Blockade of PDGFR-β activation eliminates morphine analgesic tolerance

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For centuries, opioid drugs have been the mainstay of chronic pain treatment. However, over time analgesic tolerance develops, leaving few treatment options. Here we show that platelet-derived growth factor receptor-β (PDGFR-β)-mediated signaling plays a key role in morphine tolerance. PDGFR-β inhibition selectively eliminates morphine tolerance in rats. PDGFR-β inhibitors are widely used and well tolerated, suggesting that clinical translation of our findings could reduce the suffering endured by individuals with intractable pain.

PDGFR is a receptor tyrosine kinase that profoundly affects the function of the N-methyl-D-aspartate receptor (NMDAR)1. NMDARs have a mechanistic role in opioid tolerance2, but, clinically, NMDAR antagonists have been ineffective or neurotoxic3. The μ-opioid receptor (MOR) transactivates PDGFR-β (ref. 4) and other receptor tyrosine kinases5, but the clinical relevance of this effect remains unknown. Clinical PDGFR inhibitors do not cross the blood-brain barrier6. We reformulated imatinib (Gleevec) to improve brain penetration and then determined whether PDGFR-mediated signaling modulated opioid tolerance.

We treated MOR-transfected C6 glioma cells with 1 and 10 µM morphine for various times and performed immunoprecipitation and immunoblotting (IP-IB) to quantify PDGFR phosphorylation (see Supplementary Methods for details). Morphine did not induce PDGFR-α phosphorylation (data not shown) but did activate (phosphorylate) PDGFR-β 40 min after treatment (Supplementary Fig. 1). Morphine substantially increased phosphorylation of the PDGFR-β at 10 nM–1 µM concentrations (Supplementary Fig. 2). Activation did not follow a standard dose-response curve but seemed to be threshold based. The MOR agonist fentanyl induced a similar magnitude and pattern of PDGFR-β activation (Supplementary Fig. 3). Morphine did not activate PDGFR-β in nontransfected cells (Supplementary Fig. 4).

We then treated rats with either 0.6 nmol morphine, 10 µg imatinib or both drugs intrathecally (i.t.) and collected spinal cords 40 min later. We microdissected the substantia gelatinosa and analyzed it by IP-IB. Morphine caused a 47% increase in PDGFR-β phosphorylation, which was blocked by imatinib (Fig. 1a,b) and naloxone (Supplementary Fig. 5).

Imatinib administered from day 1 completely eliminated morphine tolerance. Initiation of imatinib treatment on days 3 or 5 of morphine treatment reversed tolerance within 2 d (Fig. 1c), demonstrating that imatinib can reverse established tolerance. On day 7, all rats received morphine alone. To our surprise, all rats were tolerant to morphine, indicating that imatinib only temporarily reversed the processes that cause tolerance. Systemic imatinib also reversed opioid tolerance (Fig. 1d). Neither imatinib nor vehicle (a 1:1 mixture of artificial cerebrospinal fluid (aCSF) and 20% sulfobutylether-7-β-cyclodextrin (Capsol; CyDex)) was analgesic (Fig. 1d). In addition, prolonged administration of imatinib or vehicle did not interfere with morphine analgesia (Fig. 1d).

We then investigated whether imatinib reversed tolerance after continuous, high-dose morphine. We determined morphine dose-response curves by subcutaneous (s.c.) injection of escalating morphine doses in naive rats (Fig. 2a) and thenimplanted 150 mg of continuous-release morphine pellets or placebo (pellet matrix alone) pellets6. Five days later, dose-response curves were determined again. Thirty minutes before testing, half of the morphine-pelleted rats received 5 mg per kg body weight imatinib s.c., whereas the other half and placebo-pelleted rats received vehicle (a 1:1 mixture of 0.9% normal saline with 20% Capsol). Notably, imatinib reversed morphine tolerance (ratio of half-maximal effective dose (ED50) for morphine-pelleted rats to ED50 for placebo-pelleted rats, 6.1–7.8 (95% confidence interval, CI); ED50 for morphine-pelleted and imatinib-treated rats/ED50 for placebo-pelleted rats, 1.4–1.8 (95% CI); Fig. 2b). This procedure was repeated the following day. Similarly to i.t. administration (Fig. 1c), s.c. imatinib completely reversed morphine tolerance (morphine-pelleted ED50/placebo-pelleted ED50, 6.9–8.9 (95% CI); morphine-pelleted and imatinib-treated ED50/placebo-pelleted ED50, 0.8–1.0 (95% CI); Fig. 2c).

Another possible explanation for this effect is that opioid tolerance unmasked a latent analgesic effect of imatinib. To test for this, we treated rats with 10 mg per kg body weight morphine s.c. twice daily for 2, 5, 8 or 10 d. In the first three treatment groups, after morphine was discontinued, rats received 5 mg per kg body weight imatinib alone to complete a 10-d course. Imatinib was not analgesic (Supplementary Fig. 6).

Opioids act through the MOR, a Gαi/0-activating G protein–coupled receptor (GPCR)8. α-2 adrenoreceptor agonists activate Gαi, activating GPCRs and can cause analgesia9, Therefore, we hypothesized that imatinib would inhibit tolerance to clonidine. We administered 5 µg clonidine or clonidine and 10 µg imatinib i.t. for 10 d. Imatinib did not inhibit clonidine analgesic tolerance (Supplementary Fig. 7), suggesting that tolerance inhibition is opioid specific.

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A representative

IP-IB experiment is shown, with irrelevant lanes removed. (b) Quantification of PDGFR-β phosphorylation from IP-IB experiments as in a. Data are shown as mean ± s.d. Overall one-way analysis of variance (ANOVA) \( F_{3,19} = 13.8; \ P < 0.0001; \ \ast P < 0.05 \) versus all other treatment groups by Bonferroni multiple comparison post-tests. \( n = 5 \) or 6 independent rats per treatment group. (c) Rats were treated daily with i.t. injection of either 0.6 nmol morphine alone or morphine plus 10 µg imatinib, with imatinib begun on day 1, 3 or 5 of morphine treatment (morphine + imatinib-1, morphine + imatinib-3 or morphine + imatinib-5, respectively). On day 7, all rats received morphine alone (indicated by discontinuous lines between days 6 and 7). Analgesic responses were monitored using tail flick latency. Treatment \( F_{3,32} = 18.5, \ day F_{6,224} = 160, \ interaction F_{21,224} = 22.0; \ all P < 0.0001 \) (two-way ANOVA). \( n = 9 \) rats per treatment group. (d) Rats were treated for 4 d with s.c. injection of either 3.5 mg per kg body weight morphine, 5 mg per kg imatinib body weight, both morphine and imatinib, or vehicle (a 1:1 mixture of 0.9% normal saline with 20% Captsol). On day 5, all rats received morphine alone. Treatment \( F_{3,32} = 90.2, \ day F_{5,160} = 44.5, \ interaction F_{15,160} = 41.2. \) Data in c,d are shown as mean ± s.e.m.; all \( P < 0.0001 \) (two-way ANOVA); \( n = 9 \) rats per treatment group.

Although imatinib is selective, it is not PDGFR specific. Also, the time to peak PDGFR activation was longer than that seen in previous examples of transactivation, suggesting another mechanism could be involved, such as induction of PDGF release by morphine. We treated rats i.t. for 4 d with 0.6 nmol morphine alone or with morphine plus 10 ng of a fusion construct of PDGFR-β and antibody Fc portion (PDGFR-β–Fc), which scavenges released PDGF subunit B (PDGF-B). Morphine with PDGFR-β–Fc completely reversed tolerance without enhancing the analgesic effect of a given dose of morphine (Fig. 2d). PDGFR-β–Fc or vehicle (a 1:1 mixture of aCSF with 20% Captsol) alone was not analgesic. On day 5, morphine-treated rats received morphine and PDGFR-β–Fc, whereas all other groups received morphine alone. Rats that received morphine alone after 4 d of morphine and PDGFR-β–Fc were profoundly tolerant. However, PDGFR-β–Fc completely restored analgesia in tolerant rats, and PDGFR-β–Fc or vehicle did not alter the analgesic effect of a subsequent morphine dose. PDGFR-β–Fc also blocked morphine-induced PDGFR-β phosphorylation in stably transfected C6 cells (Supplementary Fig. 8), further supporting the idea that tolerance inhibition is PDGFR-β selective and is due to opioid-induced release of PDGF-B.

It is possible that PDGF release causes ‘apparent’ tolerance either by decreasing morphine analgesia or decreasing basal response latencies (that is, by inducing thermal hyperalgesia). To investigate this, we treated rats i.t. for 4 d with either 0.6 nmol morphine, 10 pmol PDGF subunit B homodimer (PDGF-BB), vehicle (a 1:1 mixture of aCSF with 20% Captsol), morphine plus 10 µg imatinib, morphine plus PDGF-BB, or morphine plus imatinib and PDGF-BB. On day 5, vehicle- or PDGF-BB–treated rats received morphine, whereas all others received morphine plus PDGF-BB–Fc fragment, morphine plus PDGFR-β–Fc, or vehicle (a 1:1 mixture of aCSF with 20% Captsol). On day 5, the groups treated with PDGFR-β–Fc, vehicle, and morphine plus PDGFR-β–Fc received morphine alone, whereas the morphine group received morphine and PDGFR-β–Fc (indicated by discontinuous lines between days 4 and 5). Treatment \( F_{3,192} = 84.8, \ day F_{6,192} = 64.4, \ interaction F_{15,160} = 37.8; \ all P < 0.0001 \) (two-way ANOVA). \( n = 6 \) rats for PDGFR-β–Fc and vehicle groups; \( n = 12 \) rats for morphine and PDGFR-β–Fc groups. (c) Rats received daily i.t. injections for 4 d of either 0.6 nmol morphine; 10 pmol PDGF-BB; morphine and 10 µg imatinib; morphine and 10 pmol PDGF-BB; morphine, imatinib, and PDGF-BB; or vehicle (a 1:1 mixture of aCSF with 20% Captsol). On day 5, the PDGF-BB and vehicle groups received morphine alone, and all other groups continued their previous treatments (indicated by discontinuous lines between days 4 and 5). Treatment \( F_{3,31} = 236, \ day F_{5,155} = 73.8, \ interaction F_{25,155} = 20.4; \ all P < 0.0001 \) (two-way ANOVA). \( n = 5–8 \) rats per group. Data in a–e are shown as mean ± s.e.m.
continued on previous treatments (Fig. 2e). Analgesic responses were similar for rats receiving morphine alone or morphine and PDGF-BB, indicating that PDGF-BB did not interfere with morphine analgesia or become antianalgesic over time. However, PDGF-BB completely abolished tolerance inhibition by imatinib. Conversely, rats given PDGF-BB for 4 d were tolerant when challenged with morphine, even though they had not received opioids, indicating that PDGF-β activation could directly cause morphine tolerance.

We replicated this finding by administering vehicle or 10 pmol PDGF-BB i.t. for 4 d and then measuring paw-withdrawal latency. Baselines remained stable. On day 5, rats received 0.6 nmol morphine. Vehicle-treated rats showed robust analgesia, whereas PDGF-BB–treated rats were completely tolerant (Supplementary Fig. 9). To determine whether this effect was opioid specific, rats were given 10 pmol PDGF-BB or vehicle i.t. for 4 d, then challenged with 5 μg clonidine on day 5. Both groups had robust analgesic responses (Supplementary Fig. 10), suggesting that tolerance induction by PDGF-BB is opioid specific.

Our findings show that PDGF-β antagonism eliminates morphine tolerance. When PDGF-β activation was blocked, tolerance was reversed, whereas PDGF-BB administration alone caused tolerance, indicating that phosphorylation of PDGF-β causes morphine tolerance. The scavenging experiments in Figure 2d and Supplementary Figure 8 show that morphine-induced PDGF release, not direct trans-activation, stimulated PDGF-β. Our finding that opioid-induced PDGF-β activation in vitro seems to be uniform above a threshold concentration is consistent with the hypothesis that tolerance is mediated by opioid-induced PDGF release.

PDGF-β activation inhibits NMDARs1. Therefore, if common signaling pathways mediated morphine tolerance induced by both PDGF activation and NMDAR activation, PDGF-β-agonists, rather than inhibitors, might block tolerance. However, the behavioral effects of these signals are quite different. PDGF-BB does not alter morphine analgesia or baseline responses, and it does not alter the rate of these signals are quite different. PDGF-BB does not alter morphine tolerance development (see Fig. 2e). NMDA reduces morphine analgesia, induces thermal hyperalgesia and accelerates the development of morphine tolerance12,13. Unlike PDGF-β inhibition, NMDAR antagonists alone can cause analgesia14 and sustained reversal of morphine tolerance2. Together, these findings suggest that NMDAR and PDGF-β modulate tolerance independently.

If the NMDAR is not involved, then what are possible explanations for the effect of PDGF-β inhibition? On the basis of our findings, we postulate that PDGF-β inhibition blocks tolerance using two mechanisms: a rapid effect causing most of the reversal and a slower process that completely restores analgesia (see Figs. 1c and 2a–c). The initial reversal may be due to rapid post-translational modification of analgesic effectors after PDGF-β antagonist administration, whereas changes in transcriptional or translational regulation of effector molecules could account for delayed effects. This hypothesis is outlined in Supplementary Figure 11. Given the widespread use of imatinib and morphine, it may seem surprising that tolerance inhibition by imatinib has not been previously observed. We hypothesize that current imatinib treatments do not achieve the level of imatinib in the central nervous system needed to inhibit tolerance.

Opioids and PDGF-β have opposing effects on several putative analgesic mediators. For example, opioids increase current amplitudes of voltage-sensitive calcium channels and voltage-activated potassium channels, whereas PDGF-β decreases them15–17. Conversely, opioids decrease the nonselective cation current, whereas PDGF-β increases it17,18. Logically, PDGF-β antagonism could reverse tolerance by acting on some (or all) of these effectors. Opioids and PDGF-β also activate some common intracellular signaling molecules, such as PI3K, PLCγ and PKC, and mitogen-activated protein kinase cascades17,19. If one or more of these substrates cause tolerance, PDGF-β inhibitors could induce rapid changes in the post-translational modification of relevant targets. Opioids and PDGF-β activate many transcription factors, such as CREB, AP-1, STAT and NF-κB, and also modulate translational machinery20. We propose that transcriptional or translational modulation of these targets could underlie the delayed phase of tolerance reversal.

In conclusion, we have shown that inhibiting PDGF-β signaling selectively eliminates morphine analgesic tolerance without altering acute analgesic effects of morphine in rats. We also found that morphine-induced PDGF-β signaling plays a key role in morphine tolerance. These findings could have profound clinical implications for the millions of people suffering from chronic intractable pain.

All animal study protocols were approved by the MD Anderson Cancer Center Institutional Animal Care and Use Committee.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturemedicine/.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

Y.W., K.R.S., M.D. and B.M. performed experiments and analyzed data. H.B.G. conceived and designed all experiments, interpreted results and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Valenzuela, C.F. et al. J. Biol. Chem. 271, 16151–16159 (1996).
2. Trujillo, K.A. & Akil, H. Science 251, 85–87 (1991).
3. Rice, A.S.C. & Hill, R.G. Annu. Rev. Med. 57, 535–551 (2006).
4. Chen, C., Farooqui, M. & Gupta, K. Curr. Neurovasc. Res. 3, 171–180 (2006).
5. Belcheva, M.M., Szucs, M., Wang, D., Sadee, W. & Coscia, C. J. Biol. Chem. 276, 33847–33853 (2001).
6. Dai, H., Marbach, P., Lemaire, M., Hayes, M. & Elmquist, W.F. J. Pharmacol. Exp. Ther. 304, 1085–1092 (2003).
7. Gold, L.H., Stinus, L., Inturrisi, C.E. & Koob, G.F. Eur. J. Pharmacol. 253, 45–51 (1994).
8. Gutstein, H. & Akil, H. in Goodman & Gilman’s The Pharmacological Basis of Therapeutics (eds. Brunton, L., Lazo, J. & Parker, K.) 547–590 (McGraw-Hill, New York, 2006).
9. Aghajanian, G.K. & Wang, Y.Y. Neuropharmacology 26, 793–799 (1987).
10. Karaman, M.W. et al. Nat. Biotechnol. 26, 127–132 (2008).
11. Herrlich, A. et al. Proc. Natl. Acad. Sci. USA 95, 8895–8990 (1998).
12. Kolesnikov, Y., Jain, S., Wilson, R. & Pasternak, G.W. J. Pharmacol. Exp. Ther. 284, 455–459 (1998).
13. Malmberg, A.B. & Yaksh, T.L. Pain 54, 291–300 (1993).
14. Codere, T.J. & Van Empel, I. Pain 59, 345–352 (1994).
15. Black, M.J., Woo, Y. & Rane, S.G. J. Neurosci. Res. 74, 23–36 (2003).
16. Timpe, L.C. & Fantl, W.J. J. Neurosci. 14, 1195–1201 (1994).
17. Williams, J.T., Christie, M. & Manzoni, O. Physiol. Rev. 81, 299–343 (2001).
18. Frace, A.M. & Gargus, J.J. Proc. Natl. Acad. Sci. USA 86, 2511–2515 (1989).
19. Andrae, J., Gallini, R. & Betsholtz, C. Genes Dev. 22, 1276–1312 (2008).
20. Ginsg, A.C., Raught, B. & Sonenberg, N. Annu. Rev. Biochem. 68, 913–963 (1999).