Venoms to Drugs

DOI: 10.1002/anie.201209851

# Peptides from Mamba Venom as Pain Killers**

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ion channels · pain · peptides · toxins · venoms

Venomous snakes inject a cocktail of toxins to kill or rapidly immobilize their prey, or to defend themselves against predators. The life or death nature of these prey/predator encounters has meant that, by necessity, toxins have evolved to be fast-acting and potent molecules. For this reason many toxins have attracted the attention of chemists and pharmacologists for use as drug leads and pharmacological tools. Peptides are particularly prevalent components of animal venoms. Recently Diochot et al.[1] reported two peptides from the venom of the black mamba snake (*Dendroaspis polylepis*), which they surprisingly found to be potent analgesics in a mouse pain model. They named them mambalgins-1 and -2, and because they differ only by one amino acid they focussed mainly on just one of them, mambalgin-1, as a model peptide to probe their pharmacological effects.

Black mamba venom is very fast-acting. If not treated with antivenom a human may die within 30 min of envenomation, with various components of the venom disrupting the central nervous system and causing respiratory paralysis, as well as blocking a wide range of other physiologically important receptors. Rather than examining this broad range of biological actions, Diochot et al. screened black mamba venom specifically looking for peptides that would target acid-sensing ion channels (ASICs), which had earlier attracted attention as interesting targets in pain research.

ASICs were discovered in 1997[2] and are thought to be primary sensors of pain and are associated with a range of physiological conditions including inflammation, ischemia, and physical trauma.[3,4] Diochot et al. reported that mambalgins are members of the three-finger toxin family (Figure 1a), but share no sequence homology with either PcTx1[5] or APETx2,[6] two three-finger toxins previously reported by them to act on ASICs. The mambalgins are potent, rapid, and reversible inhibitors with nanomolar potency at recombinant homomeric and heteromeric rat ASIC subtypes ASIC1a, ASIC1a + ASIC2a, and ASIC1a + ASIC2b normally expressed in the central nervous system (Figure 1b) and ASIC1b and ASIC1a + ASIC1b found in primary nociceptors (Figure 1c). They act as gating modifiers and thus bind to the closed and/or inactivated state of the channel. The mambalgins are reported to also inhibit human ASICs.

Unlike other three-finger toxins, the mambalgins do not appear to evoke neurotoxic effects in animals, such as motor

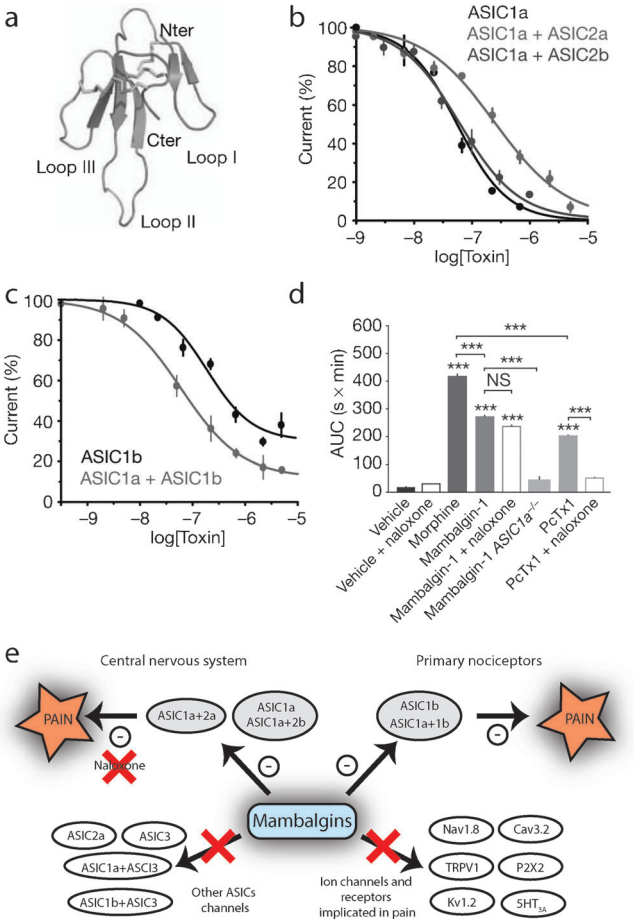


***Figure 1.*** a) Model three-dimensional structure of mambalgin-1.[1] b) Electrophysiological recordings showing the activity of the peptide on ion currents for ASIC channels located in the central nervous system and c) in peripheral nociceptors for rat ASIC channels expressed in COS-7 cells. d) Analgesia in mice following intrathecal injection of mambalgins is not affected by naloxone, unlike PcTx1.[5] (Pain responses measured by AUC area under the curve in tail flick latency following thermal pain). e) Mambalgins are highly selective for a subset of ASICs and are not active on a range of non-ASIC ion channels and receptors also reported to be involved in producing pain. Portions of this figure were reproduced with permission from Nature Publishing Group (Ref. [1]).

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[**] Work in the authors' laboratory on toxins and pain is supported by grants from the Australian Research Council (DP1093115) and the National Health and Medical Research Council (631457, 1010552, and 1026501.

dysfunction, convulsion, or death upon injection. On the contrary, the observed analgesic effects of the peptides against acute and inflammatory pain were in some cases as potent as the effect of morphine. The analgesic effect was reported not to be blocked by naloxone, a morphine antagonist, and intrathecal administration of the peptides did not lead to respiratory distress or death in mice (Figure 1 d). The mambalgins were found to be highly selective for ASICs and did not affect a range of other ion channels or receptors reported to be involved in analgesia (Figure 1 e). The selectivity of these molecules is perhaps their greatest strength. In an illustration of this selectivity, another toxin from the Texas coral snake acts on other peripheral ASICs and actually triggers, rather than relieves, pain,[7] more akin to what would be expected of components of a defensive chemical armory.

Chemistry has the potential to play a role in exploiting the mambalgins. At only 57 amino acids in size they are amenable to solid-phase peptide synthesis, or potentially could be expressed in bacteria using recombinant methods. Both approaches would face the challenge of successfully forming the native disulfide bond connectivity of the eight Cys residues from the 105 possible disulfide isomers. However, other toxins containing eight Cys residues, including chlorotoxin, from scorpion venom, have recently been chemically synthesiszd in the laboratory.[8] Either chemical or recombinant methods would provide a source of the peptides that avoids the hazardous milking of the mamba and also allows for large-scale manufacture should the peptides be successful in human clinical trials. Chemical synthesis would make it possible to prepare a range of analogues to explore structure–activity relationships and in our opinion would be a fruitful topic to explore. For example, the hypothesis that their positively charged residues assist in binding to negatively charged ASIC channels could be tested by making mutants to neutralize some of these charges.

A particularly promising aspect of the mambalgins is their potential for reduced side effects compared to conventional analgesics. Although morphine is a widely used analgesic, it has a number of side effects, including respiratory depression, nausea, constipation, the development of tolerance, and the risk of addiction or drug abuse. The lack of side effects associated with the mambalgins derives from their unique target profile, and in particular their lack of action on opioid receptors, the target of morphine.

Another aspect of the work with great potential is the use of the mambalgins as neuroscience tools to probe the mechanisms that contribute to pain in humans. In the in vitro studies the researchers were able to pinpoint the activity of mambalgin-1 to a specific central nervous system ASIC known as ASIC1a. Transgenic mice lacking this channel did not experience the pain-relieving effect of mambalgins. By contrast, in primary nociceptors the peptides target ASIC1b, a channel whose role in pain had been unclear until now.

Snakes are not the only animals to use venoms, and a variety of spiders, scorpions, sea anemones, and cone snails have similarly inspired efforts in the development of pain-relieving drugs. Several of these animals have been reported to produce toxins interacting with ASICs, including PcTx1 and APETx2 from the tarantula spider and sea anemone, respectively. These peptides interact with different ASIC subtypes, making them useful for further elucidating the pharmacology of ASICs as well as potential drug leads for the treatment of a range of pain-inducing conditions. The most advanced venom-derived pain relieving peptide, surprisingly, comes not from snakes, spiders, or other terrestrial sources but from a carnivorous marine snail with a particular liking for a fish diet. Ziconotide, a 25 amino acid peptide toxin from *Conus magus* has been on the market since 2004 for the treatment of severe neuropathic pain.[9] Unlike the mambalgins, it does not target ASICs but hones in on a specific subtype of calcium channels that it uses to paralyze its fish prey. Fortuitously, the same channels are also involved in the transmission of pain signals in the spinal cord of mammals, including humans, which led to the development of ziconotide as a treatment for human neuropathic pain (i.e. pain associated with nerve injury). Like the mambalgins, this peptide must be delivered directly to the spinal cord, and in the case of human patients this is done with a surgically implanted pump.

The intrathecal route of delivery of ziconotide, and of the mambalgins is clearly less desirable than oral drug delivery because of its invasive nature, expense, and risk of infection. However, there is promise that chemists might be able overcome this limitation by re-engineering toxins to make them more stable and orally active. For example, recent work from our laboratory has shown that another cone snail venom peptide, Vc1.1, can be stabilized and made amenable to oral delivery by cyclizing the backbone.[10]

These examples of the successful translation of a venom peptide to a clinically approved drug, and the role of chemistry in improving biopharmaceutical properties provide great encouragement that other peptides including the mambalgins might one day become useful therapeutics. Although the mambalgins have not yet been tested in humans and much work remains to be done, the Diochot study is a promising start and with further unravelling of pain pathways has the potential to lead to a human therapeutic for a condition that affects millions of people worldwide. In an ironic twist of fate, mice are among the mambas' favorite prey in the wild in Africa and experience the full brunt of the venom's deadly properties, but it has been laboratory mice in France who have been the "guinea pigs" for evaluation of the beneficial facets of mamba venom. There is optimism now too that humans, many of whom have succumbed to the mambas' wrath in the past, might in the future benefit from one tiny component of its venom cocktail.

Received: December 10, 2012
Published online: February 4, 2013

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