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# Development of a novel antibody to calcitonin gene-related peptide for the treatment of osteoarthritis-related pain

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SUMMARY

*Objective:* Investigate a role for calcitonin gene-related peptide (CGRP) in osteoarthritis (OA)-related pain.
*Design:* Neutralizing antibodies to CGRP were generated *de novo*. One of these antibodies, LY2951742, was characterized *in vitro* and tested in pre-clinical *in vivo* models of OA pain.
*Results:* LY2951742 exhibited high affinity to both human and rat CGRP ($K_D$ of 31 and 246 pM, respectively). The antibody neutralized CGRP-mediated induction of cAMP in SK-N-MC cells *in vitro* and capsaicin-induced dermal blood flow in the rat. Neutralization of CGRP significantly reduced pain behavior as measured by weight bearing differential in the rat monoiodoacetate model of OA pain in a dose-dependent manner. Moreover, pain reduction with neutralization of CGRP occurred independently of prostaglandins, since LY2951742 and NSAIDs worked additively in the NSAID-responsive version of the model and CGRP neutralization remained effective in the NSAID non-responsive version of the model. Neutralization of CGRP also provided dose-dependent and prolonged (>60 days) pain reduction in the rat meniscal tear model of OA after only a single injection of LY2951742.
*Conclusions:* LY2951742 is a high affinity, neutralizing antibody to CGRP. Neutralization of CGRP is efficacious in several OA pain models and works independently of NSAID mechanisms of action. LY2951742 holds promise for the treatment of pain in OA patients.


## Introduction

The most common form of arthritis is osteoarthritis (OA). A variety of factors can lead to the development of OA, all resulting in a reduction in the mobility and normal functioning of the joint[1]. In addition to loss of function, joint pain is a major factor in reducing the quality of life for patients with OA. The exact source of pain related to OA is poorly understood though several parts of the joint, including subchondral bone, periosteum, synovium, and the joint capsule, are highly innervated and could thus be the origin of nociceptive stimuli[2–4].

Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide that is widely expressed in the central and peripheral nervous system[5]. It is primarily associated with small unmyelinated sensory neurons, which are in close proximity of blood vessels. CGRP is a potent vasodilator and local administration of CGRP causes transient increases in blood flow[6,7]. CGRP has also been associated with pain transmission, pain modulation, and neurogenic inflammation[8]. CGRP can be released from sensory neurons *via* activation of the transient receptor potential cation channel V1 (TRPV1) using capsaicin[9]. Laser Doppler Imaging (LDI) has been used to detect the resulting changes in dermal blood flow and this has been shown to be predominantly caused by CGRP[10]. CGRP is

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also linked to inflammatory pain as demonstrated by attenuated responses in CGRP knock-out mice in a number of pain models[11,12]. This role in pain perception is congruent with the expression of CGRP in sensory neurons.

OA pain can be modeled pre-clinically in rats injected intra-articularly with monoiodoacetate (MIA). Injection of MIA into the knee joint produces an acute inflammatory insult and inhibits chondrocyte metabolism which then develops into chronic degeneration of tissues in the injected joint[13,14]. Similarly OA pain can also be modeled in a less inflammatory surgical model, the rat meniscal tear (MT) model[15]. In this model of OA, joint destruction and pain occur after surgical destabilization of the knee joint by transection of the medial collateral ligament and medial meniscus. The pain resulting from the joint injury in both models can be measured *via* differential weight bearing of the hind legs using an incapacitance tester. Efficacy for treating pain in both models has been described for standards of care for OA such as the nonsteroidal anti-inflammatory drugs (NSAIDs) diclofenac, naproxen, and celecoxib, as well as opiates such as morphine[13,15,16]. Similar to human OA, both models have also been shown to become non-responsive to NSAIDs with increasing time after induction (authors' unpublished data for MT model and[17]).

We hypothesized that CGRP could play a role in OA pain due to dense innervation with CGRP-positive neurons in the joint[2–4] and the link between CGRP and inflammatory pain[11,12]. We describe the generation of a highly specific and potent humanized antibody to CGRP, LY2951742. The antibody binds and neutralizes both human and rat CGRP *in vitro*. In addition, it prevents capsaicin-induced dermal vasodilatation *in vivo*, a phenomenon known to depend on the release of endogenous CGRP from sensory neurons. Neutralization of CGRP is efficacious in a number of pre-clinical models of OA pain, providing prolonged pain relief with only a single injection of the antibody. Pain involving CGRP is independent of prostaglandins, as shown by additive effects of CGRP neutralization and prostaglandins in the MIA model, and by efficacy with CGRP neutralization in a NSAIDs non-responsive model. Our findings demonstrate a role for CGRP in OA-related pain and suggest potential therapeutic approaches to treat this pain in patients.

## Method

### *Generation of antibodies to CGRP*

In order to generate antibodies specific to human CGRP, BALB/c mice were immunized with human CGRP conjugated to ovalbumin. Antibodies were screened for the ability to bind human CGRP and several were selected and subsequently humanized, optimized for affinity and developability, and expressed as human IgG4[18]. Here we describe the characteristics of one of these molecules, LY2951742.

### *Binding affinity and specificity*

Binding affinity and stoichiometry was determined using a surface plasmon resonance (SPR) assay on a Biacore T100 instrument primed with HBS-EP+ (GE Healthcare, 10 mM HEPES pH 7.4 + 150 mM NaCl + 3 mM EDTA + 0.05% surfactant P20) running buffer and analysis temperature at 37°C. A CM5 chip containing immobilized protein A was used to capture LY2951742. Briefly, antibody samples were prepared at 2 μg/mL and human or rat CGRP (Bachem) were prepared at 5.0, 2.5, 1.3, 0.63, 0.31, and 0 (blank) nM. Data were processed using standard double-referencing and fit to a 1:1 binding model to determine the association rate ($k_{on}$, $M^{-1}$ $s^{-1}$ units), dissociation rate ($k_{off}$, $s^{-1}$ units), and Rmax (RU units). The equilibrium dissociation constant ($K_D$) was calculated from the relationship $K_D = k_{off}/k_{on}$. Tests for binding to human adrenomedullin, intermedin, amylin, and calcitonin (Bachem) at concentrations up to 500 nM were performed using this same basic procedure. For some peptides that exhibited higher levels of non-specific binding to the chip, binding was tested in buffer containing higher levels of NaCl (500 mM) to reduce non-specific binding.

### *In vitro activity assay*

The human neuroepithelioma cell line SK-N-MC naturally expresses the CGRP receptor and was used to assess whether LY2951742 was able to inhibit CGRP-induced cAMP production. SK-N-MC cells were cultured in MEM, containing 10% FBS, 1X MEM non-essential amino acids, 1 × 100 mM MEM sodium pyruvate, 1X Pen/Strep, and 2 mM L-glutamine. After harvesting, cells were washed once and resuspended in assay buffer (stimulation buffer (HBSS with Mg and Ca, 5 mM HEPES, 0.1% BSA, 100 μM ascorbic acid) diluted 1:2 with Dulbecco's PBS containing a final concentration of 0.5 mM IBMX) and plated in 96-well plates at 15,000 cells per well. LY2951742 or a control human IgG4 antibody were added (serial 4-fold dilutions in assay buffer, 10 concentrations) to the cells, followed by a fixed amount of human or rat CGRP (2 nM). Plates were incubated for 1 h at room temperature. Levels of cAMP were subsequently measured by a homogeneous time resolved fluorescence assay system (Cisbio). The percentage inhibition was calculated relative to the amount of cAMP induced by 2 nM human or rat CGRP in assay buffer alone.

### *In vivo studies*

All animal studies conformed to protocols approved by the Eli Lilly Institutional Animal Care and Use Committee. In all studies, operators were blinded to the treatments and all antibodies were prepared in PBS.

### *Laser Doppler Imaging*

Male Lewis Rats ($n = 8$/group) were dosed subcutaneously (sc) with either 4 mg/kg of LY2951742 or an isotype control antibody 5 days prior to capsaicin challenge. Rats were fasted overnight prior to capsaicin challenge. On the day of the experiment, the rat abdomens were shaved and the rats were placed in a heated air chamber on a heating pad under the LDI instrument. A rectal probe was used throughout the study for temperature monitoring. Anesthesia was induced with 5% isoflurane in 1.5 L/min $O_2$ and the rat was stabilized under 2.5% isoflurane anesthesia for approximately 20 min prior to scanning. The scan series began with two baseline scans after which 8 μl of capsaicin solution (50 mg capsaicin in a solution of 60 μl EtOH, 40 μl Tween 20 and 100 μl purified $H_2O$) was applied to each of three o-rings placed on the abdomen. Scanning continued with a scan every 2.5 min for an additional 25 min. Data were analyzed using Moor v.5.2 software for region of interest analysis and Microsoft Excel worksheets were used for averaging the signal from the regions of interest at each time point. Data are reported as percent change from baseline (the average of two baseline scans).

### *MIA model*

For all studies, ~8-week old male Lewis rats ($n = 6$/group) were used in two different versions of the MIA model. For MIA injection, rats were anesthetized using 5% isoflurane in 1.5 L/min $O_2$. In the standard version, the right knee of each rat was injected with 0.3 mg MIA in 50 μl of saline and the left knee with 50 μl of saline. In our experience, this dose (0.3 mg MIA) provides a consistent pain-