Pentoxifylline reduces chronic post-ischemia pain by alleviating microvascular dysfunction

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Abstract

Background—Microvascular dysfunction and ischemia in muscle play a role in the development of cutaneous tactile allodynia in chronic post-ischemia pain (CPIP). Hence, studies were designed to assess whether pentoxifylline (PTX), a vasodilator and hemorrheologic agent, relieves allodynia in CPIP rats by alleviating microvascular dysfunction.

Methods—Laser Doppler flowmetry of plantar blood flow was used to examine the effects of PTX on CPIP-induced alterations in post-occlusive reactive hyperemia (reflecting microvascular dysfunction), and von Frey testing was used to examine its effects on CPIP-induced allodynia. Time course effects of PTX on allodynia and microvascular dysfunction were assessed early (2–8 days) and late (18–25 days) post-ischemia/reperfusion (I/R) injury, and its effects on allodynia were also tested at 30 days post-I/R injury.

Results—PTX (25 mg/kg) produced significant anti-allodynic effects throughout the 21-day time course, but was not effective 30 days post-IR injury. In laser Doppler studies, the reduced reactive hyperemia in early CPIP rats was significantly improved by PTX (25 mg/kg). Conversely,

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Authors' contributions

J.V.R. and A.L. completed most of the experimental work, with assistance in the behavioral studies by M.K. J.V.R. and A.L. performed the statistical analysis and prepared the figures with assistance from T.J.C. J.V.R. wrote the manuscript, with editorial assistance from T.J.C. and A.L.

treatment with PTX at the same dose did not affect reactive hyperemia in late CPIP rats, likely since reactive hyperemia was not significantly reduced pre-drug in these animals.

Conclusion—Since, poor tissue perfusion underlies early stages of CPIP pain, the ameliorative effect of PTX on microvascular dysfunction might account for its anti-allodynic effect in our experimental model of CRPS-I.

1. Introduction

Complex Regional Pain Syndrome type I (CRPS-I) is a disabling pain syndrome characterized by spontaneous and stimulus-evoked pain, edema, motor dysfunction, vasomotor and sudomotor abnormalities, in the absence of major peripheral nerve injury (Stanton-Hicks et al., 2003; Schwartzman et al., 2006). Patients with chronic CRPS-I exhibit atrophy of skin, muscles, and bones and often present with a cold-affected extremity (Baron and Janig, 2004). Increased vasoconstriction, tissue hypoxia and metabolic tissue acidosis have also been demonstrated in the affected extremity (Birklein et al., 2000).

Using a rat model of CRPS-I, called chronic post-ischemia pain (CPIP), we showed that rats developed signs of CRPS-I, namely, early swelling, hyperemia, hind paw warmth, and long-lasting allodynia, after an injury produced by 3 hours of hind paw ischemia followed by reperfusion (I/R injury) (Coderre et al., 2004). Recently, we showed that CPIP rats have microvascular dysfunction that leads to persistent muscle ischemia (Laferrière et al., 2008). Microvascular dysfunction was accompanied by increases in lipid peroxidation (an indicator of oxidative stress), and in the levels of nuclear factor kappa B (NFκB) and pro-inflammatory cytokines in the CPIP hind paw muscle, and allodynia was relieved by agents that inhibit oxidative stress, NFκB and cytokine activity (Laferrière et al., 2008).

In this study, we investigated whether pentoxifylline (PTX), a vasoactive agent can alleviate pain in CPIP rats by improving microvascular function. PTX is a methylxanthine derivative possessing a variety of effects that alleviate microvascular dysfunction (Ward and Clissold, 1987). These properties include the ability to increase red cell deformability (Bradbury et al., 1993), attenuate the release of pro-inflammatory cytokines (Strieter et al., 1988), block interactions between leukocytes and endothelial cells (Samlaska and Winfield, 1994), and increase blood flow (Bowton et al., 1989). Hence, PTX has been preferentially used in the treatment of peripheral arterial disease and intermittent vascular claudication conditions (Jacoby and Mohler, 2004). Furthermore, it has been reported to promote the oxygenation of ischemic areas and reduce metabolic disturbances associated with I/R injury (Adams et al., 1995). Although PTX has been shown to have potent anti-allodynic and anti-hyperalgesic effects in various animal models of pain (Vale et al., 2004; Liu et al., 2007; Wordliczek et al., 2000), it has not been tested in an animal model of ischemic pain, nor has it been shown to alleviate pain by reducing microvascular dysfunction.

Hence, the aim of this study is to assess whether PTX relieves allodynia in CPIP rats by alleviating microvascular dysfunction. We used laser Doppler flowmetry to detect changes in post-occlusive reactive hyperemia before and after PTX treatment. Relative increases in hyperemic responses following a brief 2 min occlusion before and after PTX treatment, were considered indicative of the effect of PTX on microvascular function. Changes in hyperemic

responses were examined at two different time periods -- 2–8 days and 18–25 days post I/R injury.

2. Materials and methods

2.1 Animals

Male Long Evans rats (275–300 g, Charles River, Quebec) arrived a week before experiments. All treatments and testing were performed blindly using a randomized block design. Methods were approved by the Animal Care Committee at McGill University, and conformed to ethical guidelines of the Canadian Council on Animal Care and the International Association for the Study of Pain.

2.2 Drugs

Drugs used included sodium pentobarbital (Ceva Santé Animal, Libourne, France), urethane and pentoxifylline (Sigma-Aldrich, St. Louis, MO).

2.3 Hind paw ischemia/reperfusion

Chronic post-ischemia pain was generated following exposure to prolonged hind paw ischemia and reperfusion. Rats were anesthetized over a 3 h period with a bolus (55 mg/kg, i.p.) and chronic i.p. infusion of sodium pentobarbital (27.5 mg/kg/h for the first two hours). After induction of anesthesia, a Nitrile 70 Durometer O-ring (O-rings West, Seattle, WA) with 5.556 mm internal diameter was placed around the rat's left ankle joint for 3 h (Coderre et al., 2004).

2.4 Mechanical sensitivity

Rats were initially habituated for 20 min to acrylic testing chambers (12 cm wide, 18 cm long and 15 cm high) fitted with wire mesh floors (12 × 12 mm mesh size). The plantar surface of the contralateral, then ipsilateral hind paw was tested for mechanical paw withdrawal threshold. Filaments were applied in either ascending (after a negative response) or descending (after a positive response) force as necessary to determine the filament closest to the 50% threshold of response. Each filament was applied for 10 sec or until a flexion response occurred. The minimum stimulus intensity was 1.0 g and the maximum was 15.0 g. Based on the response pattern, and the force of the final filament (5th stimulus after first direction change), the 50% threshold (gram) was calculated as: $(10^{[X_f + k\delta]})/10000$, where $X_f = \log(10 * \text{filament buckling force in mg})$, k = value for the pattern of positive/negative responses and δ = mean difference in log units between stimuli (here, $\delta=0.220$, for more details see Chaplan et al., 1994). Paw withdrawal thresholds were assessed prior to (pre-drug), and at different time-points following PTX treatment (post-drug). We did not record pre-injury baselines, however, we have recorded these baselines many times in other experiments, and they are reliably between 12 g and 15 g.

2.5 Pharmacological treatment

PTX was tested at three different doses: 12.5, 25 and 50 mg/kg. The doses were selected from previously reported anti-nociceptive effects observed in various animal models of pain

(Vale et al., 2004; Liu et al., 2007; Wordliczek et al., 2000). Drug or saline vehicle were administered i.p. 2, 7, 14 and 21 days post I/R injury to two groups of rats. A time course profile was determined on each test day by testing drug- and vehicle-treated animals before and 20, 40, 90, 120 and 150 min after treatment. In a separate group of rats, PTX (25 mg/kg, i.p.) was administered only on day 30 post I/R injury and tested for its activity against mechanical allodynia. The aim of this experiment was to compare the effect of single treatment to that of repeated PTX treatment, in order to assess potential drug tolerance in the repeated drug treatment groups.

In order to determine the effect on locomotor performance, PTX was administered to a group of naïve rats and evaluated in the rotorod test. Briefly, rats were placed on a rotating cylinder constantly accelerating from 0 to 30 rpm over a period of two min (Rotorod, IITC Life Science, Woodland Hills, CA). The time for which the animal remained on the rotating cylinder was recorded. For each animal, six trials, spaced by approximately 20 min were performed daily for four consecutive days. On day 5, animals were tested again in six trials, starting 20 min after i.p. administration of the highest dose of PTX used in pain behavioral studies (50 mg/kg).

2.6 Laser Doppler flowmetry

Plantar microcirculatory blood flow and post-occlusive reactive hyperemia were assessed in a total of 69 rats using a laser Doppler perfusion and temperature monitor (DRT4, Moor Instruments, Wilmington, DE). Four experimental groups were randomly constituted from animals tested either 2–8 days (early) or 18–25 days (late) post I/R injury: CPIP-vehicle (n = 9 and 11, respectively), sham-vehicle (n = 9 and 7, respectively), CPIP-PTX (n = 6 and 10, respectively) and sham-PTX (n = 11 and 6, respectively). Briefly, rats were anesthetized with bolus (0.9 g/kg) and maintenance doses (200 mg/kg, i.p.) of urethane, as necessary to maintain anesthesia level constant (absence of tail and foot pinch responses and absence of vibrissae movement). Body temperature was monitored through a rectal thermometer coupled to a heating pad (FHC, Bowdoinham, ME), and was maintained between 37.5°C and 39.0°C throughout the experiment. With the rat placed in prone position, the plantar blood flow of the ipsilateral hind paw was monitored using a laser-emitting probe placed and maintained between the tori pads of the hind paw, along the midline. Prior to recording responses to occlusion, each rat underwent a period of stabilization lasting 20 to 50 min. At the rate of one sample per second, an initial 10 minute baseline blood flux was recorded, followed by a 2 minute occlusion induced by pressurizing and inflating a tight fitting loop of Tygon® R 3603 tubing (outside diameter = 2.38 mm, inside diameter = 0.79 mm, wall thickness = 0.79 mm) connected to an air-filled pump-driven 60 mL syringe (Model 11, Harvard Apparatus, Montreal, QC), creating a tourniquet around the ankle joint. Ischemia was confirmed by observing a flux reduction greater than 95% of pre-occlusion value. Two min later, the pressure was released quickly and the tourniquet loosened without disturbing the laser probe to allow reperfusion to occur. Reactive hyperemia was monitored by continuously sampling flux at the rate of 1/sec for 2 min following the onset of reperfusion. PTX (25 mg/kg, i.p.) or vehicle (saline) was administered five min post-reperfusion onset. Beginning 20 min after drug/vehicle administration, a second 2 min occlusion was induced

by the above procedure, and was followed by a 2 min recording of hyperemic responses at the rate of 1 sample/sec (see Fig. 1 for a sample trace).

2.7 Data analysis

Estimates of 50% von Frey threshold were grouped by dose, day and time of testing and analyzed by two-way repeated measures analysis of variance (ANOVA). Significant interaction effects were further analyzed by *post hoc* comparisons using Bonferroni's tests. The area under the curve (AUC) of individual time-course plots of the effects of PTX (as a difference from the pre-drug measure) was calculated using the trapezoid method. Individual AUCs were then grouped by dose (pre- and post-drug) and day of testing and analyzed by repeated measures ANOVA, followed by Fisher's LSD comparisons when appropriate. For rotorod testing, the running time for each trial was averaged across test days, and analyzed by one-way repeated measures ANOVA.

For reactive hyperemia measures, the data from each animal were first normalized to baseline (as a percent of mean baseline value, computed over the 120 sec preceding the onset of each occlusion), and then grouped into 10 sec bins defining a total of 12 consecutive time segments over the first 120 sec after reperfusion. For 2–8 days and 18–26 days post-CPIP animals, binned data were averaged by treatment group (sham-PTX, sham-vehicle, CPIP-PTX and CPIP-vehicle) and subjected to two-way ANOVA. The Greenhouse-Geiser correction was applied to the estimation of ANOVA repeated factor effects. Post-hoc pairwise comparisons were made by Bonferroni's tests when appropriate. In addition, the AUC of individual time-course plots of the hyperemic responses (expressed as a percent of the pre- and post-drug baseline measure) was calculated using the trapezoid method. Individual AUCs were then averaged by group and drug treatment (pre- and post-drug or vehicle) and analyzed by two-way repeated measures ANOVA, followed by Fisher's LSD comparisons when appropriate. Statistical computations were performed with Statistica, version 6 (Statsoft, Tulsa, OK) or Prism 5 (GraphPad Software, La Jolla, CA). Significance was defined as $p < 0.05$.

3. Results

3.1 Anti-allodynic effect of PTX

The effects of systemic treatment with three doses of PTX on paw withdrawal thresholds on days 2, 7, 14 and 21 are depicted in Figs. 2A–D, respectively. On each day of testing, PTX reduced mechanical allodynia depending on dose and time after drug administration, as indicated by the presence of a significant interaction between PTX dose and the time of PWT measurement (Day 2: $F(15,180) = 6.055$, $p < 0.0001$; Day 7: $F(15,180) = 2.456$, $p = 0.0027$; Day 14: $F(15,180) = 7.194$, $p < 0.0001$; Day 21: $F(15,180) = 6.621$, $p < 0.0001$). The lowest dose of 12.5 mg/kg was observed to be devoid of anti-allodynic effect when tested 2, 7, 14 or 21 days post I/R injury. In contrast, PTX given at 25 mg/kg exhibited significant anti-allodynic effects with respect to the pre-drug baseline PWTs (PRE) on day 2 at 20 and 40 min ($p = 0.010$ and $p = 0.0003$, respectively), on day 7 at 40 min ($p = 0.027$), on day 14 at 20 min ($p = 0.0001$), and on day 21 at 20 min ($p = 0.022$) post treatment. On day 14, PWTs recorded at 150 min post-PTX were lower than those recorded in vehicle-

treated animals ($p = 0.025$). The increases in PWT occurred between 20 and 40 min after drug administration, but never observed beyond the latter time point. When tested at the highest dose of 50 mg/kg, PTX attenuated mechanical allodynia from 20 to 90 min post-treatment on day 2 ($p = 0.033$, $p = 0.017$ and $p = 0.042$, respectively), but exhibited anti-allodynic activity only at the first time-point of testing (20 min) on days 14 and 21 post IR injury ($p = 0.0037$ and $p = 0.002$, respectively).

3.2 Time course of the magnitude of PTX effect

Fig. 2E shows the calculated δ AUCs for all doses across treatment days. δ AUCs varied significantly over days of testing and according to PTX dose (main effect of test day: $F(3,108) = 6.544$, $p = 0.0004$; main effect of dose: $F(3,36) = 27.178$, $p < 0.0001$). As observed, the δ AUC for 12.5 mg/kg was identical to that of vehicle administration. However, the δ AUCs for both the higher doses (25 and 50 mg/kg), calculated on day 2 post I/R injury, were significantly higher than those calculated on subsequent days of testing. For 25 mg/kg, the δ AUC calculated on day 21 was significantly less than that obtained on day 2 ($p = 0.018$). Similarly, the δ AUCs for the highest dose (50 mg/kg) on days 7 and 21 were less than that obtained on day 2 ($p = 0.011$ and $p = 0.0005$, respectively). Thus, the magnitude of PTX effect was greater when tested early after I/R injury and decreased over the 21-day test period following the induction of I/R injury.

A decrease in drug effect at later time points post I/R injury might either be due to drug tolerance owing to repeated drug administration until day 21, or differences in the pathological mechanisms underlying allodynia early and late post I/R injury. Hence, to rule out the possibility that the observed decrease in the anti-allodynic effect of PTX was due to drug tolerance, we administered PTX (25 mg/kg) to a group of previously untested/untreated CPIP animals on day 30 post I/R injury, and assessed the drug effect until 150 min post-treatment. As observed in Fig. 2F, PTX showed no anti-allodynic effect at any time-point, as there was no significant effect of drug or interaction between drug and time.

3.3 Rotorod test

Fig. 2G depicts mean running duration over the first four training days and after PTX treatment on day 5 in the rotorod test. Rats displayed a significant improvement in performance on the fifth day compared to the first day of testing (effect of day: $F(4,20) = 7.239$, $p = 0.0009$). However, PTX at 50 mg/kg did not have any detectable effect on performance compared to the previous day.

3.4 Effect of PTX on post-occlusive reactive hyperemia

Fig. 1 shows laser Doppler flux measures recorded at a frequency of 1/sec from a sham-treated and a 2-day CPIP rat. Note that the pre-drug reactive hyperemia seen in the first 2 min post-reperfusion is dramatically reduced in the CPIP rat as compared to the sham rat. The two traces also illustrate that PTX administration did not produce either large or sustained shifts in measured flux in sham or CPIP rats. Importantly, there was a marked increase in the peak value of the post-occlusive reactive hyperemic response in CPIP animals after PTX administration, compared to the pre-drug peak hyperemic response.

Figs. 3A–D and 3F–I show time-averaged variations in normalized flux (expressed as a percent of baseline) for 120 sec after reperfusion, before and after PTX or vehicle treatment. In rats tested between 2 and 8 days after I/R injury (Figs. 3A–D), flux varied significantly during the course of the reperfusion period (effect of time: $F(11,341) = 28.747$, $P < 0.0001$). The magnitude of the pre-drug post-occlusive reactive hyperemic response was initially smaller in CPIP animals. PTX administration resulted in a significant increase in reactive hyperemia in CPIP animals, but not in sham-treated rats, thus producing a significant drug by group interaction ($F(3,31) = 4.283$, $p = 0.0122$). Rats tested between 18 and 25 days after I/R injury did not show PTX-induced increases in reactive hyperemia (Figs. 3F–I). Figs. 3E and 3J display the mean AUCs calculated over the hyperemic measurement period in 2–8 day and 18–25 day CPIP rats respectively. For early CPIP rats, the pre-drug AUC calculated from CPIP animals was significantly lower than those calculated in either sham-PTX or sham-vehicle rats ($p = 0.031$ and $p = 0.047$, respectively). PTX treatment significantly increased the AUC in CPIP animals ($p = 0.0045$, Fig. 3E). No comparable effects of PTX could be observed in CPIP rats tested between 18 and 25 days post I/R injury (Fig. 3J). This finding of a differential effect of PTX on plantar blood flow could well be considered as a causal mechanism for its significant anti-allodynic effect observed early after I/R injury.

4. Discussion and conclusion

Deep tissue ischemia resulting from prolonged microvascular dysfunction may play a cardinal role in the inflammatory processes of CRPS. Using the CPIP model of CRPS-I, we had earlier shown that arterial vasospasms, endothelial cell injury and capillary slow flow/no-reflow after IR injury leads to persistent allodynia that was observed to be dependent on oxygen free-radicals, NF κ B, pro-inflammatory cytokines and levels of lactate in muscle (Laferrière et al., 2008). Hence, the primary aim of this study was to specifically assess if anti-allodynic effects of PTX in CPIP rats were paralleled by PTX-induced increases in post-occlusive reactive hyperemia (indicating alleviation of microvascular dysfunction).

Post-occlusive reactive hyperemia is the transient and compensatory increase in blood flow that occurs after a brief period of tissue ischemia. We decided to measure relative changes in hyperemic responses for the following reasons: 1) It has widely been reported in the vascular literature as a direct measure of microvascular function since the magnitude of the reactive hyperemic responses correlates well with the vascular flow reserve (Bollinger et al., 1996); 2) Although evaluated at the beginning of reperfusion, post-occlusive reactive hyperemia has been considered to be a fundamental indication of the preservation of vessel muscle function (Rochetaing and Kreher, 2003); and 3) Since measurement of reactive hyperemic blood flow can be used to evaluate the development of a functional collateral circulation in response to ischemic insults, reactive hyperemia has been used as a measure of effective tissue perfusion in various animal models of peripheral ischemia (Corcoran et al., 2009). Moreover, since this vascular event involves innate vasodilatory mechanisms that include local production of endothelium-derived nitric oxide and various other mediators (Dakak et al., 1998), we used post-occlusive reactive hyperemia as a measure of microvascular function before and after treatment with PTX.

PTX is a nonselective PDE-4 inhibitor, and prevents the cAMP metabolizing action of PDE-4 in inflammatory and immune cells. PDE-4 inhibition leads to an increase in cAMP in the vascular endothelia and smooth muscle cells resulting in anti-inflammatory and vasodilatory effects (Eckly-Michel et al., 1997). PTX has also been reported to possess actions that improve blood rheology and tissue perfusion (Accetto, 1982). The results of our study show consistent anti-allodynic effects for the 25 mg/kg dose of PTX, observed early post I/R injury, the magnitude of which decreased significantly in late CPIP rats. In order to determine if the incidence of mechanical allodynia in CPIP rats depends on microvascular dysfunction, we assessed changes in the magnitude of post-occlusive reactive hyperemic responses before and after systemic PTX treatment. This was achieved by specifically measuring both the peak value and duration of hyperemic responses observed following brief occlusion periods. A relative increase in the peak intensity of the hyperemic response after PTX treatment observed in early CPIP rats was considered to be indicative of an improvement in microvascular function. Our results show that reactive hyperemia was significantly increased by 25 mg/kg of PTX in rats tested early after I/R injury. Conversely, PTX did not significantly affect reactive hyperemia in late CPIP rats. However, the peak hyperemic response had fully recovered to near sham levels in late CPIP rats, even before they received PTX. Hence, our study documents the parallel effects of PTX on allodynia and post-occlusive reactive hyperemia in CPIP rats.

In addition, the single dose drug trial (day 30 testing) showed that the decrease in the anti-allodynic effect of PTX observed over the repeated drug treatment period was not due to the development of drug tolerance. We speculate an involvement of a non-vascular component underlying CPIP allodynia during the later stages, which remains unresponsive to PTX treatment. It is possible that early muscle ischemia stimulates afferent activity that induces a lasting central sensitization (Wall and Woolf, 1984), or alternatively that early persistent nerve ischemia produces subsequent neuronal injury leading to prolonged neuropathic pain.

With regard to regulatory effect on blood flow, most *in vitro* and *in vivo* experiments show that PTX induces vasodilation in the skeletal muscle vascular bed. Consequently, perfusion in the microcirculatory vascular bed is improved after PTX treatment (Dinn et al., 1990; Kamphuis et al., 1994). These PTX-induced vasodilatory effects have been reported to be due to inhibition of phosphodiesterase activity leading to an increase in cAMP levels (Wu et al., 1984).

Furthermore, PTX has also been reported to possess various other activities, namely inhibition of the production of pro-inflammatory cytokines (Strieter et al., 1988; Wei et al., 2009), reduction of neutrophil adhesiveness to endothelial cells, inhibition of platelet aggregation and free radical production (Horton and White, 1993), all of which contribute to its palliative effect on IR injury, most of which can be attributed to its effects on cAMP in vasculature endothelia and smooth muscle cells. Indeed, PTX has been reported to reduce IR injury in hind paw skeletal muscle after partial ischemia induced by aortic clamping (Kishi et al., 1998).

The dose response profile of any given drug will depend on the specific drug and its route of administration among many other factors. It has been shown that PTX produces dose-

dependent inhibition of increase in paw edema in the formalin-induced chronic inflammation model in rats (Al-Saad et al., 2012). The maximum dose of PTX used in that study was only 4 mg/kg. In the current study, we wanted to correlate the anti-allodynic efficacy of PTX with improvement in plantar microcirculation. Studies showing the alleviation by PTX of IR injury in various organ systems used PTX doses up to 50 mg/kg. Hence, we used 25 mg/kg in our studies of microvascular function. As well as the 25 mg/kg dose, we used one lower dose (12.5 mg/kg) and one higher dose (50 mg/kg) in pain behavioral studies. Surprisingly, we showed that 50 mg/kg PTX showed a lesser anti-allodynic effect than 25 mg/kg. We expect that the lack of dose dependency with these doses reflected that we are at the plateau of PTX's efficacy at 25 mg/kg, and that the highest dose was less effective due to behavioral variability, or potentially due to the initiation of receptor desensitization.

The results of our study lend support to a possible etiological role played by microvascular dysfunction and ischemia in the development of cutaneous allodynia in CPIP rats. Thus, slow flow or no-reflow (consequent to IR injury), results in a significant reduction in the hyperemic response in early CPIP rats, which is improved after treatment with PTX, and the improved reactive hyperemia is correlated with alleviated allodynia. It is possible that some of the effects of PTX may be due to a central action, since there are studies that report inhibitory effects of PTX on TNF- α synthesis, pro-inflammatory cytokine expression and microglial cell activation in the spinal cord (Mika et al., 2009). However, we have specifically shown in our experiments that at the same systemic anti-allodynic dose tested, PTX also produced an increase in reactive hyperemia in the affected hind paw; and importantly, for late CPIP rats, where PTX did not affect the hyperemic response, it also did not reduce allodynia.

With regard to clinical significance, it is well known that CRPS is a syndrome involving a host of multifarious pathophysiological mechanisms that result in a complex range of clinical manifestations. Although many of these signs and symptoms differ at each stage of the syndrome, microvascular dysfunction has been shown to be present throughout the various stages. In particular, persistent tissue ischemia in skin, and particularly deep tissue, is clinically evident in many CRPS patients, regardless of whether they are classified as having hot or cold CRPS (Birklein et al., 2000; Koban et al., 2003; Kurvers et al., 1995). Hence, there is growing evidence that targeting microvascular dysfunction and ischemia can lead to the formulation of effective therapeutic strategies against CRPS (Zollinger et al., 1999; Perez et al., 2003).

In summary, we show that the anti-allodynic effects of PTX observed early after I/R injury are paralleled by ameliorative effects of PTX on reactive hyperemia (an improvement in microvascular function), while PTX does not relieve allodynia in late CPIP rats that have normal hyperemic responses. Thus, since poor tissue perfusion underlies early stages of CPIP pain, the effects of PTX on the mediators of microvascular dysfunction might account for its early anti-allodynic effect in our experimental model of CRPS-I.

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What's known

Allodynia associated with early, but not late, chronic post-ischemia pain depends on microvascular dysfunction in the affected limb.

What's new

Systemic pentoxifylline relieves microvascular dysfunction and allodynia in rats with early, but not late, chronic post-ischemia pain.

Figure 1.

Laser Doppler flux measurements (in arbitrary units – AU) from a sham rat (blue) and a rat tested two days post I/R injury (red), illustrating the protocol followed for the collection of post-occlusive reactive hyperemia measures before and after PTX treatment (25 mg/kg, i.p.). Note that the CPIP animal displayed markedly lower reactive hyperemia after the initial period of ischemia compared to the sham animal, but a noticeably elevated response during the second reperfusion period, post-PTX administration. The dotted lines are means of baseline values of the sham and CPIP rat.

Figure 2.

Effects of PTX on mechanical allodynia and locomotor performance. Time course of anti-allodynic effects of three doses of pentoxifylline (PTX) or vehicle (saline) evaluated 2, 7, 14 and 21 days after induction of chronic post-ischemia pain (CPIP) (panels A, B, C, D, respectively). Paw-withdrawal thresholds to mechanical stimulation with von Frey hairs were measured before (PRE) and 20, 40, 90, 120 and 150 min after systemic treatment with PTX. Asterisks (*) denote the presence of a significant difference between vehicle- and drug-treated groups (Bonferroni's test, $p < 0.05$). The calculated areas under the time-effect curve (AUC) of the anti-allodynic effect of PTX, averaged by dose over days following

induction of I/R injury is shown in panel E. Asterisks (*) denote the presence of a significant difference between vehicle- and drug-treated groups (Fisher's LSD test, $p < 0.05$). Daggers (†) indicate that the average AUC differs from that calculated on day two within the same group (Fisher's LSD test, $p < 0.05$). In order to determine if the decreasing trend in the anti-allodynic effect of PTX is due to drug tolerance, a single dose of PTX (25 mg/kg) was administered to PTX- naïve rats, 30 days after the induction of I/R injury. Paw withdrawal thresholds to mechanical stimulation were assessed before (PRE) and 20, 40, 90, 120 and 150 min after systemic PTX treatment (panel F). The locomotor impairment effect of PTX was assessed in the rotarod test. Panel G shows the average time (sec) spent on the rotarod (average of six trials) on the first four consecutive training days, and on the following day when PTX (50 mg/kg) was administered 20 min before testing. Asterisk indicates a significant difference from day 1 performance.

Figure 3.

Effects of PTX on plantar microcirculatory blood flow. Time course of changes in post-occlusive reactive hyperemia (expressed as percent of pre-occlusion baseline blood flux) measured every sec over 120 sec after the onset of reperfusion in CPIP rats that received vehicle (A, F) or PTX (B, G) and sham rats that received vehicle (C, H) or PTX (D, I) tested between 2 and 8 days (panels A–D) or 18 and 25 days (panels F–I) following I/R injury. Pre- (filled circles) and post-drug (open circles) Reactive hyperemia measurements were averaged over successive 10 sec segments of the reperfusion period. Asterisks indicate the presence of a significant difference between pre- and post-drug measures within each

treatment group (Bonferroni's test, $p < 0.05$). PTX effects could only be observed in rats tested between 2 and 8 days post I/R injury. Panels E and J depict the calculated AUCs of the hyperemic responses calculated over the measurement period, averaged by treatment in rats tested 2–8 or 18–25 days post I/R injury respectively. Asterisk (*) denotes the presence of a significant difference between pre- and post-drug flux measures within the treatment group (Fisher's LSD test, $p < 0.05$). Dagger (†) indicates that the average AUC differs from the pre-drug value of the sham-vehicle group (Fisher's LSD test, $p < 0.05$). Double dagger (‡) indicates a significant difference between the pre-drug values of CPIP-PTX and sham-PTX groups (Fisher's LSD test, $p < 0.05$). Section sign (§) indicates a significant difference between the post-drug values of CPIP-vehicle and CPIP-PTX groups (Fisher's LSD test, $p < 0.05$). No differences were observed in rats tested between 18 and 25 days post I/R injury.