

Nonopioid analgesics discovery and the Valley of Death: EMA401 from concept to clinical trial

Maree T. Smith

1. Introduction

Globally, the prevalence of chronic pain is high at 10% to 50% of the adult population.^{18,23,40,57,63,64} Chronic pain not only impairs the quality of life of patients and their caregivers^{40,75} but also has a large socioeconomic cost comprising direct healthcare costs and indirect costs due to reduced workforce participation.^{10,25}

Chronic pain is often difficult to alleviate adequately with clinically used analgesic and/or adjuvant agents because of lack of efficacy and/or dose-limiting side effects.¹²³ Thus, there is a large unmet medical need for novel, efficacious, and well-tolerated nonopioid analgesics for the relief of chronic pain.¹²³ This unmet need is a powerful driver of research on chronic pain mechanisms as well as on novel analgesics discovery.¹¹⁴ In the past 3 decades, numerous receptors, ion channels, and enzymes have been identified at multiple levels of the somatosensory nervous system in neurons and/or nonneuronal cells as potential targets for novel analgesics discovery. Based on the huge collective effort to date to reveal chronic pain mechanisms, a reasonable prediction 20 years ago would have been that by 2022, multiple, novel highly efficacious nonopioid analgesics would have been added to the armamentarium for front-line clinicians to prescribe. That this is not the case prompts the question “Why not?” Reasons in common with other therapeutic areas include toxicity and/or poor tolerability, poor pharmaceutical properties, and unsuitable pharmacokinetics of the clinical candidate. However, the major impediment to successful preclinical to clinical research translation in the novel nonopioid analgesics field is lack of efficacy in early phase clinical trials.²⁸

2. Root cause analysis of failed clinical trials of novel nonopioid analgesics

Factors contributing to poor efficacy in clinical trials of investigational nonopioid analgesics for the relief of chronic

pain include incomplete knowledge of the underlying pain mechanisms, inappropriate analgesic drug target selection, insufficient drug target engagement at clinically tolerable therapeutic drug concentrations, use of rodent pain models that do not adequately recapitulate the pathobiology of the chronic pain conditions that will be targeted in human clinical trials and in the clinical setting, pain behaviours in rodent models that do not adequately model ongoing pain in patients, between-sex differences, lack of robust and validated pain biomarkers, and problems with clinical trial design and execution including patient selection (**Fig. 1**). These factors are addressed in the next few sections of this review.

2.1. Analgesic drug target—which to choose?

According to the mouse pain gene and human pain gene databases curated by Jeff Mogil⁸⁵ and the Pain Research Forum⁷⁸ (<https://www.painresearchforum.org/resources/pain-gene-resource>; accessed March 16, 2022), respectively, there are currently 430 mouse and 94 human genes implicated in the pathobiology of pain. However, the precise nature of how these pain genes and their encoded targets interact in the pathophysiology of individual chronic pain conditions in patients is unclear. It is thus unsurprising that modulation of a single target receptor, enzyme, or ion channel to evoke promising pain relief in rodent models of chronic pain has in most instances not translated to significant pain relief relative to placebo in early phase clinical trials.^{121,124} Examples of “failed” novel analgesics include neurokinin-1 receptor antagonists,⁹⁹ chemokine receptor-2 antagonists,⁴⁴ fatty acid amide hydrolase inhibitors,³⁸ Na_v1.7 inhibitors,⁴⁹ and p38 mitogen-activated protein kinase (MAPK) inhibitors,⁷⁶ to name but a few.¹²¹ The overall poor preclinical to clinical translation of novel analgesics for relief of chronic pain suggested that between-species differences in biology between humans and rodents may have been underestimated.⁶⁵ However, this notion is countered by the fact that pain-related behaviours are phylogenetically ancient and widespread.⁶⁶

2.2. Drug–target engagement at tolerable doses

Once a target is selected and lead molecules are synthesized that modulate the target, another factor to consider is how much target engagement is necessary to alleviate chronic pain and how much is needed to safely block a pain response without blocking “protective” pronociceptive signalling in response to acute insults such as major trauma or surgery. It is plausible that clinical trial “failures” may have been underpinned, at least in part, by doses that were too low to ensure adequate target engagement to block pathological pronociceptive signalling and alleviate chronic pain.⁴⁹

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Centre for Integrated Preclinical Drug Development, School of Biomedical Sciences, Faculty of Medicine, The University of Queensland, Brisbane, Queensland, Australia

Corresponding author. Address: Centre for Integrated Preclinical Drug Development (CIPDD), School of Biomedical Sciences, Faculty of Medicine, The University of Queensland, St Lucia Campus, Brisbane, QLD, Australia 4072. Tel.: +61 7 33652554; fax: +61 7 33467391. E-mail address: maree.smith@uq.edu.au (M.T. Smith).

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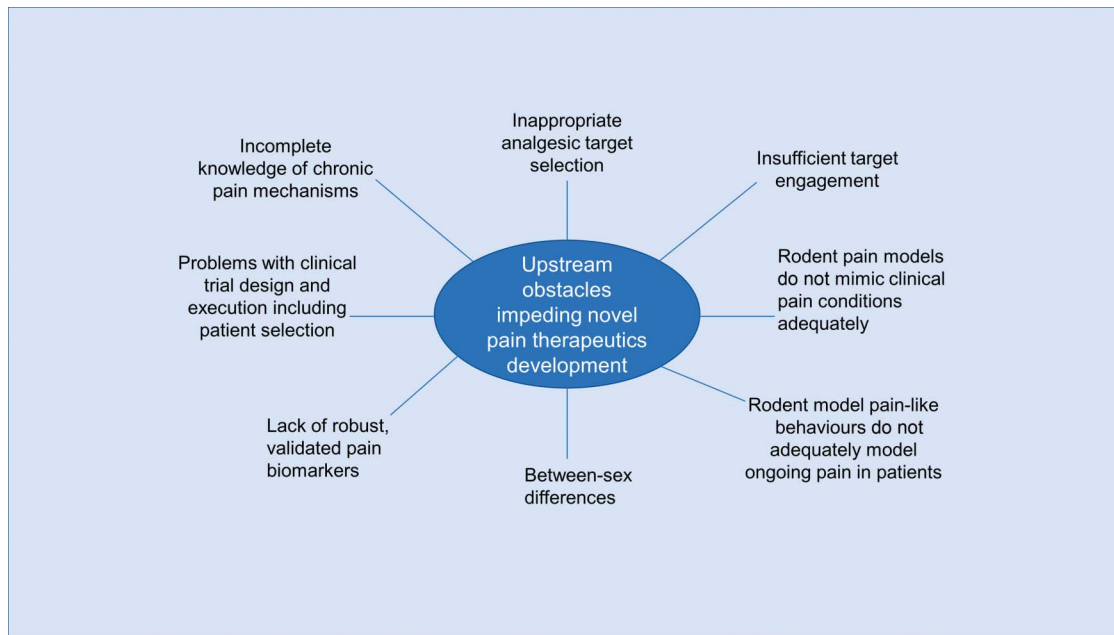


Figure 1. Schematic diagram of obstacles impeding the translation of promising preclinical efficacy data to successful clinical trials in patients suffering from various chronic pain conditions.

2.3. Discovery approaches for novel therapeutics to alleviate chronic pain

There are 2 overarching approaches for discovery of novel nonopioid analgesics for alleviating chronic pain in humans. The first is to discover molecules that cross the blood–brain barrier and enter the brain and spinal cord to reduce central sensitization and/or augment descending inhibitory neurotransmission, with the net result being relief of chronic pain. The second is to discover agents that are peripherally restricted and so excluded from the brain and spinal cord to avoid central nervous system side effects. The latter modulate targets in peripheral components of the somatosensory nervous system to reduce pronociceptive input into the spinal dorsal horn, thereby leading to indirect attenuation of central sensitization that underpins the development and maintenance of various chronic pain conditions over time.¹¹⁶

2.4. Predictive validity of rodent pain models

Another factor potentially contributing to poor preclinical to clinical research translation in the novel pain therapeutics field is that the animal models used to assess efficacy may inadequately recapitulate the pain pathobiology in patient populations recruited into early phase clinical trials.¹²⁴ For example, preclinical efficacy may have been assessed in mice or rats exhibiting pain-like behaviour because of a mechanically induced peripheral nerve injury, whereas clinical trials may have been conducted in patients with painful diabetic neuropathy as a consequence of a prolonged period of poorly controlled type 2 diabetes. To address this issue, multiple research groups, including my own, have invested considerable resources in establishing optimised rodent pain models with back-translated pharmacological data, with the aim to more closely mimic individual chronic pain conditions in humans.¹¹ Examples include rat models of breast cancer–induced bone pain⁹⁵ and prostate cancer–induced bone pain,^{39,69} rat models of antiretroviral toxic neuropathy (ATN)^{53,117} and HIV-associated neuropathy,¹¹⁸ the chronic phase of the monoiodoacetate rat model of knee osteoarthritis,³⁴ a rat model of mechanical low back pain,^{73,79} a mouse model of chronic low back

pain,⁶² mechanical nerve injury,⁴³ an EAE mouse model of central neuropathic pain,^{45,46,48} a genetic model of painful diabetic neuropathy in the type 2 diabetic Zucker diabetic fatty rat,^{24,77,93} as well as models of chemotherapy-induced peripheral neuropathy in both rats^{32,33,60} and mice.^{41,92,110} However, it is too early to draw conclusions on the effectiveness of this strategy to identify novel analgesic drug leads that will be more likely to achieve clinical trial success.

2.5. Pain behavioural end points

Identification of pain-like end points other than evoked reflexive behaviours has attracted research attention aimed at better mimicking spontaneous ongoing pain in patients. To this end, burrowing,^{4,72,90,100,119} gait analysis,^{34,111,122} facial grimacing,^{20,56,67} and electroencephalography^{52,58} have been proposed as nonevoked surrogate measures of pain in rodents. However, for translational success, reliable pain biomarkers as translatable end points from the preclinical to the clinical research setting are needed and this is addressed in the next section.

2.6. Pain biomarkers

The dearth of robust, validated pain biomarkers in animal models that have utility for use in human clinical studies has hindered mechanistically driven development of novel pain therapeutics to alleviate chronic pain.^{86,114} Despite 3 decades of effort, the extent to which individual chronic pain conditions are underpinned by shared mechanisms remains unclear.⁸⁶ In 2017, a task force concluded that quantitative sensory testing, skin biopsy, and neuroimaging have potential applicability as diagnostic, prognostic, predictive, and pharmacodynamic biomarkers.¹⁰⁷ Other techniques proposed to have pain biomarker potential include microneurography, biochemical (eg, nerve growth factor [NGF], cytokines, and chemokines) and genetic signatures, patient-derived neurons, electroencephalography/magnetoencephalography,¹¹⁴ exosomes,¹⁷ and hyperspectral autofluorescence

imaging.²⁹ However, acceptance of “chronic pain biomarkers” as valid and reliable by international regulatory agencies such as the U.S. Food and Drug Administration and the European Medicines Agency (EMA) will be conditional on their demonstrated utility in clinical trials of analgesic drug candidates in patients recruited into these trials.^{86,114} As yet, there are no chronic pain biomarkers that have reached this level of validation.

2.7. Challenges in the conduct of novel analgesic clinical trials

Estimates are that only 57% of novel analgesic candidates tested in phase 3 clinical trials progress to regulatory approval.²⁸ This implies that many novel investigational analgesics with phase 2 clinical trial data sufficiently promising to progress to phase 3 trials do not go on to produce positive phase 3 results.²⁸ Reasons for this high level of attrition in late-stage clinical development include (1) false positive phase 2 trial results; (2) incorrect dosage selection based on phase 2 trial data; (3) phase 2 and phase 3 clinical trial designs had features compromising assay sensitivity, eg, entry criteria that were too heterogeneous or inappropriate outcome measures; (4) insufficient increase in sample size between phase 2 and phase 3 to allow for potentially increased heterogeneity in the target population in larger phase 3 trials; and (5) low-quality execution of the phase 2 or phase 3 trials.²⁸ To address these issues, the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials has made a concerted effort to develop and publish recommendations on the design of phase 2 and phase 3 clinical trials in patients with chronic pain to optimise clinical trial validity, assay sensitivity, and clinical trial execution.^{21,22,27,28}

2.8. Navigating obstacles impeding novel pain therapeutics development

From the foregoing, there are multiple obstacles impeding success in the development of novel nonopioid pain therapeutics that are efficacious, safe, and well-tolerated for relief of chronic pain in patients. The high degree of difficulty in achieving success has led several large pharmaceutical companies to exit the novel pain therapeutics field because it is “too hard.” Nevertheless, others in industry and academia continue to persist despite the long (15+ years) and arduous path to success.

3. Angiotensin II type 2 receptor antagonists for alleviating peripheral neuropathic pain

As noted in preceding sections, most novel nonopioid analgesics with promising preclinical pain relief data have not translated to successful proof-of-concept (POC) phase 2a clinical trials in patients.¹²¹ An exception is EMA401, a highly selective, orally active, peripherally restricted, small molecule, angiotensin II type 2 (AT₂) receptor antagonist.⁸⁷ The preclinical and clinical development of EMA401 as a novel, first-in-class, nonopioid analgesic for the relief of peripheral neuropathic pain^{71,88,106} is elaborated in the remaining sections of this review.

3.1. Classical renin-angiotensin system

Angiotensin II (Ang II) is the major bioactive peptide of the classical renin-angiotensin system (Fig. 2) that was first discovered >120 years ago.¹¹² Angiotensin II is formed by a multistep enzymatic process commencing with formation of the inactive precursor peptide, angiotensinogen in the liver, which is secreted into the

bloodstream for transport to the kidney.¹¹³ Angiotensinogen is cleaved by the enzyme, renin, which is secreted by the juxtaglomerular apparatus in the kidney, to form the inactive decapeptide, angiotensin I.¹¹³ This in turn is cleaved predominantly by the enzyme, angiotensin-converting enzyme (ACE), to the biologically active octapeptide, Ang II.^{55,113} Angiotensin II binds with nM affinity at both the angiotensin II type 1 (AT₁) receptor and the AT₂ receptor.¹⁹ Both receptors are members of the superfamily of seven-transmembrane domain G-protein-coupled receptors (GPCRs), but their sequence homology is only 34%¹⁹ and they have tissue-specific expression and functional effects.⁸¹ The well-known pressor effect of Ang II is mediated by the AT₁ receptor in the cardiovascular system, and this led to the development of AT₁ receptor blockers and ACE inhibitors for the treatment of hypertension.⁵⁵ By contrast, AT₂ receptor function was long regarded as enigmatic with the Ang II-AT₂ receptor signalling axis thought to antagonize some, but not all, of the cellular actions of Ang II-AT₁ receptor signalling.^{55,91}

3.2. Angiotensin II type 2 receptor: crystal structure and biochemical pharmacology

It was not until 2017 that the crystal structure of the AT₂ receptor was first described¹²⁸ which confirmed its enigmatic nature.⁹¹ Unlike most GPCRs, helix 8 of the AT₂ receptor was not in the canonical position parallel to the membrane pointing outside of the TM7 bundle.¹²⁸ Instead, helix 8 adopted an unusual conformation by flipping over and interacting with the intracellular ends of transmembrane (TM) 3, TM5, and TM6.¹²⁸ Consistent with earlier work by others,^{16,115} neither Ang II nor its agonist analogue, [Sar¹-Ile⁸]-Ang II, induced G-protein or β -arrestin signal transduction at the AT₂ receptor.⁶ This may be due to the increased polarity of intracellular loop 1 to impede engagement of classical GPCR effectors such as G proteins or β arrestins.¹⁶ Another possible explanation is that the AT₂ receptor requires additional effectors for activity.³⁶ This notion is supported by earlier work whereby Ang II signalling via the AT₂ receptor induced neurite outgrowth and morphological differentiation of cultured neuroblastoma x glioma hybrid NG108-15 cells of neuronal origin.^{15,84} Angiotensin II signalling was underpinned at least in part by increased nitric oxide (NO) production,¹⁵ inhibition of p21^{ras}, and sustained activation (phosphorylation) of p44/p42 MAPK, an absolute mediator of neurite outgrowth.^{26,84} In addition, Ang II/AT₂ receptor signalling induced sustained activation of p44/p42 MAPK (also called ERK1/2) and the corresponding neurite outgrowth was mediated by phosphorylation of the high-affinity NGF receptor, TrkA.⁸⁴

3.3. Nitric oxide has a pathobiological role in neuropathic pain

Augmented NO production due to persistent activation of the N-methyl-D-aspartate-NO synthase-nitric oxide signalling cascade at multiple levels of the somatosensory nervous system has long been implicated in the pathobiology of neuropathic pain.^{108,129} This led to multiple drug discovery programs aimed at producing neuronal and/or inducible NO synthase inhibitors for relief of neuropathic pain (reviewed by Mukherjee and colleagues).⁶⁹ As an alternative strategy, I hypothesized that AT₂ receptor antagonism may reduce elevated NO generation in neuropathic pain and so evoke pain relief. I pitched this idea to the inaugural “Trailblazer Challenge Ideas Competition” held towards the end of 2003 by UniQuest, the technology transfer company of The University of Queensland (UQ). I was awarded an AU\$8000 prize which I gave to my UQ chemistry collaborator (Professor Craig Williams) to synthesize for me a batch of PD123,319, an AT₂ receptor antagonist used as a tool

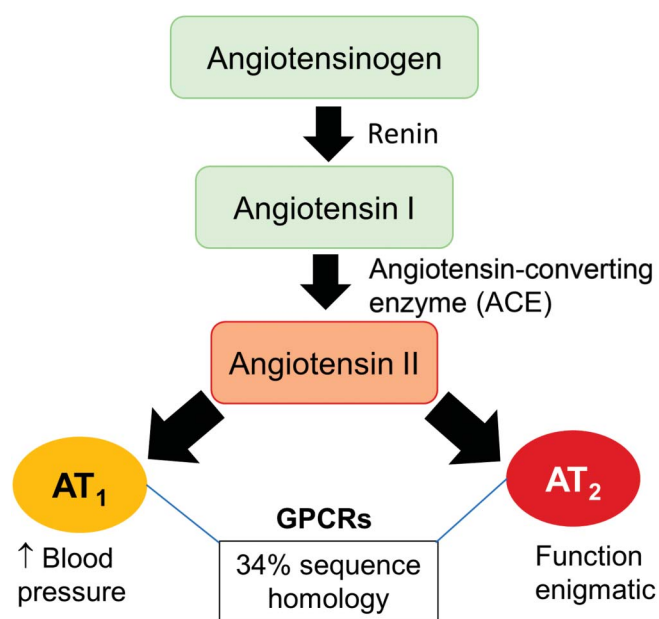


Figure 2. Schematic diagram of the classical renin–angiotensin system.

compound in the cardiovascular field. About a year later, my laboratory tested the pain relief efficacy of PD123,319 (which I renamed EMA200) in a widely used rat model of neuropathic pain. The positive animal efficacy data led to the filing of a provisional patent that was published 18 months later.¹⁰¹ A UQ spin-out company, Spinifex Pharmaceuticals, was formed in mid-2005 to commercialize this discovery with initial investment capital of AU\$3.25m. In the ensuing year, my research team generated efficacy data for multiple AT₂ receptor antagonists in 2 rat models of peripheral neuropathic pain for patent enablement, together with *in vitro* radioligand binding data and pharmacokinetic (PK) data in rats. Research on the mechanism of action was also initiated. An overview of our work, extended by others in more recent years, is outlined below.

3.4. Angiotensin II type 2 receptor antagonists: nonclinical data

3.4.1. *In vitro* binding assays

The radioligand binding data (Table 1) for the small-molecule AT₂ receptor antagonists of interest, namely, EMA200 (also called PD123,319), and its structural analogues, EMA300 (also called PD121,981) and EMA400 (also called PD126,055) (Fig. 3), showed they have >1000-fold binding selectivity at the cloned rat AT₂ receptor relative to the cloned rat AT₁ receptor.¹⁰³ Of these, EMA400 had the highest AT₂ relative to AT₁ receptor binding selectivity at >30,000.¹⁰³ As EMA400 is a racemic mixture of the *S*- and *R*-enantiomers (EMA401 and EMA402, respectively), these were assessed individually, and the *S*-enantiomer (EMA401) was found to have the highest binding specificity at both rat and human AT₂ receptors (>10,000).¹⁰³ In more recent work by others, the nM binding affinity of EMA401 at the AT₂ receptor was confirmed along with the binding affinities of a new series of EMA401 analogues.³⁰

3.4.2. *In vivo* pain relief in rodent models of chronic pain

Highly selective, small molecule AT₂ receptor antagonists alleviated pain-like behaviour in multiple rodent pain models, and these data are briefly described in the following paragraphs and summarized in Table 2.

3.4.2.1. Neuropathic pain

Single intraperitoneal (i.p.) bolus doses of each of EMA200, EMA300, and EMA400 evoked dose-dependent relief of mechanical allodynia in the ipsilateral hind paws of rats with a chronic constriction injury (CCI) of the sciatic nerve, a widely used rat model of peripheral neuropathic pain.¹⁰³ The mean ED₅₀ values were 3.22, 0.78, and 0.013 mg/kg for EMA200, EMA300, and EMA400, respectively (Table 2), and at the doses tested, there were no discernible side effects in these animals.¹⁰³ As the antiallodynic efficacy of EMA300 was abolished in CCI mice null for the AT₂ receptor with intermediate effects in the hemizygotes, the AT₂ receptor was confirmed as the target.¹⁰⁴ More recently, oral (p.o.) EMA401 (10 mg/kg) given as a single dose or by once-daily dosing for 7 days in CCI rats according to an intervention protocol alleviated thermal hyperalgesia in the ipsilateral hind paws (Table 2).⁵² In addition, in CCI rats (Table 2), single doses of EMA401 (30 mg/kg p.o.) alleviated mechanical allodynia in the ipsilateral hind paws with a potency similar to that of an orally active 6-cyano-substituted benzoxazole analogue of EMA401 (compound 15) and orally administered pregabalin.³⁰

The efficacies of single i.p. doses of EMA200 (Smith Laboratory, Australia) and twice-daily i.p. dosing of EMA300 for 3 consecutive days (Rice Lab, United Kingdom) were assessed in a rat model of ATN induced by dideoxycytidine (ddC). In brief, single doses of EMA200 evoked dose-dependent antiallodynia in the bilateral hind paws with an ED₅₀ of 3.2 mg/kg (Table 2),¹⁰⁵ mirroring the ED₅₀ for EMA200 in CCI rats.¹⁰³ Twice-daily dosing of EMA300 at 30 mg/kg (but not 1 or 10 mg/kg) for 3 days alleviated mechanical hyperalgesia in the bilateral hind paws (Table 2).¹⁰⁵ The antihyperalgesic potency of EMA300 did not differ from that of i.p. gabapentin 30 mg/kg dosed twice daily for 3 days, and importantly, these effects were fully reversed by 1 week after dosing cessation.¹⁰⁵

In the spared sciatic nerve injury (SNI) mouse model of peripheral neuropathic pain, single doses of EMA200 (10 mg/kg i.p.) alleviated mechanical and cold allodynia in the ipsilateral hind paws (Table 2) that did not differ between males and females.⁹⁶ This dose of EMA200 also evoked pain relief in SNI mice when assessed using 2 nonevoked pain behavioural assays, namely, the mechanical conflict-avoidance test and the cold preference/avoidance test.⁹⁸ These findings are aligned with the antiallodynia evoked by EMA200 in both CCI rodents^{103,104} and a rat model of ATN.¹⁰⁵ As perisciotic application of EMA200 (30 nmol) evoked antiallodynia in SNI mice but intrathecal EMA200 (30 nmol) did not (Table 2), this implicated a peripheral mechanism of action.⁹⁶ In SNI mice, the AT₁ receptor blocker, losartan (10 mg/kg i.p.), lacked efficacy which excluded a role for AT₁ receptor signalling in evoking allodynia in SNI mice.⁹⁶ Importantly, EMA200-evoked antiallodynia in SNI mice was independent of any hemodynamic changes because systemic dosing did not influence blood pressure in contrast to the AT₁ receptor blocker, losartan.⁹⁶

In a mouse model of acute paclitaxel-induced neuropathic pain, pretreatment with intravenous (i.v.) EMA200 (20 mg/kg) or oral EMA401 (10 mg/kg) prevented the development of mechanical and cold allodynia (Table 2) that otherwise developed in the hind paws of saline pretreated mice.¹²⁷ On the contrary, pretreatment with either ACE inhibitors or the AT₁ receptor blocker, losartan, lacked antiallodynic efficacy¹²⁷ mimicking the lack of losartan efficacy in SNI mice.⁹⁶

3.4.2.2. Mixed neuropathic and chronic inflammatory pain

Prostate cancer-induced bone pain (PCIBP) is underpinned by both inflammatory and neuropathic mechanisms⁶⁹ and so EMA200 pain relief efficacy was assessed in a rat model of PCIBP. In brief, single

Table 1

Radioligand binding affinity and angiotensin II type 2/AT₁ binding selectivity at cloned rat and human angiotensin II type 2 and AT₁ receptors (adapted from Smith and colleagues with permission¹⁰³).

Cloned rat receptors*	AT ₂ R		AT ₁ R		Binding affinity selectivity AT ₂ R/AT ₁ R
	Mean	SEM	Mean	SEM	
Ligand	K _D (nM)		K _D (nM)		
Angiotensin II	1.6	1.0	14.9	1.1	9.3
	IC ₅₀ (nM)		IC ₅₀ (μM)		
EMA200	71.7	14.5	210.5	121.5	~3000
EMA300	46.5	3.3	49.9	10.2	>1000
EMA400	75.2	10.0	2918	1270	>30,000
EMA401	39.5	5.2	408	335	>10,000
	26 ³⁰	12			
EMA402	804		85.4		106
Cloned human receptors					
Ligand	IC ₅₀ (nM)		IC ₅₀ (nM)		
Saralasin ^{##}	0.19		0.48		0.40
	Mean IC ₅₀ (nM)		Mean IC ₅₀ (μM)		
EMA401 [#]	39		n.c.		>10,000 but n.c.
EMA402 [#]	1100		59		53.6
Compound 15 ³⁰	25	9	>10		>400

* n = 3 to 4 separate experiments performed in duplicate except for EMA402 (R-enantiomer of EMA400), where n = 2 separate experiments each performed in duplicate.

For the human receptors, n = 1[#] or 2^{##} performed in duplicate.

AT₂R, angiotensin II type 2 receptor; AT₁R, angiotensin II type 1 receptor; SEM, standard error of the mean; n.c., not calculable.

i.v. doses of EMA200 evoked dose-dependent relief of mechanical allodynia and thermal hyperalgesia in the bilateral hind paws (Table 2).⁷⁰ The ED₅₀s for alleviating mechanical allodynia and thermal hyperalgesia in the ipsilateral hind paws were 0.8 and 3.9 mg/kg, respectively, and the corresponding ED₅₀s for the contralateral hind paws were 1.8 and 5.5 mg/kg, respectively (Table 2).⁷⁰

3.4.2.3. Chronic inflammatory pain

In the Freund complete adjuvant (FCA)-induced rat model of chronic inflammatory pain in 1 hind paw, a 7-day infusion of EMA200 (5 mg/kg/day i.p.) given as a prevention protocol blocked the development of mechanical and thermal hyperalgesia in the ipsilateral hind paws (Table 2) that otherwise developed in FCA rats given a 7-day saline infusion.¹³ In addition, for FCA rats with fully developed hyperalgesia in the ipsilateral hind paws, a single dose of EMA200 (10 mg/kg i.p.) on day 7 fully alleviated thermal hyperalgesia with a partial effect on mechanical hyperalgesia (Table 2).¹³ However, contrary results were reported in FCA mice because single doses of EMA200

(10 mg/kg i.p.) administered on day 2, day 4, or day 7 of the model lacked efficacy (Table 2), as did the AT₁ receptor blocker, losartan (10 mg/kg i.p.).⁹⁶

Vestibulodynia is a chronic inflammatory pain condition characterized by perivaginal mechanical allodynia, hyperinnervation, and abundant inflammatory cell infiltration.¹⁴ In an FCA-induced rat model of this condition, a chronic 7-day infusion of EMA200 (5 mg/kg/d i.p.) given as a prevention protocol attenuated mechanical allodynia in the vestibular tissue by day 3 and this effect was maintained until at least day 6 of the infusion (Table 2).¹⁴

3.4.2.4. Noninjured mice

In noninjured male and female mice, intraplantar (i.pl.) Ang II evoked dose-dependent mechanical allodynia, but not heat hyperalgesia, in the ipsilateral hind paws.⁹⁷ As mechanical allodynia was attenuated by i.pl. EMA200 (10 pmol) (Table 2) but not i.pl. losartan (10 pmol) and it was abolished in AT₂ receptor knockout (KO) but not AT₁ receptor KO mice, a key

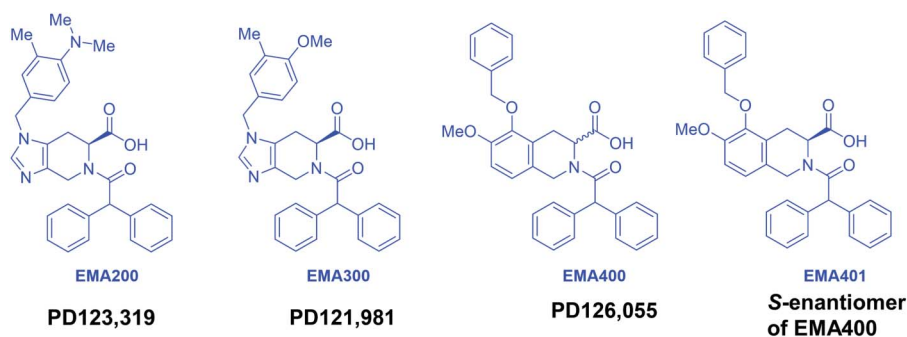


Figure 3. Chemical structures of the small molecule angiotensin II type 2 receptor antagonists—EMA200, EMA300, EMA400, and EMA401.

Table 2

Selective, small molecule, angiotensin II type 2 receptor antagonists alleviate pain-like behavior in rodent models of neuropathic and chronic inflammatory pain.

Pain type	Rodent model and pain-like behavioral end points	AT ₂ R antagonist(s) evaluated and dosing regimen	ED ₅₀ (95%CI) (mg/kg)			Ref
			EMA200	EMA300	EMA400/EMA401 or analogue	
Neuropathic	CCI of the sciatic nerve in the rat; mechanical allodynia	Single i.p. bolus doses; EMA200 1-10 mg/kg; EMA300 1-10 mg/kg; EMA400 0.003-0.03 mg/kg	3.22 (2.02-5.14)	0.78 (0.08-7.68)	0.013 (0.008-0.021)	103
		Single oral dose; EMA401 (30 mg/kg)	NA	NA	✓	30
		Single oral dose; compound 15 (EMA401 analogue)	NA	NA	✓	30
Neuropathic	ddC-induced ATN in the rat; mechanical allodynia (single doses); mechanical hyperalgesia (repeat doses)	Single i.p. bolus doses; EMA200 at 0.3–10 mg/kg	3.2 (1.43-7.0)	NA	NA	105
		Twice-daily i.p. doses; EMA300 at 1, 10, and 30 mg/kg for 3 days	NA	✓	NA	105
		Single i.p. dose; EMA200 at 10 mg/kg	✓	NA	NA	
		Single perisciatic dose of EMA200 (30 nmol)	✓	NA	NA	96
Neuropathic	SNI of sciatic nerve in mice; mechanical and cold allodynia	Single intrathecal dose of EMA200 (30 nmol)	Inactive	NA	NA	96
		Single i.v. doses as prevention protocol; i.v. EMA200 at 20 mg/kg	✓	NA	✓	123
		Oral EMA401 at 10 ng/kg				
Inflammatory	Unilateral i.pl. FCA-induced inflammatory pain in the rat; mechanical and thermal hyperalgesia	Single i.p. dose of EMA200 at 10 mg/kg on day 7; chronic i.p. dosing of EMA200 at 5 mg/kg/d for 7 consecutive days as a prevention protocol	✓	NA	NA	97
			✓			
		Single i.p. dose of EMA200 at 10 mg/kg on day 2, day 4, or day 7	X			96
Inflammatory	FCA-induced monoarthritic pain in the rat knee joint	Single i.p. bolus doses of EMA300 and EMA400 at 0.1-10 mg/kg.	NA	✓	✓	102
		Chronic i.p. doses of EMA200 at 5 mg/kg/d for 7 consecutive days as a prevention protocol	✓	NA	NA	14
Inflammatory and neuropathic	Prostate cancer–induced bone pain in the rat; mechanical allodynia and thermal hyperalgesia in ipsilateral and contralateral hind paws	Single i.v. doses of EMA200 at 0.3–10 mg/kg	0.8 (0.61-1.03) ipsi; 1.8 (1.2-2.8) contra;	NA	NA	70
			3.9 (3.0-5.2) ipsi; 5.5 (3.9-7.7) contra			
Acute pain	i.pl. or intrathecal Ang II in noninjured mice; mechanical allodynia	Single i.pl. dose of EMA200 (10 pmol)	✓	NA	NA	97
		Single intrathecal dose of EMA200 (10 pmol)	X			

Ang II, angiotensin II; AT₂, angiotensin II type 2; ATN, antiretroviral neuropathy; CCI, chronic constriction injury; CI, confidence interval; contra, contralateral; ddC, dideoxycytidine; FCA, Freund complete adjuvant; i.p., intraperitoneal; ipsi, ipsilateral; i.v., intravenous; NA, not assessed; SNI, spared nerve injury.

pathobiological role for the AT₂ receptor was implicated.⁹⁷ Observations that intrathecal Ang II did not induce hind paw hypersensitivity in noninjured mice affirmed a peripheral mechanism.⁹⁷

3.5. Angiotensin II type 2 receptor antagonists in rodent neuropathic pain models: Ex vivo mechanistic insights

3.5.1. Angiotensin II

Angiotensin II immunofluorescence (IF) expression levels are elevated in the lumbar dorsal root ganglia (DRG) from rat and mouse models of peripheral neuropathic pain as well as in a rat model of mixed neuropathic/inflammatory pain. In brief, for CCI rats with fully developed mechanical allodynia in the ipsilateral hind paws, Ang II IF levels were increased ~2-fold to 5-fold in the ipsilateral lumbar DRG relative to the respective levels in the sham controls.^{47,104} Angiotensin II IF was colocalized with that for a subset of neurons (NeuN), as well as CD3⁺ T cells and glial fibrillary acidic protein (GFAP) as a marker of satellite glial cell activation.⁴⁷ In the ipsilateral lumbar DRG of vehicle-treated CCI rats, there was an ~10-fold increase in the number of Ang II-expressing CD3⁺ T cells and an ~5-fold increase in the total number of T cells relative to the respective levels in the sham group.⁴⁷ At the time of peak effect (1 hour postdose) of EMA300 in CCI rats, the otherwise augmented ipsilateral lumbar DRG levels of Ang II IF were reduced to match the respective levels in the sham controls.^{47,104} In particular, the number of Ang II-expressing CD3⁺ T cells and total CD3⁺ T cells were reduced to match the corresponding numbers in the sham group,⁴⁷ thus implicating a mechanism whereby AT₂ receptor antagonism blocks autocrine and paracrine activation of Ang II/AT₂ receptor-expressing CD3⁺ T cells in the ipsilateral lumbar DRG.³⁵ This notion is supported by earlier work where Ang II-dependent influx of CD3⁺ T cells into the rat kidney was inhibited by an AT₂ receptor antagonist.¹²⁰ In the ipsilateral lumbar DRG of these CCI rats, the increased GFAP expression was not attenuated by a single dose of EMA300.⁴⁷ A role for augmented Ang II signalling via the AT₂ receptor to increase GFAP expression is supported by work from others whereby a 7-day infusion of a subpressor dosing regimen of Ang II potentiated hind paw hypersensitivity in CCI rats which was accompanied by satellite glial cell activation in the ipsilateral lumbar DRG of the same animals.⁸² Interestingly, expression levels of individual NGF isoforms (pro-NGF and mature NGF) were significantly reduced in the ipsilateral lumbar DRG of CCI rats to match the corresponding sham group, when assessed by western blot.⁴⁷ At the time of peak effect of a single dose of EMA300 in these animals, the otherwise reduced mature NGF expression levels were fully restored to match the corresponding levels in the sham controls.⁴⁷ As NGF is also expressed by satellite glial cells, future work directed at defining temporal changes in NGF expression patterns in both neuronal and nonneuronal cells in the lumbar DRG in multiple peripheral neuropathic pain conditions is warranted to gain detailed insight into the interplay between augmented AT₂-mediated and NGF-mediated pronociceptive signalling.⁴⁷

For PCIBP rats exhibiting bilateral hind paw hypersensitivity, there were similar findings in the lumbar DRG whereby Ang II IF levels were increased in both the ipsilateral (6.5-7.0-fold) and the contralateral (3.5-4.0-fold) DRG, relative to the respective levels for the sham controls.⁷⁰ Similarly, in SNI mice, Ang II levels quantified using a quantitative enzyme-linked immunosorbent assay were markedly elevated (8-10-fold) in the ipsilateral, but not the contralateral, sciatic nerve or in sciatic nerves from the sham controls.⁹⁶ A peripheral mechanism is implicated because Ang II levels were unchanged in the spinal cords of both SNI and sham-operated mice.⁹⁶

3.6. Angiotensin II type 2 receptor

There are conflicting reports on AT₂ receptor expression by DRG neurons. Immunohistology with an Abcam antibody (ab19134) showed that AT₂ receptor IF levels did not differ between the ipsilateral lumbar DRG from CCI and sham rats.^{47,104} This was mirrored in PCIBP rats and confirmed using quantitative real-time polymerase chain reaction that showed that the total lumbar DRG levels of AT₂ receptor mRNA in PCIBP rats did not differ from that of the sham group.⁷⁰ Because of questions raised on the specificity of commercially available AT₂ receptor antibodies,³¹ *Agtr2*^{GFP} reporter mice were used to gain more insight. Somewhat unexpectedly, the GFP signal was undetectable in the DRG and the superficial layers of the spinal dorsal horn in these mice.⁹⁶ This was unlikely due to low GFP signal intensity as a subset of NF200⁺ fibres (but not CGRP⁺ fibres) were GFP-stained in the sciatic nerves, as were NF200⁺ and NeuN⁺ somata in deeper laminae of the spinal dorsal horn and the ventral horn.⁹⁶ In *Agtr2*^{GFP} SNI mice, the GFP signal was undetectable in DRG neurons or microglia and/or MΦs but there was a large and sustained infiltration of MΦs to the site of nerve injury in both sexes, but not in the spared sural nerve fibres.⁹⁶ In SNI *Agtr2*^{GFP} mice, overlap of the macrophage marker, F4/80, with the GFP signal suggested that MΦs at the site of nerve injury express the AT₂ receptor, whereas the lack of a GFP signal in the spinal cord indicated that microglia do not.⁹⁶

Macrophage Fas-induced apoptosis (MaFIA)/SNI mice were used by 2 groups independently^{96,125,126} to assess a pathobiological role for peripheral MΦs in SNI-induced hind paw hypersensitivity. Two different protocols were used to deplete peripheral MΦs in MaFIA/SNI mice, and this may underpin the differing conclusions drawn as outlined below.^{96,125,126}

Angiotensin II type 2 receptor-expressing peripheral MΦs in MaFIA/SNI mice were depleted by Shepherd and colleagues⁹⁶ over a 5-day period, and this was accompanied by progressive attenuation of allodynia in the ipsilateral hind paws. This was reversible because allodynia redeveloped in a temporal manner in the ipsilateral hind paws on cessation of peripheral MΦ depletion, and this was accompanied by repopulation of the injured sciatic nerves with infiltrating MΦs.⁹⁶ The depletion protocol did not reduce the expected increase in MΦ and/or microglia density in the spinal dorsal horn of MaFIA/SNI mice which led to the conclusion that peripheral MΦs have a critical role in the development of allodynia in these animals.⁹⁶ In the depleted mice, there was a 50% reduction in blood monocyte and/or MΦs, which was accompanied by a near-complete loss of infiltrating MΦs at the site of nerve injury.⁹⁶ By contrast, Yu and colleagues^{125,126} used a milder macrophage depletion protocol in MaFIA/SNI mice to implicate an expansion of resident DRG MΦs rather than MΦs at the nerve injury site as being critical for initiation and maintenance of allodynia in the ipsilateral hind paws of MaFIA/SNI mice (**Fig. 4**). In support of this latter notion, selective removal of MΦs at the nerve injury site had no impact on the pain phenotype.^{125,126}

A critical role for peripheral MΦs in the development of hind paw hypersensitivity in SNI mice was further confirmed using radiation to deplete bone marrow haematopoietic progenitor cells in *Agtr2*-wild-type (WT) mice, which was followed by transplantation with haematopoietic progenitor cells from either *Agtr2*-KO or *Agtr2*-WT donors.⁹⁶ Eight weeks later, an SNI was induced, and 5 days later, allodynia was present in the ipsilateral hind paws of mice that had received the haematopoietic progenitors from *Agtr2*-WT mice.⁹⁶ By contrast, allodynia did not develop in those that had received haematopoietic progenitors from *Agtr2*-KO mice.⁹⁶

3.6.1. Angiotensin II–angiotensin II type 2 receptor signalling and activated mitogen-activated protein kinases in the dorsal root ganglia of rodent pain models

As noted in section 3.2, Ang II signalling via the AT₂ receptor induces neurite outgrowth in cultured cells of neuronal origin with this effect mediated by persistent activation of ERK1/2.²⁶ Notably, there are high expression levels of activated (phosphorylated) p38 MAPK and ERK1/2 in painful neuromas in humans⁹ and experimental neuromas in rats,⁸³ which develop because of abnormal sprouting of peripheral nerve fibres. Of particular interest, pp38 MAPK and/or pERK1/2 can phosphorylate multiple voltage-gated ion channels, including Na_v1.7,^{83,109} Na_v1.8,³⁷ Cav2.2 calcium channels,⁶¹ and TRPV1,⁴² all of which are expressed by DRG neurons and are implicated in the pathobiology of neuropathic pain. Based on these data, we assessed the extent to which the antiallodynic effects of small molecule AT₂ receptor antagonists were underpinned by inhibition of p38 MAPK and ERK1/2 activation in the lumbar DRG from CCI and PCIBP rats exhibiting hind paw hypersensitivity. In brief, augmented levels of pp38 MAPK and pERK1/2 in the ipsilateral lumbar DRG of CCI rats were attenuated at the time of peak effect of EMA300 (1 hour postdose) to match the respective levels in the sham controls.^{47,104} Similarly, in PCIBP rats, EMA200 at the time of peak effect attenuated the otherwise elevated levels of pp38 MAPK and pERK1/2 in the lumbar DRG to match the respective levels in the sham group.⁷⁰ These ex vivo findings are aligned with in vitro work by Anand and colleagues³ who showed that exposure of cultured rat DRG neurons to Ang II or compound 21 (AT₂ receptor agonist) increased the formation of pp38 MAPK and pERK1/2 and this was attenuated by coexposure of neurons to EMA401.³ Together, these data implicate a role for augmented Ang II signalling via the AT₂ receptor that leads to activation (phosphorylation) of p38 MAPK and ERK1/2 in the ipsilateral lumbar DRG, which contribute to the pathobiology of peripheral neuropathic pain^{47,104} and mixed neuropathic/inflammatory pain.⁷⁰

3.6.2. Angiotensin II/angiotensin II type 2 receptor signalling and TRPA1 in mice

Allodynia evoked by i.pl. Ang II injection into the ipsilateral hind paws of mice is mediated by the TRPA1 and not the TRPV1 or TRPV4 receptor because this allodynia was abolished in TRPA1-receptor KO, but not TRPV1 or TRPV4 KO mice.⁹⁷ This notion was affirmed in SNI mice as a TRPA1 antagonist (A967079), but not a TRPV1 antagonist (AMG9810), attenuated allodynia in the ipsilateral hind paws.⁹⁷ In a rat model of visceral pain, TRPA1 activation increased expression levels of pp38 MAPK and pERK1/2 in DRG neurons.^{50,51} The extent to which augmented Ang II signalling via the AT₂ receptor enhances signalling through the TRPA1 receptor in DRG neurons to increase expression levels of pp38 MAPK and pERK1/2 in the lumbar DRG of rodent models of neuropathic pain remains to be investigated.

3.7. Tissue renin–angiotensin system in peripheral components of the somatosensory nervous system

The aforementioned ex vivo data implicate the presence of a tissue renin–angiotensin system in peripheral components of the somatosensory nervous system, and this is addressed in the 2 sections below.

3.7.1. Dorsal root ganglion neurons

As noted in section 3.5.2, data on AT₂ receptor expression by DRG neurons are conflicting. In adult rat DRG, quantitative real-time polymerase chain reaction showed that mRNAs for multiple renin–angiotensin system components including the AT₂ receptor, angiotensinogen, renin, ACE, and cathepsin D were present.^{12,70,80} *In situ* hybridization confirmed the cytoplasmic presence of angiotensinogen mRNA in rat DRG neurons.⁸⁰

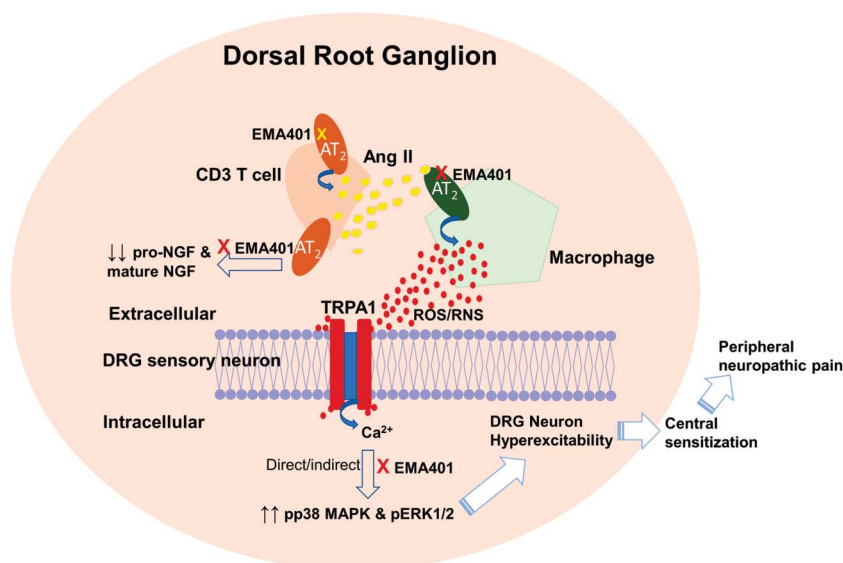


Figure 4. Schematic diagram of augmented Ang II from CD3⁺ T cells and possibly other cells signalling through the AT₂ receptor on peripheral macrophages to generate reactive oxygen species and/or reactive nitrogen species (ROS and/or RNS) that transactivates the TRPA1 receptor on DRG neurons resulting in neuronal hyperexcitability, a hallmark of peripheral neuropathic pain. Augmented Ang II signalling by the AT₂ receptor also induces activation of p38 MAPK and pERK1/2 that is reduced by a single bolus dose of small molecule AT₂ receptor antagonists at the time of peak effect to match the corresponding levels in the sham controls. Augmented Ang II signalling via the AT₂ receptor also reduces levels of pro-NGF and mature NGF in the ipsilateral lumbar DRG with levels of mature NGF restored to match the corresponding levels in the sham controls at the time of peak effect of an AT₂ receptor antagonist. The precise interplay between TRPA1 receptor activation, increased expression levels of pp38 MAPK and pERK1/2, and reduced expression levels of pro-NGF and mature NGF in the ipsilateral lumbar DRG in rodent pain models is currently unclear and remains to be defined. Ang II, angiotensin II; AT₂, angiotensin II type 2; DRG, dorsal root ganglia; MAPK, mitogen-activated protein kinase; NGF, nerve growth factor.

Semiquantitative RT-PCR showed that exposure of female postnatal day 15 (P15) cultured DRG neurons to an inflammatory soup for 24 hours increased AT₂ receptor mRNA expression.⁷ In both rat and human DRG, intracellular Ang II staining in neurons and their processes was colocalized with pronociceptive neuropeptides (substance P and calcitonin gene-related peptide) in some neurons.^{7,80} In human DRG extracts, high-performance liquid chromatography and radioimmunoassay confirmed the presence of angiotensin peptides.⁸⁰ In human nerve tissue extracts, Ang II was the major angiotensin peptide because Ang III levels were below the lower limit of detection of the enzyme-linked immunosorbent assay.³ Using immunohistology with a goat AT₂ receptor antibody (sc-48452), AT₂ receptor IF was colocalized with that of both Ang II and the transient receptor potential cation channel subfamily V member 1 (TRPV1) in a large proportion of cultured small-diameter adult human DRG neurons.^{2,3} Using the same antibody with cultured P15 primary female rat DRG neurons showed that AT₂ receptor IF was colocalized with isolectin B4, a marker of nonpeptidergic neurons.⁷ Angiotensin II type 2 receptors on cultured adult rat DRG neurons were functional as exposure of these neurons to either Ang II or an AT₂ receptor agonist (compound 21) increased neurite outgrowth that was attenuated by the AT₂ receptor antagonist, EMA401, without producing neurotoxicity.^{2,3} There were similar findings for AT₂ receptor-mediated neurite outgrowth for P15 female rat DRG neurons.⁷

On the contrary, *Agtr2* mRNA in mouse DRG neurons was undetectable using RT-PCR and high-throughput RNAseq in agreement with a lack of detectable GFP in DRG sections from *Agtr2*^{GFP} reporter mice and insignificant expression of *AGTR2* mRNA by next-generation deep sequencing of total RNA from human DRG.⁹⁷ Thus, caution is required when interpreting AT₂ receptor IF data due to questions raised on the specificity of commercially available AT₂ receptor antibodies³¹; additional investigation is warranted.

3.7.2. Peripheral macrophages

A role for AT₂ receptor-expressing peritoneal MΦs in the pathogenesis of peripheral neuropathic pain is supported by high-throughput mRNA expression data in the National Center for Biotechnology Information-Gene Expression Omnibus database whereby mouse and human MΦs express *Agtr2* and *AGTR2* mRNA, respectively.⁹⁷ Angiotensin II type 2 receptors on mouse peritoneal MΦs are functional as their *in vitro* stimulation with Ang II or an AT₂ receptor agonist (CGP42112A) induced ERK1/2 phosphorylation that was sensitive to EMA200, but not losartan, and it was abolished in MΦs from AT₂ receptor KO, but not AT₁ receptor KO mice.⁹⁷

3.8. Angiotensin III/angiotensin II type 2 receptor signalling induces ROS/RNS formation by peritoneal macrophages, but not dorsal root ganglion neurons

The presence of functional AT₂ receptors on mouse peritoneal MΦs was confirmed by live cell imaging.⁹⁷ In brief, their exposure to either Ang II or CGP42112A induced time-dependent and concentration-dependent generation of reactive oxygen species/reactive nitrogen species (ROS and/or RNS) that did not differ between the sexes.⁹⁷ ROS and/or RNS formation was sensitive to EMA200, abolished in MΦs from AT₂-KO mice, and attenuated by the free radical scavenger, N-acetylcysteine.⁹⁷ Primary cultures of mouse and human DRG neurons did not seem to express functional AT₂ receptors as their Ang II exposure did not elicit ROS/

RNS generation.⁹⁷ However, contrary findings were reported by Anand et al.^{2,3} such that Ang II-induced neurite outgrowth by cultured adult rat DRG neurons was inhibited by EMA401 without producing neurotoxicity.^{2,3} These differences require additional investigation.

3.8.1. Angiotensin II type 2 receptor and transactivation of TRPA1

Functional AT₂ receptors on MΦs have a critical role in MΦ to DRG neuron transactivation of neuronal TRPA1 on DRG neurons as Ang II exposure induced a sustained increase in intracellular calcium signalling in cocultures of AT₂ receptor KO mouse DRG neurons with WT mouse peritoneal MΦ, but not the converse.⁹⁷ Similar findings were produced in cocultures of human DRG neurons and a human monocyte and/or MΦ cell line.⁹⁷

Taken together, the *in vitro* data implicate a critical role for augmented Ang II-induced activation of the AT₂ receptor on peripheral MΦs to increase ROS and/or RNS generation which transactivates TRPA1 on DRG neurons to induce neuronal hyperexcitability and central sensitization in the spinal dorsal horn, a process that underpins the pathobiology of peripheral neuropathic pain.⁹⁷ These effects were all attenuated by AT₂ receptor antagonists *in vivo* resulting in pain relief in rodent neuropathic pain models (Table 2) and analgesia in a POC clinical trial in patients with postherpetic neuralgia, a type of peripheral neuropathic pain that is often intractable.⁸⁷

4. Angiotensin II type 2 receptor antagonists: preclinical pharmacokinetics

It is essential to assess the PK and oral bioavailability of novel analgesics from drug discovery to inform selection of drug candidates that will have sufficient systemic exposure over a suitably long period to enable optimal target engagement without evoking dose-limiting toxicity. Hence, the preclinical pharmacokinetics of each of EMA200, EMA300 (sodium salt), EMA400, and EMA401 (sodium salt) were assessed in naive rats after bolus dosing by the *i.v.* and oral routes.¹⁰³ The PK parameters derived from the plasma concentration vs time curve data for each molecule of interest are summarized in Table 3 for between-analogue comparison.¹⁰³ In brief, the dose-normalized systemic exposure of oral EMA401 was 20 to 40-fold greater than that for EMA200 and EMA300 (Table 3). The volume of distribution (*V_d*) of EMA401 at 3.7 L/kg was an order of magnitude lower than that for EMA200 and EMA300 (Table 3), consistent with the fact that EMA401 does not cross the blood-brain barrier.^{30,52,71} The plasma clearance of EMA401 at 1.1 L/h/kg was 9-fold and 6-fold lower than that for EMA200 and EMA300, respectively, and the oral bioavailability of EMA400 and EMA401 was ~30%, which is acceptable, whereas that for EMA200 and EMA300 at <10% was not (Table 3).

Overall, the antiallostatic potency rank order of EMA400 > EMA300 > EMA200 in male CCI rats is consistent with the corresponding dose-normalized systemic exposure rank order in rats.¹⁰³ The suitability of EMA401 as the drug candidate for progression to formal development was supported by its good potency, its >10,000-fold binding selectivity over the AT₁ receptor, and its favourable PK.¹⁰³ The poor ability of EMA401 to cross the blood-brain barrier^{30,52,103} suggested that central nervous system side effects may be minimal in human clinical trials. *In vitro* metabolism of EMA401 in hepatocytes from mouse, rat, dog, monkey, and human showed that it had relatively low

Table 3

Summary of mean pharmacokinetic parameters and oral bioavailability of EMA200, EMA300, EMA400, and EMA401 (S-enantiomer of EMA400) in adult male SD rats (adapted from Smith and colleagues¹⁰³ with permission).

Parameter	EMA200	EMA300 (sodium salt)	EMA400	EMA401 (sodium salt)
$t_{1/2}$ (h)	3.6	8.2	5.9	2.3
V_d (L/kg)	47.3	76.8	6.0	3.7
Cl (L/h/kg)	9.3	6.1	0.71	1.1
Oral T_{max} (h)	0.23	0.36	0.33	2.0
Dose-normalized oral C_{max} (kg/mL)	7.3	6.8	130.4	88.5
Dose-normalized oral $AUC_{0-\infty}$ (hour.kg/mL)	6.5*	13.9	398.6	301
F (%)	5.9	7.1	28	33.2

$AUC_{0-\infty}$ = area under the plasma concentration vs time curve from time zero extrapolated to infinity; Cl = systemic clearance; C_{max} = peak plasma concentration after oral dosing; F (%) = oral bioavailability; $t_{1/2}$ = terminal elimination half-life; T_{max} = time of C_{max} after oral dosing; V_d = volume of distribution; *value based on AUC_{0-t} , where t is the last measurable plasma concentration.

metabolic stability and that glucuronidation was a major metabolic pathway.⁷⁴

4.1. Toxicity and safety studies

After completing an Investigational New Drug (IND)-enabling toxicology and safety pharmacology program of EMA401 in animals, oral EMA401 (sodium salt) was progressed by Spinifex Pharmaceuticals into phase 1 clinical trials in healthy young and healthy older volunteers according to single ascending dose and multiple ascending dose paradigms. The respective maximum tolerated doses were 2000 mg (single dose) and 800 mg twice daily.¹⁰⁶

5. Early phase clinical trials

Based on the favourable safety, tolerability, and PK properties of oral EMA401 in healthy human subjects, it was progressed by Spinifex into a Phase 2a POC clinical trial in patients with postherpetic neuralgia (PHN), a type of peripheral neuropathic pain that is notoriously difficult to treat.⁸⁷ This was the first randomized controlled trial reported in any disease where the therapeutic potential of AT₂ receptor antagonism was shown.⁸⁸

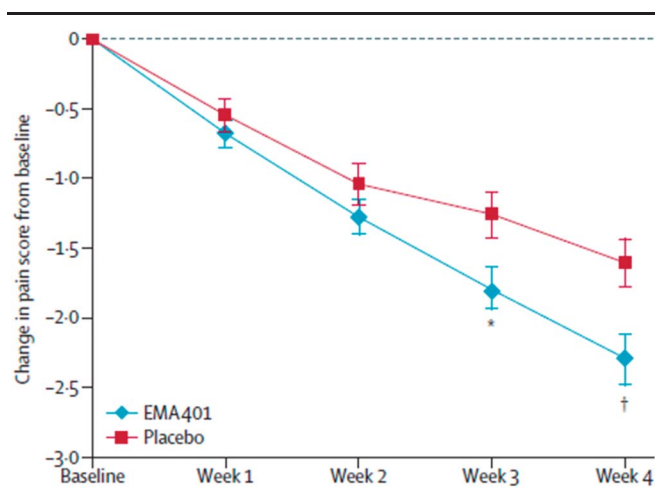


Figure 5. In a randomized, double-blind, parallel group, placebo-controlled, POC clinical trial in patients with postherpetic neuralgia (PHN) of at least 6-month duration, twice-daily dosing with oral EMA401 (sodium salt) at 100 mg for 4 weeks evoked superior relief of PHN relative to placebo at the end of 28 days of treatment with significant differences between the 2 groups evident by day 21 of treatment. Reproduced from Ref. 87, with permission from Elsevier.

5.1. Proof-of-concept clinical trial of EMA401 in patients with postherpetic neuralgia

This POC trial (ACTRN12611000822987) was designed as a randomized, double-blind, parallel-group study to assess the efficacy, safety, and PK of twice-daily oral doses (100 mg) of either EMA401 or placebo in 183 informed, consenting patients with PHN of at least 6-month duration.⁸⁷ EMA401 and placebo were administered for a 4-week period in accordance with recommendations for POC study designs for novel analgesics.²⁷ This trial was conducted at 29 study sites across 6 countries with 92 patients randomized to EMA401 and 91 patients randomized to placebo.⁸⁷ Randomization was according to a centralised randomization schedule and blocked by site to increase assay sensitivity.^{22,28} There were 87 and 84 completers in the EMA401 and placebo groups, respectively.⁸⁷ The primary efficacy end point was change in mean pain intensity between baseline and the final (fourth) dosing week measured using an 11-point numerical rating scale.⁸⁷ Encouragingly, EMA401 provided superior relief of PHN relative to placebo at the end of 28 days of treatment with significant differences between the 2 groups evident by day 21 of treatment (**Fig. 5**).⁸⁷ Importantly, there were no serious adverse events in the EMA401 group.⁸⁷ Based on these data, the number needed to treat to obtain $\geq 50\%$ pain relief for EMA401 was estimated at 6.7, which was comparable to pregabalin 300 mg/day (5.2), pregabalin 600 mg/day (4.0), gabapentin ≥ 1200 mg/day (7.7), and topical capsaicin 8% (7.1).¹⁰⁶ In 2015, Spinifex Pharmaceuticals was acquired by Novartis.

5.2. Phase 2 dose-ranging clinical trials of EMA401

The positive POC phase 2a clinical trial results in PHN and the minimal treatment emergent adverse effects evoked by EMA401 administered at 100 mg twice daily for 4 weeks justified the initiation by Novartis of two 13-week, dose-ranging, efficacy, and safety phase 2b clinical trials of oral EMA401 in patients with PHN (NCT03094195) and painful diabetic neuropathy (PDN; NCT03297294). Unfortunately, these 2 trials were terminated prematurely in 2019 because of unexpected adverse histopathologic findings in the livers of cynomolgus monkeys in a 39-week preclinical toxicity study.⁸⁹ In both phase 2b trials, the mean reduction in the pain score was numerically in favour of EMA401 dosed at 100 mg twice daily at the end of week 12. However, no firm conclusions could be drawn because of the premature termination and that the 300-mg twice-daily dosing regimen was not assessed.⁸⁹ Importantly, for patients dosed with oral EMA401

at 25 or 100 mg twice daily for up to 13 weeks, EMA401 was well-tolerated and there were no changes in liver biochemical parameters during or after these trials were ceased.⁸⁹ A possible mechanism for the unexpected hepatotoxicity in cynomolgus monkeys may involve acyl migration of EMA401 glucuronide to produce products with potential to form covalent adducts with liver proteins that may act as a hapten to the immune system, but this remains to be determined.

6. Conclusions

Discovery and development of novel nonopioid analgesics for the relief of chronic pain is a high-risk, arduous, and lengthy process (>15 years), with many obstacles to be navigated (Fig. 1). In the past 3 decades, an enormous collective global effort has been directed at enhancing knowledge on chronic pain mechanisms and/or identification of potential pain biomarkers to facilitate preclinical to clinical research translation, and this is ongoing. My research team and others have invested considerable resources aimed at improving the extent to which rodent pain models mimic individual chronic pain conditions experienced by patients. Use of these optimised models has the potential to also assist with identification of mechanisms in common between various chronic pain subtypes. In the clinical trial arena, the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trial consortium has contributed in a major way to the development and dissemination of recommendations on the design of phase 2 and phase 3 clinical trials of novel analgesic agents aimed at optimising clinical trial validity, assay sensitivity, and clinical trial execution.^{21,22,27,28} These are all essential steps for navigating obstacles that impede the translation of promising preclinical efficacy data of novel pain therapeutics to positive clinical trial outcomes. A target with positive POC clinical trial data is the AT₂ receptor.⁸⁷ However, phase 2b trials of the clinical candidate, EMA401, were terminated prematurely because of unexpected hepatotoxicity in cynomolgus monkeys in a 39-week toxicity study.⁸⁹ Nevertheless, in work by others, there are second generation small-molecule AT₂ receptor antagonists in development for the relief of peripheral neuropathic pain.

Conflict of interest statement

M.T. Smith is named inventor on The University of Queensland (UQ) patents for the use of AT₂ receptor antagonists in neuropathic pain and chronic inflammatory pain. This discovery was commercialized by the UQ spin-out company, Spinifex Pharmaceuticals Pty Ltd that was formed in 2005. Spinifex was acquired by Novartis in 2015, and clinical development was terminated by Novartis in 2019 because of unexpected hepatotoxicity in a long-term animal toxicity study. M.T. Smith is undertaking contract research in collaboration with Boehringer Ingelheim Pharma GmbH & Co. KG and with Argenica Pharmaceuticals.

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