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Effects of tanezumab on satellite glial cells in the cervicothoracic ganglion of cynomolgus monkeys: A 26-week toxicity study followed by an 8-week recovery period

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ABSTRACT

Tanezumab, a humanized monoclonal anti-NGF antibody, has demonstrated efficacy and safety profiles in Phase III clinical trials of chronic pain. In a 24-week study in non-human primates, morphological observations of sympathetic ganglia showed decreased ganglia volume, decreased neuronal size, and increased glial cell density compared with controls after 3 tanezumab treatments. Using stereological techniques to quantify glial cells, the present 26-week study found no significant difference after weekly treatments in total cervicothoracic ganglia satellite glial cell number between placebo- or tanezumab-treated cynomolgus monkeys. These findings suggest that tanezumab treatment does not result in a true gliosis in sympathetic ganglia.

1. Introduction

During nervous system development, nerve growth factor (NGF) mediates the survival and proper differentiation of specific populations of sensory and sympathetic neurons through the binding of its receptors tropomyosin-related kinase A (TrkA; high affinity) and p75 neurotrophin receptor (low affinity). However, in the adult, NGF becomes important for pain signaling pathways. NGF levels increase during inflammation and other painful conditions, and signaling pathways downstream of NGF receptor activation result in the activation of transcription factors that control nociceptive gene expression (Mantyh et al., 2011).

Tanezumab is a highly selective and specific humanized IgG2Δa monoclonal anti-NGF antibody that prevents NGF binding to its receptors, and is in development for the treatment of chronic pain (Mantyh et al., 2011). Tanezumab (or its murine precursor, muMab911) reduced pain-related behaviors in preclinical animal models of pain (Shelton et al., 2005; Zahn et al., 2004). In Phase II and Phase III human clinical trials, tanezumab demonstrated efficacy over placebo in chronic pain conditions such as osteoarthritis and chronic lower back pain (Brown et al., 2012; Katz et al., 2011).

In studies performed to investigate the effects of tanezumab-induced NGF inhibition on sympathetic ganglia, stereologic and morphologic analyses of cynomolgus monkey sympathetic ganglia after three subcutaneous (SC) 1.2 mg/kg tanezumab injections 8 weeks apart revealed diffusely small neurons compared with controls with no indication of neuronal cell death. Glial cell density appeared increased, likely a result of the decreased average neuronal cell size (Belanger et al., 2017). In the present study, stereologic analyses of cervicothoracic ganglia (CTG) from tanezumab-treated adult cynomolgus monkeys were conducted to determine if there was an absolute increase in satellite glial cell (SGC) number (*i.e.* a true gliosis).

2. Materials and methods

2.1. Animal care and use

Male and female cynomolgus monkeys were kept in a temperature- and humidity-controlled environment. Animals were housed individually in cages that complied with the Animal Welfare Act requirements and suggested guidelines (National Research Council, 1996). Animals were offered PMI's LabDiet Laboratory Fiber-Plus biscuits twice daily unless fasted for study procedures; water was provided *ad libitum*. A total of 35 male animals (including 3 spares) weighing 4.0 to 10.8 kg and aged 4.1 to 9.0 years and a total of 38 female animals (including 6 spares) weighing 2.7 to 3.6 kg and aged 5.0 to 6.0 years were selected at the pre-study physical examination. Animals were randomized to groups in a weight-stratified manner to achieve similar

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Table 1
Study design.

Group	Tanezumab dose (mg/kg/week)[a]	Route	Number of animals	
			Males	Females
1	0	SC	6[b] + 4[c]	6[b] + 4[c]
2	4	IV	5[b]	6[b]
3	30	IV	5[b]	6[b]
4	30	SC	5[b] + 4[c]	6[b] + 4[c]

SC, subcutaneous injection; IV, intravenous injection.
[a] Animals were administered vehicle (group 1) or tanezumab (groups 2–4) once a week for 26 consecutive weeks.
[b] Post-dosing necropsy, Day 177.
[c] Recovery necropsy, Day 232.

group body weight distributions.

2.2. Tanezumab administration and study design

Tanezumab, a humanized IgG2Δa monoclonal antibody against NGF, was manufactured and supplied by Pfizer, Inc. Animals were dosed weekly for 26 weeks with tanezumab at: 0 mg/kg (vehicle), 4 mg/kg intravenous (IV), 30 mg/kg IV, or 30 mg/kg SC. Six females and five or six males in each group were terminated and necropsied on Day 177 (the day after the final [26th] dose; post-dosing necropsy). Four females and four males from the control and 30 mg/kg SC groups were followed for 8 weeks following completion of scheduled dosing and were terminated and necropsied on Day 232 (recovery necropsy; Table 1).

2.3. Toxicokinetic analyses

Toxicokinetic (TK) analyses were conducted in animals administered tanezumab IV or SC (n = 6/sex/dose group for IV administration, n = 10/sex/dose group for SC administration). Systemic exposure (as assessed by the highest drug concentration observed in plasma [C_{max}] and the area under the plasma drug concentration-time curve from 0 to 168 h post dose [AUC_{168}]) was similar in males and females across dose groups, therefore, group mean TK parameters are discussed and presented using combined data from both male and female cynomolgus monkeys.

2.4. Section preparation

Tissues for stereology investigations were prepared as previously described (Butt et al., 2014). Briefly, at necropsy, the monkeys were whole-body perfused with 4% methanol-free formaldehyde. The CTG (stellate ganglia; typically formed by fusion of the inferior cervical and the first thoracic sympathetic ganglia) were initially harvested along with the spinal column and stored in 4% methanol-free formaldehyde. For stereology, CTG were removed from the sympathetic chain, embedded in paraffin and sectioned completely through using an 18-μm block advance. Every 20th section was mounted to a glass slide and stained with hematoxylin and eosin (H&E). Stereologic counts of the satellite glial cells were performed on the post-dosing necropsy control, 30 mg/kg IV, and 30 mg/kg SC males and females; counts were not performed on the animals that received 4 mg/kg IV. Prior to the satellite glial cell analysis, a stereologic assessment of neurons was performed (unpublished data). The satellite glial cell assessment was performed on all animals for which sections were deemed suitable (as part of the blinded evaluation performed by the reviewing pathologist) for the prior neuronal analysis. Table 2 reflects the number of animals in each of the target groups analyzed.

Table 2
Summary data for the stereologic evaluation of cervicothoracic ganglia satellite glial cells from cynomolgus monkeys.

Dose and route	Dosing period			
	0 mg/kg SC	4 mg/kg IV	30 mg/kg IV	30 mg/kg SC
Males, n	4	4	4	4
Mean number of SGC	6,490,086	NP	6,523,645	5,780,055
p value	–	–	0.9863	0.7099
Females, n	5	4	3	5
Mean number of SGC	5,031,581	NP	4,813,857	4,677,406
p value	–	–	0.8028	0.6136

SC, subcutaneous injection; IV, intravenous injection; SGC, satellite glial cells; NP, stereologic evaluation not performed.

2.5. Stereologic evaluations

Stereologic investigations were performed blinded (with the operator/technician and the reviewing pathologist completely unaware of the treatment status of the various animals) using Stereologer® software (Stereology Resource Center, Chester, MD). Sections were analyzed using a systematic random approach: every 40th section through the entirety of the ganglion was examined (section sampling fraction of 1/40) beginning with a randomly selected starting section. Ganglion volume was based on the Cavalieri method (Gundersen et al., 1999). The total satellite glial cell count was estimated using the optical fractionator method (Mouton, 2002) counting satellite glial cells in a subset (area sampling fraction of approximately 0.529) of each section evaluated. SGCs were only counted if they came into focus within the frame height and only if the center of the cell nucleus was within the dissector frame or touching an inclusion line. SGCs were identified based on their morphologic characteristics on H&E-stained sections.

2.6. Statistical analysis

To determine if the differences between groups were statistically significant, a two-tailed, independent *t*-test was performed. Tanezumab-treated animals were compared to concurrent controls of the same sex.

3. Results

3.1. Stereologic evaluations

3.1.1. Ganglion volume

Differences in ganglion volume were not statistically significant across treatment groups (males and females; data not shown). Although not statistically significant, average male ganglion volume was lower in the IV 30 mg/kg and SC 30 mg/kg tanezumab groups compared with controls. These results are congruent with those of a previous study, which demonstrated a significant decrease in ganglion volume in male cynomolgus monkeys treated with SC 1.2 mg/kg tanezumab (Belanger et al., 2017).

3.1.2. Satellite glial cell counts

The average total number of SGCs in the ganglia of the control, 30 mg/kg IV tanezumab, and 30 mg/kg SC tanezumab-treated animals (both male and female) was similar for animals evaluated after the dosing phase (Table 2). There were no statistically significant differences between the control groups and the tanezumab-treated groups.

3.2. Toxicokinetic analyses

There were no quantifiable concentrations of tanezumab in plasma samples collected and analyzed from control dose group animals. Mean overall C_{max} on Day 169 was 233, 1480 and 1080 μg/mL for the 4 (IV),

30 (IV) and 30 (SC) mg/kg/week dose groups, respectively (Supplemental Table 1). Mean overall AUC_{168} on Day 169 was 26,000, 160,000 and 162,000 μg·hour/mL for the 4 (IV), 30 (IV) and 30 (SC) mg/kg/week dose groups, respectively (Supplemental Table 1). For the IV dose groups, mean systemic exposure increased with increasing dose in an approximately dose-proportional manner on Days 1, 22, 85 and 169. For the IV and SC dose groups, mean systemic exposure was similar on Days 22, 85 and 169; exposure on these days was higher compared to Day 1. The mean time at which C_{max} was first observed (T_{max}) was 3.0 h post-dose on all Days for the 4 (IV) and 30 (IV) mg/kg/week dose groups. For the 30 (SC) mg/kg/week dose group, mean T_{max} was observed at 67, 37, 33 and 45 h post dose on Days 1, 22, 85 and 169, respectively (Supplemental Table 1).

4. Discussion

Prior morphologic studies in sympathetic ganglia have reported an increased density of satellite glial cells in the CTG of tanezumab-treated cynomolgus monkeys compared with controls (Belanger et al., 2017). At the end of the current study's dosing period, there was no significant difference in total calculated SGCs between sex-matched controls and male or female monkeys treated with 30 mg/kg IV or 30 mg/kg SC tanezumab. The observed (at the morphologic evaluation) increased density of SGCs was therefore not due to an increase in absolute numbers of SGCs; a true gliosis was not present. The current investigation represents, to the knowledge of the authors, the only quantitative investigation concerning SGCs in animals that have received NGF antibody therapy.

Changes in sympathetic ganglia, including decreased ganglion volume and decreased neuron size/area, have been reported to occur in association with NGF antibody therapy in animal models (Andrews and Cowen, 1994; Hendry and Campbell, 1976; Rich et al., 1987; Ruit et al., 1990). The current investigation indicates that these changes are not caused by and do not elicit a SGC response. The observed increased SGC density that may be associated with some NGF antibody therapies does not appear to be indicative of a true morphologic change, but rather an apparent change possibly due both to decreased ganglion volume and to decreased average neuron size (Belanger et al., 2017), bringing existing cells closer together than normal. Indeed, in the current study, both a trend towards lower ganglion volume and a significant decrease in neuron size/area were observed in tanezumab-treated males compared with placebo (data not shown).

The results of this study are limited by the criteria used to count SGCs. Satellite glial cells were identified as cells with round to oval, distinct/dark nuclei. Cells with oval nuclei with a long axis more than twice the length of the short axis were not counted since most of these cells were likely either endothelial cells or fibroblasts. The counts may have included some Schwann cells (another glial cell population in sympathetic ganglia), as these cells are not always reliably distinguished from SGCs on paraffin/H&E sections, especially when they occur in close proximity to neurons. Nonetheless, the present study findings were reproducible both within and across treatment groups. Therefore, this possible limitation does not change the overall study conclusion.

5. Conclusions

Tanezumab treatment in non-human primates was not associated with an increase in the absolute number of SGCs in sympathetic (cervicothoracic) ganglia as determined using stereologic methods. The apparent observational increase in SGC density in sympathetic ganglia was not considered to represent a true gliosis, as the number of SGCs was similar (not significantly different) between placebo- and tanezumab-treated animals. These results add to the growing body of evidence regarding the preclinical safety profile of tanezumab (Belanger et al., 2017; Bowman et al., 2015; Butt et al., 2014; Gropp et al., 2018; Zorbas et al., 2011).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.autneu.2019.02.004.

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Disclosures

PB, JG, and MZ are employees of Pfizer and own stock and/or stock options in Pfizer. ME and TC are former employees of Pfizer, Inc. ME owns Pfizer shares. MB is an employee of Tox Path Specialists who were paid consultants to Pfizer in connection with the development of this manuscript.

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