



## Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans

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### Abstract

The vanilloid receptor TRPV1 has been identified as a molecular target for the treatment of pain associated with inflammatory diseases and cancer. Hence, TRPV1 antagonists have been considered for therapeutic evaluation in such diseases. During Phase I clinical trials with AMG 517, a highly selective TRPV1 antagonist, we found that TRPV1 blockade elicited marked, but reversible, and generally plasma concentration-dependent hyperthermia. Similar to what was observed in rats, dogs, and monkeys, hyperthermia was attenuated after repeated dosing of AMG 517 (at the highest dose tested) in humans during a second Phase I trial. However, AMG 517 administered after molar extraction (a surgical cause of acute pain) elicited long-lasting hyperthermia with maximal body temperature surpassing 40 °C, suggesting that TRPV1 blockade elicits undesirable hyperthermia in susceptible individuals. Mechanisms of AMG 517-induced hyperthermia were then studied in rats. AMG 517 caused hyperthermia by inducing tail skin vasoconstriction and increasing thermogenesis, which suggests that TRPV1 regulates vasomotor tone and metabolic heat production. In conclusion, these results demonstrate that: (a) TRPV1-selective antagonists like AMG 517 cannot be developed for systemic use as stand alone agents for treatment of pain and other diseases, (b) individual susceptibility influences magnitude of hyperthermia observed after TRPV1 blockade, and (c) TRPV1 plays a pivotal role as a molecular regulator for body temperature in humans.

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### 1. Introduction

The vanilloid receptor TRPV1, a non-selective cation channel expressed in multiple locations within the pain

pathways, is activated by diverse cross-sensitizing painful stimuli, which include, heat (>42 °C), protons (pH < 5.7), capsaicin, resiniferatoxin, components of the “inflammatory soup,” products of lipoxygenase, and G-protein coupled receptor signaling, making it a molecular integrator of pain transduction [1,14,17,29]. TRPV1 is considered as a promising target for an analgesic agent because: (a)

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agonists of TRPV1 cause pain in humans and pain behavior in preclinical species [16,28], (b) TRPV1 expression is up regulated in painful inflammatory conditions in rodents and humans [15,19], (c) TRPV1 knockout mice display attenuated inflammation-induced pain behaviors [2,4,18], and (d) TRPV1 antagonists reverse pain behavior in rodent models of inflammation [8,11,22,32] and cancer [9], reviewed in [14,29]).

AMG 517 was identified as a clinical candidate because it is a potent ( $IC_{50}$  value  $<10$  nM), selective ( $IC_{50}$  value of  $>20$   $\mu$ M against closely related TRP channels, TRPA1, TRPM8, TRPV2, TRPV3 and TRPV4), and orally bio-available antagonist of all modes of TRPV1 activation [5,6]. AMG 517 reverses inflammation-induced pain behavior in rats (the minimally effective dose for reversing CFA-induced thermal hyperalgesia was 0.3 mg/kg in rats), and has a predicted long half-life in humans that may be amenable to once-a-week dosing [5,6]. In addition to blocking pain behavior in rodents, TRPV1 antagonists elicited hyperthermia in rodents, dogs and monkeys but not in TRPV1 knockout mice, suggesting that TRPV1 is tonically activated in vivo and involved in body temperature regulation [6,7,26,27]. Interestingly, hyperthermia evoked by TRPV1-selective antagonists was attenuated after repeated dosing of these antagonists to rats, dogs and monkeys [6], and TRPV1 knockout mice did not exhibit an impairment of thermoregulation [13,30]. Together, these observations supported the initiation of clinical trials with AMG 517. Here, we describe the results of three independent clinical studies of AMG 517 in healthy adults and mechanisms by which AMG 517 elicits hyperthermia.

## 2. Methods

### 2.1. Clinical trials

These studies were conducted in accordance with Medicines and Healthcare products Regulatory Agency (MHRA) and US Food and Drug Administration (FDA) guidelines, and regulations and guidelines provided by the International Conference on Harmonization (ICH) Good Clinical Practice (GCP).

#### 2.1.1. Single dose safety and pharmacokinetics study

This was a double-blind, placebo-controlled, randomized, single dose, dose-escalation sequential-cohort study. Healthy adults received a single oral dose of placebo, or 1, 2, 5, 10, 20, or 25 mg AMG 517 (Table 1). The primary endpoints were the number and incidence of treatment-emergent adverse events including multiple daily (while in residence) and study visit oral and tympanic body temperature measurements.

#### 2.1.2. Multiple dose-temperature study

This was a double-blind, placebo-controlled, randomized, multiple-dose, dose-escalation, sequential-cohort study. Healthy adults were randomized to receive a single daily dose over 7 days of either placebo or 2, 5 or 10 mg AMG 517 (see

Table 1  
Summary of healthy subjects who received AMG 517 and their plasma concentration, across Phase I clinical studies

Dose (mg)	Number of subjects who received AMG 517	$C_{max}$ (ng/mL) (means $\pm$ SD)
<i>First in human (1st Phase I trial)</i>		
1	6	10.9 $\pm$ 1.76
2	6	22.2 $\pm$ 3.61
5	6	52.7 $\pm$ 8.82
10	6	105 $\pm$ 9.61
20	8	217 $\pm$ 56.8
25	7	211 $\pm$ 36.5
<i>Multiple dose (2nd Phase I trial)</i>		
2	5	45.2 $\pm$ 7.85
5	6	105 $\pm$ 11.8
10	6	199 $\pm$ 36.1
<i>Molar extraction (Phase Ib trial)</i>		
2	3	21.5 $\pm$ 5.1
8	4	103.8 $\pm$ 50.2
15	2	104.2 $\pm$ NA

Vehicle used for all studies is 2% Poloxamer 338 in OraPlus™. NA, not available. The plasma concentration of AMG 517 was determined at  $\sim$ 10–12 h post-dosing in 1st Phase I and Phase Ib trials and on day 8th in 2nd Phase I trial.

Table 1). The primary endpoints were treatment-emergent adverse events and the difference in maximum body temperature after administration of AMG 517 on day 1 versus subsequent days, through day 7.

#### 2.1.3. Molar extraction study

This was a double-blind, placebo-controlled, randomized, parallel-group, multi-center study with ibuprofen as the positive control. Single doses of 2, 8 or 15 mg of AMG 517, 400 mg of ibuprofen, or placebo were administered orally in a double-dummy design. Each subject who experienced moderate to severe pain after extraction of 2 or more 3rd molars, with at least 1 partially or completely impacted in bone, was randomized to one of the five treatments. A total of 17 subjects, including one female and 16 males ranging in age from 18 to 34 years were randomized to five groups. The primary endpoints for this study were treatment-emergent adverse events and relief from pain during an 8-h period after administration of the investigational products.

#### 2.1.4. Pharmacokinetic analyses

Pharmacokinetic (PK) endpoints included area under the plasma concentration time curve [AUC], maximum observed plasma concentration [ $C_{max}$ ], time of  $C_{max}$  [ $T_{max}$ ] and the terminal phase half-life [ $t_{1/2,z}$ ].

#### 2.1.5. Study inclusion and exclusion criteria

Primary inclusion criteria for healthy subjects included the presence of good general health with normal or clinically acceptable ECG and laboratory values, and a body mass index from 18 to 29 kg/m<sup>2</sup> inclusive at screening. Molar extraction subjects needed to report moderate or severe post-operative pain, rated on a 4-point pain scale (0 = none, 1 = mild, 2 = moderate, 3 = severe) and a score of at least 50 mm on the 100 mm VAS after surgical extraction of two or more 3rd molar teeth.

### 2.1.6. Investigational products, dose and mode of administration

AMG 517 was administered as an oral dose of 1, 2, 5, 10, 20 or 25 mg as a suspension in a 100 mL of 2% Pluronic 108 (Poloxamer 338) in OraPlus<sup>®</sup> followed by two 75 ml water washes of the investigational product bottle. Placebo was administered orally as 100 mL of the suspension vehicle (2% Pluronic in OraPlus<sup>®</sup>) plus followed by 75 ml water washes of the investigational product bottle. The total volume of solution administered was 250 mL.

### 2.1.7. Statistical analyses

Descriptive statistics for each dose group included selected demographic data, baseline characteristics, temperature, safety, and PK data. Continuous variables were summarized using means, medians, standard deviations (SD), and ranges. Frequency counts and percentages were used for categorical variables.

## 2.2. Rat thermometry studies

### 2.2.1. Surgery and preparation

The experiments were conducted in rats under protocols approved by the St. Joseph's Hospital Animal Care and Use Committee. Male Wistar rats were obtained from Harlan (Indianapolis, IN). They were housed in cages kept in a rack equipped with a Smart Bio-Pack ventilation system and Thermo-Pak temperature control system (Allentown Caging Equipment, Allentown, NJ); the temperature of the incoming air was maintained at 28 °C. Standard rat chow and tap water were available *ad libitum*. The room was on a 12:12 h light–dark cycle (lights on at 7:00 AM). Each rat was extensively handled and habituated to staying inside wire-mesh conical confiners (used later in the thermocouple-respirometry setup). At the time of the experiments, the rats weighed 310–380 g. Five to seven days before the experiment, each rat was anesthetized by ketamine–xylazine–acepromazine (55.6, 5.5, and 1.1 mg/kg; *i.p.*), treated prophylactically with an antibiotic (enrofloxacin, 1.1 mg/kg, *s.c.*), and had a silicone catheter (ID 0.5 mm, OD 0.9 mm) filled with heparinized (10 U/ml) saline implanted in the right jugular vein. The catheter was flushed with heparinized saline (10 U/ml) the day after surgery and every other day thereafter.

### 2.2.2. Experimental setup, protocols, and data processing and analysis

As reported in the past [25,26] rats were placed in confiners and equipped with copper-constantan thermocouples for recording colonic temperature and tail skin temperature. The thermocouples were connected to a data logger (Cole-Parmer, Vernon Hills, IL). Each rat in the confiner was then placed inside a cylindrical Plexiglas chamber (Sable Systems, Las Vegas, NV), which was sealed and continuously ventilated; the airflow was maintained at 600 ml/min with the help of a mass flow controller (Sierra Instruments, Monterey, CA). The air leaving each chamber was automatically sampled, dried, and passed through an oxygen analyzer (Sable Systems). The Plexiglas chamber containing the rat was kept inside a climatic chamber (Forma Scientific). A venous catheter was connected to a PE-50 extension filled with saline. The extension from the catheter was passed through a port of the Plexiglas chamber, and the port was sealed with paraffin. The extension was then passed through a port of

the climatic chamber and connected to a syringe. Using the Romanovsky et al. [24] method, we found that  $T_{as}$  of 23–29 °C were neutral for rats in this setup.  $T_a$  was set to 26 °C during the experiments. Working solutions of AMG 517 (300 µg/ml) in 50% ethanol were prepared before the experiment. The solutions were infused via the jugular catheter at a rate of 167 µl/kg/min for 2 min; the dose of AMG 517 infused was 100 µg/kg. Control rats were infused with the vehicle (50% ethanol in saline). Deep body (colonic) temperature, tail skin temperature, and oxygen consumption were monitored for at least 4 h after the infusion. Tail skin temperature was used to calculate the heat loss index [26]; a decrease in this index indicates vasoconstriction. Oxygen consumption was determined as described in our recent paper [26]. The responses were compared by two-way ANOVA using Statistica AX'99 (StatSoft, Tulsa, OK). Results are reported as means ± SE.

## 3. Results

### 3.1. AMG 517 elicited a generally plasma concentration-dependent hyperthermia in humans

Administration of single oral doses ranging from 1 to 25 mg of AMG 517 to healthy adults demonstrated rapid absorption ( $T_{max}$  of 1–2 h), dose-dependent increase in  $C_{max}$  (peak plasma concentration), and  $AUC_{0-\infty}$  (area under the plasma-time curve from zero to infinite time) (Table 1; Fig. 1A). Consistent with the long half-life of this molecule in rats (31 h), dogs (41 h), and monkeys (62 h) [5], a long mean  $t_{1/2,z}$  range of 13 to 23 days was observed in humans across the dose range. No safety issues were observed upon AMG 517 administration except moderate, generally plasma concentration-dependent increases in body temperature (Fig. 1B). Body temperature typically increased between 1 and 4 h after AMG 517 administration and returned to baseline values within 24 h, suggesting the transient nature of hyperthermia induced by AMG 517. By contrast,  $C_{max}$  was observed at 1–2 h post-dosing (Fig. 2A), usually earlier than the peak increase in body temperature, suggesting that the development of hyperthermia in healthy subjects was elicited by TRPV1 blockade. During this study, an exception to plasma concentration-dependent hyperthermia was observed in one subject in the 25 mg dose group whose body temperature was increased to 39.9 °C, although this person did not have the highest  $C_{max}$  (Fig. 1B). Thus, despite overall plasma concentration-dependent hyperthermia, the magnitude of temperature elevation after exposure to single doses of AMG 517 also reflected individual susceptibility to the hyperthermic effect of TRPV1 blockade.

### 3.2. AMG 517-induced hyperthermia was attenuated after repeated dosing in humans

When AMG 517, or its closely related analogue, AMG8163 was administered daily to rats, dogs, or mon-

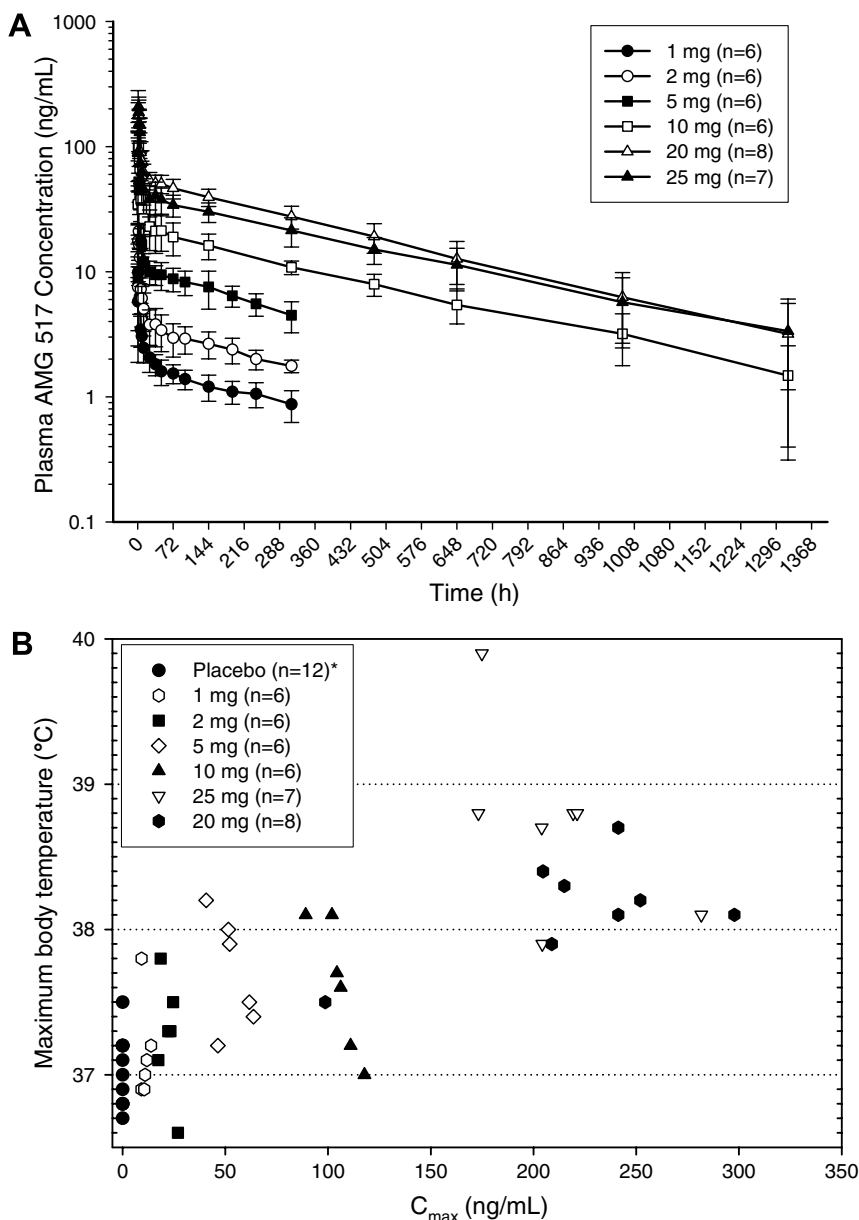


Fig. 1. (A) Pharmacokinetic profile of several doses of AMG 517 in human volunteers. (B) Mean of the maximum body temperature (tympanic) vs. maximum observed plasma concentration following oral administration of single doses of AMG 517 (1–25 mg).

keys, hyperthermia was attenuated within 2–4 days to values indistinguishable from the vehicle-treated groups [6]. This habituation or attenuation to the hyperthermic effects of TRPV1 antagonists supported the initiation of a Phase I multiple dose study in humans. When subjects were exposed to daily single oral doses of AMG 517 (2, 5, or 10 mg), for 7 days, they had dose-dependent increases in body temperature with the mean maximal temperature reaching  $38.3 \pm 0.1^\circ\text{C}$  on the first day of drug administration (Fig. 2B). There was a statistically significant attenuation of hyperthermia at the highest dose (10 mg dose of AMG 517) on days 2 through 7. However, mean maximal body temperatures of all sub-

jects administered with all doses of AMG 517 were significantly higher compared to placebo-treated group over the 7 day dosing period.

### 3.3. AMG 517 elicited marked and persistent hyperthermia in subjects undergone molar extraction

Although AMG 517 caused a generally plasma concentration-dependent hyperthermia, it was unknown what plasma concentration of AMG 517 would produce analgesia in humans and whether that concentration could be reached without triggering hyperthermia. Since TRPV1 is expressed in the dental pulp and believed to

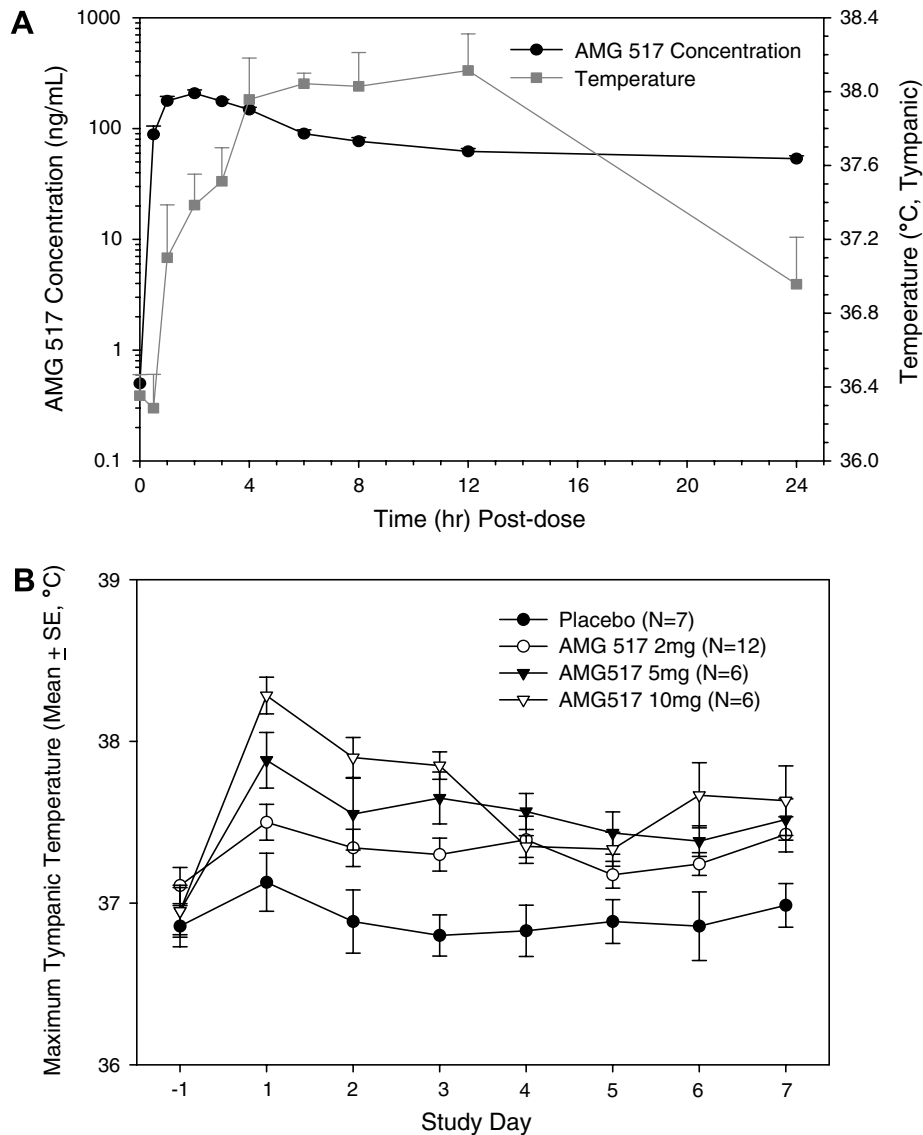


Fig. 2. (A) Mean body temperature and log AMG 517 concentration vs. time (h) after oral administration of a single dose of AMG 517 (25 mg). (B) Mean of the maximum body temperature (tympenic) after daily oral administration of multiple doses of AMG 517 (2, 5, 10 mg) for 7 days. Maximum tympanic temperature on day 1 for 10 mg AMG 517 dose group was significantly higher compared to days -1, and 2-7 ( $P < 0.05$ ). Maximum tympanic temperature on day 1 for 5 mg AMG 517 dose group was significantly higher compared to days -1, and 2, 4-7 ( $P < 0.05$ ). Mean maximum body temperatures of all doses of AMG 517 were significantly greater than placebo ( $P < 0.05$ ) on days 1-7. There is no significant temperature change for placebo group.

contribute to pain after molar extraction [20,21], it was decided to proceed to an efficacy study to treat acute pain after molar extraction.

In this Phase Ib study, subjects were administered a single dose of placebo or 2, 8, or 15 mg of AMG 517, as soon as they experienced moderate to severe post-operative pain. Body temperature elevations were observed in all subjects who received AMG 517 in this study. A 2 mg (or approximately 29  $\mu\text{g}/\text{kg}$ ) dose of AMG 517 triggered hyperthermia that exceeded 40 °C in one subject, with hyperthermia of >39 °C that persisted for three days despite multiple doses of anti-pyretic medication (Fig. 3A). Similar marked hyperthermia

that persisted for two or more days was also observed in two subjects who received 15 mg dose of AMG 517 after molar extraction (Fig. 3B). Hyperthermia of >38 °C was observed in three out of four subjects who received 8 mg of AMG 517. In summary, body temperatures of 39–40.2 °C persisted for 1–4 days in 33% of the subjects who received AMG 517 after molar extraction with no other observed cause for this marked and persistent hyperthermia. These studies also indicated that the largest-magnitude hyperthermia (>40 °C) occurred in one out of three subjects who received 2 mg of AMG 517 was appeared to be related to individual susceptibility.

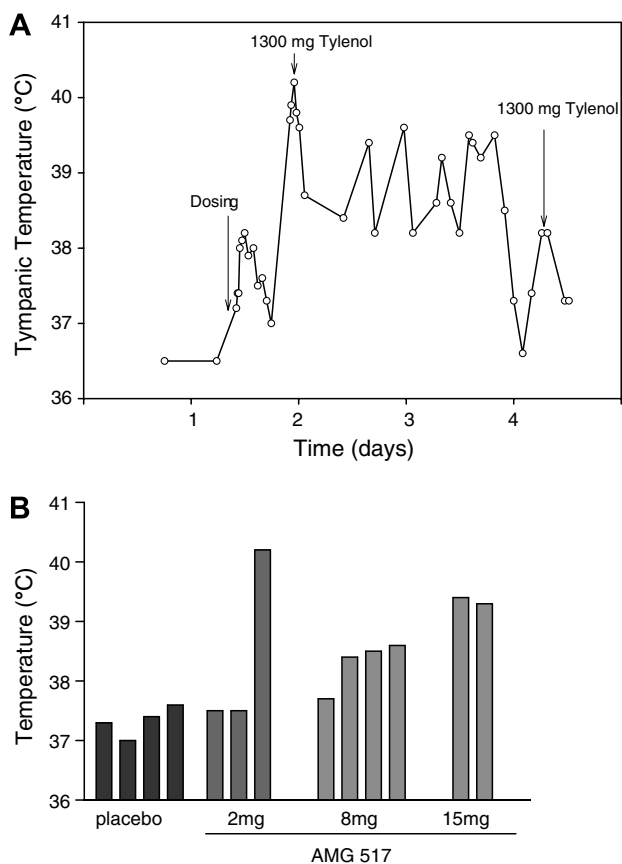


Fig. 3. (A) Tympanic body temperature measurements over 3 days in a single subject exposed to 2 mg AMG 517 after 3rd molar extraction. The times of administration of Tylenol<sup>®</sup> (acetaminophen) administered for anti-pyresis, showing incomplete reversal of hyperthermia, are also shown (arrows). Detailed log of various medicines given to this subject is shown in Supplementary information. (B) Tympanic body temperature measurements of subjects exposed to placebo or different doses of AMG 517 after 3rd molar extraction are shown.

### 3.4. AMG 517 elicits hyperthermia by skin vasoconstriction and increased thermogenesis

The mechanism(s) underlying AMG 517 elicited hyperthermia in humans are unknown. To determine candidate mechanism(s) for this effect, a thermocouple-respirometry setup was used to monitor body temperature, skin temperature and oxygen consumption in loosely restrained rats kept at a neutral ambient temperature of 26 °C. Under these conditions body core temperature exclusively depends on the autonomic effectors, and rats exhibit modest vasodilation and low thermogenesis [23]. Similar to what was observed with another TRPV1 antagonist, AMG0347 [26], intravenous administration of 100 µg/kg of AMG 517 elicited tail skin vasoconstriction (a heat conservation mechanism), and elevated thermogenesis measured by increased oxygen consumption (Fig. 4). Whereas the increased oxygen consumption predominantly represents non-shivering thermogenesis in rats, shivering thermogenesis contrib-

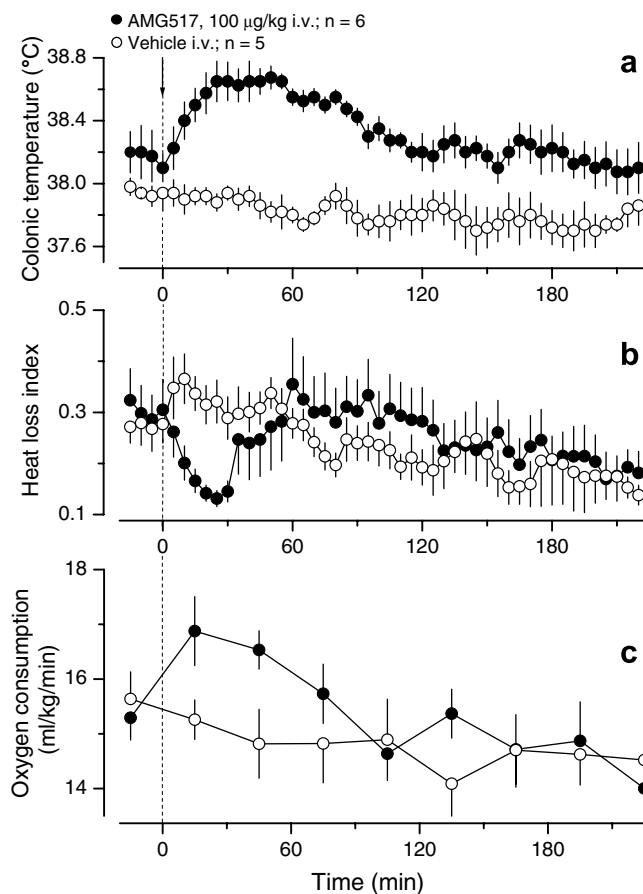


Fig. 4. AMG 517 causes hyperthermia by activation of thermoeffectors. Effects of AMG 517 or vehicle on (a) the colonic temperature, (b) heat loss index, and (c) oxygen consumption of rats at an ambient temperature of 26 °C. The colonic temperature is an index of deep body temperature; the heat loss index is a measure of skin vasodilation; oxygen consumption is an index of metabolic heat production.

utes equally to heat production in primates including humans. It is relevant to note here that many subjects who received AMG 517 during clinical trials (Section 3.1) described ‘feeling cold’ and had visible shivering prior to the onset of hyperthermia, suggesting that shivering thermogenesis might also have contributed to AMG 517-induced hyperthermia in humans.

## 4. Discussion

AMG 517, a highly selective TRPV1 antagonist was considered suitable for clinical evaluation because of its favorable selectivity, pharmacokinetic, and safety profile [5,6]. AMG 517 is a potent antagonist of all modes of human TRPV1 activation (IC<sub>50</sub> value range: 5–20 nM) and is highly selective against closely related TRP channels (IC<sub>50</sub> value >5–20 µM). In rats, the minimally effective dose (p.o.) of AMG 517 required to block capsaicin-induced flinching, and to reverse CFA-induced thermal hyperalgesia was 0.3 mg/kg (plasma

concentration of  $\sim 100$  ng/ml [6]). The minimally effective dose (p.o.) of AMG 517 to cause hyperthermia was 0.1 mg/kg (plasma concentration of  $\sim 30$  ng/ml [6] and data not shown), suggesting that hyperthermia after exposure to AMG 517 appears prior to anti-hyperalgesia in rats. However, this AMG 517-induced hyperthermia was attenuated after repeated dosing in rats, dogs, and monkeys [6]. Furthermore, TRPV1 knockout mice did not exhibit an impairment of thermoregulation [13,30]. Taken together, these observations supported evaluation of AMG 517 for its potential analgesic utility.

#### 4.1. AMG 517's effects on body temperature in humans

Based on the preclinical studies, it was predicted that AMG 517 might cause a dose-dependent hyperthermia in humans but that hyperthermia would attenuate after repeated dosing of TRPV1 antagonists [6,7]. Although AMG 517 elicited a generally plasma concentration-dependent hyperthermia in first-in-human pharmacokinetic and safety study, one individual also showed a high-magnitude hyperthermia despite having the lowest plasma concentration of AMG 517 in this dose (25 mg) group. These results suggest that there is individual susceptibility to developing high-magnitude hyperthermia after TRPV1 blockade.

In the second Phase I study, during administration of AMG 517 for 7 days, hyperthermia was attenuated after repeated dosing of the 10 mg dose, but not the 2 or 5 mg doses of AMG 517, suggesting that attenuation of hyperthermia is dose-dependent. However, mean maximal body temperatures remained higher in all drug-treated subjects when compared with placebo-treated subjects over the 7 days of dosing. The reasons for unambiguous attenuation of hyperthermia after repeated exposure to all doses of AMG 517 in preclinical species [6] but not in humans are unclear. One possibility is the differences in the doses used in preclinical species and in human studies. Rats and monkeys received 3 mg/kg and 30–500 mg/kg, respectively [6]; whereas the maximum repeated dose for humans was 10 mg or approximately 0.14 mg/kg ( $199 \pm 36$  ng/ml plasma concentration), which is about 20-fold lower than the lowest dose used in rats. Doses greater than 0.14 mg/kg were not used for repeated dosing studies in humans because of the undesirable hyperthermia observed at the higher doses in the first Phase I study, and because the long half-life of AMG 517 in humans was expected to cause an accumulation during repeated dosing. The plasma concentration of 10 mg (or 0.14 mg/kg) dose of AMG 517 on day 8 was  $199 \pm 36$  ng/ml, which is approximately equivalent to the  $C_{\max}$  obtained at 20 and 25 mg dose used in the first Phase I study (Table 1).

A major hurdle for the clinical development of AMG 517 was the emergence of marked and persistent hyper-

thermia observed in subjects undergoing molar extraction. Although the plasma concentrations of AMG 517 observed in this Phase Ib study were similar to those obtained in the first pharmacokinetic and safety study (Table 1), such large magnitude and persistent hyperthermia was not observed after exposure to AMG 517 in healthy subjects. The surgical procedure [31] and TRPV1 blockade [7,26,27] may have acted additively or synergistically to produce an undesirable, marked and persistent hyperthermia in susceptible individuals. Alternately, access of AMG 517 in solution to normal or sensitized TRPV1 receptors [20,21] in the surgical wound may have contributed to the differences in hyperthermia produced by AMG 517 in these two populations.

The appearance of marked hyperthermia at low doses (and plasma concentrations) of AMG 517 in humans, without similarly discernible analgesia, suggests that the target occupancy requirement for hyperthermia in humans may be low. Previous studies in animals have clearly demonstrated that hyperthermia is an on-target effect and represents target coverage *in vivo* [7,26]. Although, AMG 517-induced hyperthermia in humans does not indicate the level of target coverage or the location of target coverage, coverage of visceral TRPV1 channels in rats appears to be sufficient for inducing hyperthermia [26]. It appears that a plasma concentration of 20–30 ng/ml of AMG 517 represents the target coverage to cause hyperthermia in humans. However, the target coverage or plasma concentration of AMG 517 required for analgesia in humans remains unknown.

Clinical studies of AMG 517 were discontinued because of hyperthermia after exposure to single and multiple doses of this molecule in healthy volunteers, and due to marked and persistent hyperthermia after exposure to single doses of AMG 517 in subjects who underwent molar extraction. Due to the early termination of this study, too few subjects were evaluated to determine the potential analgesic effect of AMG 517.

#### 4.2. Mechanisms of TRPV1 blockade-induced hyperthermia

It is known that the TRPV1 agonists capsaicin and resiniferatoxin cause an increase in heat loss by skin vasodilation and also reduce metabolic heat production [12]. We report that TRPV1-selective antagonists such as AMG 517 (this study) and AMG0347 [26] elicit opposite effects. Taken together, these results suggest that tonically active TRPV1 channels regulate thermoeffector mechanisms such as vasomotor tone and thermogenesis. The agonist-induced hypothermia and antagonist-induced hyperthermia are abolished in TRPV1 knockout mice [2,26], suggests that effects on body temperature by agonists and antagonists are exclusively mediated by TRPV1. The fact that TRPV1 knockout

mice do not display severe impairment in thermoregulation [13,30] indicates the involvement of TRPV1 in thermoregulation may be developmentally compensated, perhaps by other thermoTRP channel(s).

The results of this study clearly establish, for the first time in humans, that a thermoTRP channel, TRPV1 is a physiologically important, tonic molecular regulator for body temperature. These results also show that blockade of tonically active TRPV1 elicits an undesirable, high-magnitude, and persistent hyperthermia in susceptible individuals. TRPV1-selective antagonists causing hyperthermia in rodents, dogs, monkeys and humans, indicate that the thermoregulatory function of this channel is evolutionarily conserved. Importantly, the mechanism by which tonically active TRPV1 controls body temperature is through constant suppression of thermoeffectors such as vasomotor tone and thermogenesis ([26]; this study). We hypothesize that TRPV1 is one of the several regulators of body temperature maintenance in which one or more of other tonically active thermoTRP channels may counterbalance the constant suppression of thermoeffectors by TRPV1 to maintain the normal body temperature.

#### 4.3. Perspectives

Recent reports unequivocally demonstrated that antagonists of TRPV1 cause hyperthermia in rodents, dogs, monkeys [6,7,26,27], and humans (this study). It was also reported that TRPV1 antagonists cause the same magnitude of hyperthermia in normal rats and in rats with ongoing inflammation, thus suggesting that inflammation-associated fever and TRPV1 antagonist-induced hyperthermia act additively (Cortright D, 2006, Spring Pain Research Conference, Apr 22–29, Grand Cayman, BWI). Despite these observations, several pharmaceutical companies have started or completed Phase I clinical trials with their TRPV1 antagonists (SB-705498, NGD 8243, and GRC 6211) and are moving forward into Phase II trials in dental pain and other indications (reviewed in [29]). No information is available regarding whether these molecules cause hyperthermia in preclinical species and humans. Furthermore, selectivity profiles of these molecules have not been published.

Antagonists such as JYL1421 and AMG8562 that block capsaicin activation of rat TRPV1 potentiated low pH activation of the channel and did not cause hyperthermia in rats ([7] Lehto et al., unpublished observations), suggesting the feasibility of eliminating hyperthermia with differential modulation of TRPV1 activation. However, it is not known if a molecule can modulate both rat and human TRPV1 similarly, and such modulators will be effective as anti-hyperalgesics or analgesics. Detailed characterization of TRPV1 antagonists for their effects on distinct modes of TRPV1

activation, selectivity profile against closely related TRP channels, effects on body temperature, and effects on hyperalgesia induced by inflammation should reveal the potential of developing hyperthermia-free TRPV1 antagonist therapy. Although SB-705498, a molecule that blocks both TRPV1 and TRPM8 [10] was recently reported to reduce capsaicin-evoked flare, and increased heat pain tolerance at the site of UVB-evoked inflammation [3], the potential analgesic utility of TRPV1 selective antagonists as single agents still remains unknown.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.pain.2008.01.024](https://doi.org/10.1016/j.pain.2008.01.024).

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