mg/kg.

Acute Toxicity (Rat)

In a rising dose acute study, TC-5214 was administered i.v. via tail vein to male and female Sprague–Dawley rats at doses of 4.1, 8.2, 16.4, and 24.6 mg/kg. Animals were observed for 14 days post dose. Doses of 4.1, 8.2, and 16.4 mg/kg produced minimal clinical signs, including partially closed eyelids and dilated pupils that were not reactive to light. At 24.6 mg/kg, mortality was produced in two of three male and two of three female rats. The intravenous LD_{50} was estimated to be $>16.4 \text{ mg/kg}$ but $<24.6 \text{ mg/kg}$. Although a NOEL was not reported in this study, a dose of 4.1 mg/kg produced minimal observable signs, that is, partially closed eyelids. In another rising dose acute study, TC-5214 was administered by oral gavage to Sprague–Dawley rats at a single dose of 41.0, 82.1, 123.2, and 164.2 mg/kg. The only dose that produced mortality was 164.2 mg/kg. At 41.0 mg/kg, clinical signs included dilated pupils that were not reactive to light, eyelids that were partially closed, hunched posture, and decreased activity. At the higher doses, additional signs included tremors, labored breathing, and ataxia. In a second phase, rats received a single oral dose of 8.2 or 143.7 mg/kg. The LD_{50} was estimated to be between 123.2 and 164.2 mg/kg. Based on these data, 123.2 mg/kg was estimated to be the maximum tolerated dose and the NOAEL approximately 8.2 mg/kg.

Acute Toxicity (Dog)

In an acute toxicity study, TC-5214 was administered i.v. to male and female dogs at doses of 0.2, 2, 6, or 10 mg/kg. Doses ≥ 2 mg/kg were associated with relaxed nictitating membranes and partially closed eyelids. Doses ≥ 6 mg/kg produced trembling. Doses of 10 mg/kg produced dry mouth. There were no effects on body weights and no findings at necropsy. The NOEL for an acute i.v. dose of TC-5214 in this study was 0.2 mg/kg/day. In an acute oral toxicity study, TC-5214 was administered as a single dose to male and female beagle dogs at doses of 0.3, 1, 5, and 10 mg/kg. The 10 mg/kg dose produced tremors in male and female dogs. At 5 mg/kg, tremors were observed in female dogs only. Other observations associated with doses of 5 and/or 10 mg/kg included partially closed eyes, relaxed nictitating membranes, dry mouth, and red eyes/conjunctiva. There were no effects on body weight or food consumption associated with administration of acute doses of TC-5214 ≤ 10 mg/kg. The NOEL for acute administration of TC-5214 was 1 mg/kg in female dogs and 0.3 mg/kg in male dogs.

Repeat-Dose Toxicity (Rat)

TC-5214 was administered by oral gavage to male and female rats at doses of 1, 10, and 50 mg/kg for at least 30 consecutive days. There were no deaths at any treatment level. Observations considered related to TC-5214 were tremors, hunched posture, and closed eyelids at 10 and/or 50 mg/kg/day. At 50 mg/kg/day, the mean food consumption and body weight gains were lower than control in males and females during the first week and higher than control at weeks 2 through 4. There were no histomorphologic changes or ophthalmologic changes at doses ≤ 50 mg/kg/day of TC-5214. The NOEL in this study was 1 mg/kg/day.

Repeat-Dose Toxicity (Dog)

TC-5214 was administered orally by gavage to male and female beagle dogs at daily doses of 1, 5, and 10 mg/kg for 30 consecutive days. TC-5214 resulted in observations including tremors, at all dose levels. Body weights and food consumption were affected at dose levels ≥ 5 mg/kg/day. Microscopic changes in TC-5214-treated animals at the terminal necropsy were atrophy of splenic white pulp, bone marrow hypocellularity and lymphoid depletion of the lymph nodes, thymic atrophy, and fat atrophy in the mesentery and tongue. There were no effects on clinical pathology or ophthalmology parameters at any dose level. The NOEL in this study was estimated to be <1 mg/kg/day.

Reverse Mutation Test

TC-5214 was tested in the bacterial reverse mutation assay using *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* tester strain WP2 uvrA in the presence and absence of Aroclor-induced rat liver S9. In the initial toxicity-mutation assay, eight dose levels were tested ranging from 1.5 to 5000 μ g per plate and the maximum dose selected in the confirmatory mutagenicity assay was 5000 μ g per plate. In the confirmatory mutagenicity assay, no positive mutagenic response was observed. The dose levels tested were 50, 150, 500, 1500, and 5000 μ g per plate. Neither precipitate nor appreciable toxicity was observed. TC-5214 was therefore concluded to be negative in the bacterial reverse mutation assay.

Chromosome Aberration Test

TC-5214 was tested in the chromosome aberration assay using Chinese hamster ovary (CHO) cells in both the absence and presence of an Aroclor-induced S9 activation system. The dose levels selected for microscopic analysis were 500, 1000, and 1500 μ g/mL for 4-h treatment in the absence of S9 activation; 1000, 1500, and 2040 μ g/mL for 4-h treatment in the presence of S9 activation; 62.5, 250, and 750 μ g/mL for 20-h treatment in the absence of S9 activation. The percentage of cells with structural or numerical aberrations in TC-5214-treated groups was not significantly increased above that of the solvent control at any dose level ($P > 0.05$, Fisher's exact test). TC-5214 was concluded to be negative for the induction of structural and numerical chromosome aberrations in CHO cells in both the nonactivated and the S9-activated test systems.

Mouse Micronucleus Test

TC-5214 was tested in the mouse micronucleus assay. In the dose range-finding study, the mice were exposed to TC-5214 at 6.25, 12.5, 25, 50, or 100 mg/kg and the high dose for the definitive micronucleus study was set at 25 mg/kg, which was estimated to be the maximum tolerated dose. In the definitive micronucleus study, mice were treated either with the controls (vehicle or positive) or with TC-5214 at 6.25, 12.5, or 25 mg/kg and were euthanized 24 h post dose. The incidence of micronucleated polychromatic erythrocytes (MPCs) and the ratio of PCs to total erythrocytes (PCs/ECs ratio) served as an indication of clastogenicity and cytotoxicity, respectively. No mortality was observed during the course of the definitive micronucleus study. The magnitude (up to 6%) of the reductions in the PCs/ECs ratio at 24 h post dose and the lack of a reduction at 48 h post dose suggest that

Table 2 Comparison of side effects and mortality for racemic mecamylamine and its two enantiomers in mice

Dose (mg/kg salt s.c.)	Ptosis, tremors, immobility, rapid breathing		
	TC-5214 S-(+)-mecamylamine	TC-5213 R-(−)-mecamylamine	Mecamylamine racemate
1	None	None	None
10	None	Yes	Yes
30	None	Yes	Yes
Dose (mg/kg salt i.v.)		Mortality	
1	0/0	0/0	0/0
10	0/0	4/6	2/6
30	6/6	6/6	6/6

TC-5214 did not inhibit erythropoiesis. TC-5214 at doses up to and including 25 mg/kg did not induce a significant increase in the incidence of MPCEs in bone marrow of male imprinting control region (ICR) mice. Therefore, TC-5214 was concluded to be negative in the mouse micronucleus assay.

Comparison of TC-5214 Preclinical Safety Profile to R-(−) Enantiomer and Racemic Mecamylamine

The safety profile of TC-5214 (S-(+)-mecamylamine) was compared to those of the R-(−) enantiomer and racemic mecamylamine. Table 2 summarizes the results of studies in mice, comparing several side effects (ptosis, tremors, immobility, rapid breathing) and mortality of TC-5214 to its enantiomer R-(−)-mecamylamine and racemic mecamylamine. The results indicate that TC-5214 has a better overall safety profile than the R-(−) enantiomer and racemic mecamylamine, with respect to both side effects and mortality.

Discussion and Conclusions

The data presented here support the hypothesis that NNR antagonists such as TC-5214 may express therapeutic actions in depression via blockade of NNRs in the CNS. This is consistent with the original cholinergic theory of depression that postulated a CNS imbalance between cholinergic and norepinephrine tone [1,2]. In depression, it has been suggested that there is a predominance of cholinergic activity. Thus, agents that either reduce cholinergic drive or increase NE activity should have antidepressant features. As TC-5214 is an NNR antagonist, its antidepressant activity might be due to restoration of a normal balance between these CNS monoamine systems.

Previous preclinical and clinical findings with racemic mecamylamine support the potential of TC-5214 in the treatment of major depressive disorders. For example, mecamylamine has demonstrated anxiolytic effects in both the social interaction and elevated plus maze

paradigms at low doses [8]. Similarly, mecamylamine has previously shown antidepressant effects in the tail suspension and forced swim models in mice [12]. This was replicated in the present studies in the forced swim test in rats. In human clinical studies, mecamylamine improved comorbid symptoms of depression and ADHD when given to children with TS [24]. Additionally, TS patients with comorbid bipolar disorder demonstrated a mood stabilizing response to mecamylamine [23].

To help determine the particular NNRs that are important for mecamylamine's antidepressant activity, a study was conducted using knockout mice. In that study, the forced swim test of behavioral despair and the tail suspension test demonstrated that both $\alpha 4\beta 2$ and $\alpha 7$ receptors are needed for antidepressant activity [12]. Therefore, it appears to be important for the potential antidepressant efficacy of TC-5214 that antagonist activity is directed toward the predominant NNR subtypes ($\alpha 4\beta 2$; $\alpha 7$) and possibly others in the brain. Off target (i.e. non-NNR) effects are of course possible, but interactions with other receptor classes that have been studied to date seem to occur at much higher concentrations/doses than those required for antidepressant activity. For example, mecamylamine inhibits MK-801 binding to NMDA receptors and NMDA-induced NE release in hippocampus at concentrations of $> 5 \mu\text{M}$ *in vitro* and more than 10 mg/kg *in vivo*, well above doses and/or plasma and CNS levels that are clinically relevant to procognitive or antidepressant effects [reviewed by Young et al., 30].

Although the specific neurotransmitter systems modulated downstream from NNR antagonism and their role in depression have not as yet been fully defined, one hypothesis is that the therapeutic actions mediated through selective blockade of NNRs may involve the release of dopamine and NE and modulation of NNR-mediated neuroendocrine responses to stress. One of the most consistent findings in neuropsychiatry is that the patients with depression have dysfunctional neuroendocrine systems possibly resulting from prolonged responses to stress

[31]. NNRs are found in essentially all major neurotransmitter systems in the CNS, including those which have been implicated in stress responses and depressive states. For example, NNRs are abundant on the cell bodies of dopaminergic neurons both in the substantia nigra and ventral tegmental area [32]. Mecamylamine inhibits the release of dopamine resulting from NNR activation [33] and decreases dopamine metabolism in the nucleus accumbens [34]. Other studies suggest the involvement of NNRs in burst firing in dopaminergic neurons through stimulation of $\alpha 7$ nicotinic receptors whereas $\alpha 4\beta 2$ -containing NNRs appear to modulate firing frequency [35]. In addition to the dopaminergic pathways, the major NE-producing neurons comprising the locus ceruleus abundantly express NNRs [36] and receive massive cholinergic inputs from various CNS areas, such as the pontine reticular formation. NNR modulation of the secretion of NE from the synaptic terminals of these neurons in the hypothalamus has been suggested to trigger homeostatic mechanisms such as stress [37]. This is consistent with earlier studies showing that blockade of NNRs in the brainstem by the antagonist, mecamylamine, resulted in a dose-dependent reduction in NE release from terminals in the paraventricular nucleus of the hypothalamus and a concomitant reduction in plasma adrenocorticotrophic hormone (ACTH) levels [38].

The available evidence suggests that stress-induced release of acetylcholine (ACh) in the brain plays a significant role in mediating neuroendocrine, emotional, and physiological responses to stress. In the CNS, stress induces primarily cholinergic hyperactivation [39] and ACh facilitates the release of several stress-sensitive neurohormones and peptides, including corticosterone, ACTH, and corticotropin-releasing factor (CRF), which has in turn been directly linked to depressive states [40]. When the cholinesterase inhibitor physostigmine was administered to patients with affective syndromes, the symptoms of negative affect were more pronounced and longer lasting [2]. These effects can be attributed to the indirect ACh agonist effects of physostigmine, which cause an increase in heart rate and blood pressure and produce symptoms of dysphoria, depression, anxiety, irritability, aggressiveness, and hostility.

Activation of NNRs by ACh during stress is implicated by the observation that chronic stress causes a downregulation of nicotinic receptor sites in the brain [41]. In addition, it is well known that nicotine mimics the facilitatory effects of ACh on the release of NE, prolactin, corticosterone, ACTH, and CRF through selective activation of NNRs [37,38] and that ACh-induced CRF release from the hypothalamus is inhibited by NNR blockade [42]. A direct contribution of nicotinic receptor activation in the neuroendocrine responses to stress is further supported

by the finding that mecamylamine abolishes the plasma ACTH and corticosterone responses to social stress in rats [43,44]. Moreover, these effects were observed at low doses of mecamylamine (0.1 mg/kg), which may have important therapeutic implications because clinical observations indicate that low doses of mecamylamine reduce tension and anxiety in patients with TS [22].

With respect to safety, the literature supports a substantial therapeutic index for mecamylamine and it is reasonable to suggest that this is also true for its enantiomer TC-5214. Young et al. [30] have compiled a compelling argument from an extensive literature review that supports the view that procognitive and antidepressant effects of mecamylamine occur at much lower concentrations/doses than those required to elicit toxicity and side effects. For example, low doses of mecamylamine have been shown to enhance cognition and memory performance in rats (0.01–0.1 mg/kg) and monkeys (0.025–0.25 mg/kg), thus mimicking the effects of classical nicotinic agonists. Similarly, oral administration of less than 5 mg/day mecamylamine to Tourette's patients with comorbid bipolar disorder reduced anxiety, irritability and aggression, and significantly improved mood [23]. By comparison, therapeutic antihypertensive doses of mecamylamine range from 25 to 90 mg/day. At these doses parasympathetic blocking effects lead to side effects such as constipation, dry mouth, and urinary retention in addition to the well-known hypotensive effects. In addition, cognitive impairment and amnesic effects are well-documented at these higher doses, both in nonclinical studies in rodents and primates and in humans [30].

Clearly a compelling argument can be made for the therapeutic utility of NNR antagonists in treating major depression, but much work has yet to be done both preclinically and clinically to completely define the mechanistic bases. The present data suggest that TC-5214 is a promising candidate for further clinical development in depression. This is strongly supported by its activity in preclinical models of depression and anxiety. In addition, TC-5214 was well tolerated in acute and chronic toxicity studies in mice, rats, and dogs; it showed no mutagenicity and displayed safety in pharmacologic, pharmacokinetic, and metabolic profiles appropriate for therapeutic development.

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