Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 502 (2004) 233–237

www.elsevier.com/locate/ejphar

Kappa opioid agonists suppress chloroquine-induced scratching in mice

Saadet Inan*, Alan Cowan

Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140, USA

Received 11 May 2004; received in revised form 17 August 2004; accepted 1 September 2004
Available online 28 September 2004

Abstract

Chemotherapy of malaria fever with chloroquine is often associated with generalized pruritus of unknown pathogenesis. This adverse side effect leads to diminished compliance. We report that chloroquine (1.25–40 mg/kg, s.c.) elicits dose-related, compulsive, and vigorous scratching in mice. This frenzied behavior is essentially abolished when the mice are pretreated s.c. or orally with nalfurafine (TRK-820), a centrally penetrating kappa opioid agonist. Peripheral kappa receptors are involved because chloroquine-induced scratching is also antagonized by the peripherally restricted kappa agonist, ICI 204,448: *R*,*S*-*N*-[2-(*N*-methyl-3,4-dichlorophenylacetamido)-2-(3-carboxyphenyl) ethyl]pyrrolidine. We propose that combination therapy for malaria with chloroquine and a kappa agonist (probably one targeting peripheral receptors) will lead to better treatment compliance because of a reduced incidence of pruritus.

Keywords: Chloroquine; Malaria; Pruritus; Kappa opioid receptors

1. Introduction

Malaria is responsible for an estimated 1–2 million deaths annually. African children under the age of five have been particularly vulnerable (Woster, 2003). For the past 40 years, chloroquine phosphate has remained a popular antimalarial agent, especially in the endemic areas of Africa. This drug provokes an often intolerable side effect—generalized pruritus of unknown mechanism—in 8% to 20% of African patients afflicted with acute febrile malaria. Whole body itching, involving especially the palms, soles, and scalp, begins after about 6 h and fades within 2–3 days (Sowunmi et al., 1989). This adverse effect has, in turn, been responsible for diminished compliance with 4-aminoquinoline antimalarials and, perhaps, has contributed to the development of chloroquine-resistant parasites.

Antihistamines have been largely ineffective against chloroquine-induced pruritus. Prednisolone has proved more successful (Abila and Ikueze, 1989; Ajayi et al., 1991; Adebayo et al., 1997). As with pain, itching is a subjective and varied sensation, and there is no universally accepted, single antipruritic drug that can suppress this annoying symptom. Endogenous opioids have been implicated in several pruritic states, and narcotic antagonists, such as naltrexone and nalmefene, have frequently been helpful in relieving itch associated with uremia, cholestasis, and the spinal administration of certain opioids (Twycross et al., 2003). Opioid agonists and antagonists have often been tested against a range of compounds (e.g., bombesin, compound 48/80, serotonin, substance P) that induce excessive scratching behavior in rodents (Gmerek and Cowan, 1984; Cowan and Kehner, 1997; Yamaguchi et al., 1999; Lee et al., 2003; Nojima and Carstens, 2003). Low doses of agonists showing selectivity for kappa opioid receptors have been notably effective against chemically induced scratching (and therefore, perhaps itching) in rodents (Cowan et al., 1995; Togashi et al., 2002; Utsumi et al., 2004) and rhesus monkeys (Ko et al., 2003). This work has led to the successful clinical testing of nalfurafine hydrochloride (TRK-820), a 4,5-epoxymorphinan derivative and kappa agonist (Endoh et al., 1999; Sorbera et al., 2003; Suzuki et al., 2004) in hemodialysis patients experiencing

* Corresponding author. Tel.: +1 215 707 88 19; fax: +1 215 707 7068.
E-mail address: sinan@temple.edu (S. Inan).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.09.010

uremic pruritus (Kumagai et al., 2004). The exciting prospect of clinical utility (at last) for a selective kappa agonist prompted us to test nalfurafine against the pruritus associated with chloroquine in the hope of uncovering an additional therapeutic target. Onigbogi et al. (2000) reported that naltrexone (0.25 mg/kg, i.p.) antagonizes the low level of scratching elicited by chloroquine (20 mg/kg, i.p.) in rats. We chose mice to comprehensively evaluate the antipruritic activity of nalfurafine because of the much greater sensitivity of this species to the scratch-inducing effects of chloroquine (Inan et al., 2003).

In addition, we compared the centrally penetrating nalfurafine with a standard, peripherally restricted kappa opioid agonist: *R*,*S*-*N*-[2-(*N*-methyl-3,4- dichlorophenylacetamido)-2-(3-carboxyphenyl)ethyl]pyrrolidine (ICI 204,448; Shaw et al., 1989). Use of ICI 204,448 was intended to answer the following question: does exclusive activation of peripheral kappa receptors result in suppression of chloroquine-elicited scratching in mice?

2. Materials and method

2.1. Animals

Male Swiss mice (Ace Laboratories, Boyertown, PA) weighing 25–30 g were used. The animals were housed five per cage with free access to food and water. A standard light–dark cycle was maintained with a timer-regulated light period from 7 a.m. to 7 p.m. The experimental procedure was approved by Temple University Institutional Animal Care and Use Committee.

2.2. Compounds

Chloroquine phosphate and ICI 204,448 hydrochloride were purchased from Sigma (St. Louis, MO) and Tocris (Ellisville, MO), respectively. Nalfurafine hydrochloride was a generous gift from Adolor Corporation (Exton, PA). The compounds were dissolved in saline and injected at a dose volume of 0.25 ml/25 g mouse. Doses refer to the particular salt.

2.3. Quantitation of scratching

Each mouse was weighed and allowed to acclimate for at least 1 h in individual, rectangular observation boxes. The animals received chloroquine either orally (20–160 mg/kg) or s.c. (1.25–40 mg/kg into the back of the neck), and the number of hind leg scratching movements directed to the neck was counted for 30 min after latencies of 10 and 1 min, respectively. Each burst of rapid scratching activity was counted as one event.

In one set of antagonism experiments, saline or a standard, behaviorally nondepressant dose of nalfurafine (0.02 mg/kg) or ICI 204,448 (5 mg/kg) was injected s.c. into the flank of mice 20 min before challenging the animals s.c. with chloroquine (1.25–40 mg/kg into the back of the neck). In a second antagonism study, saline or nalfurafine (0.02–0.12 mg/kg) was given orally, 45 min before a fixed dose of chloroquine (10 mg/kg, s.c.). Hind leg scratching was again counted for 30 min after a latency of 1 min.

In all cases, each dose of compound was tested in groups of 10 mice with 2 mice being monitored during each observation period. Mice were used only once. Experiments took place between 2.00 p.m. and 5.00 p.m.

2.4. Statistical analysis

Group data are expressed as the mean with S.E.M. The E_{max} and A50 values with 95% confidence limits were calculated using linear regression analysis (PharmTools Pro software, The McCary Group, Elkins Park, PA). Group comparisons were performed using analysis of variance (ANOVA) and the Newman–Keuls test with this software.

3. Results

3.1. Chloroquine-induced scratching behavior

Chloroquine (1.25–40 mg/kg) caused compulsive and vigorous neck-directed scratching with both hind legs within 5 min of s.c. injection. A sigmoidal dose– response curve was obtained with an E_{max} of 360±34 scratches in the 30 min observation period (Fig. 1). The A50 value for chloroquine was 5.9 (3.3–8.4) mg/kg. At the top dose tested (40 mg/kg), scratching was uniform for 30 min and then intermittent over the following 30 min.

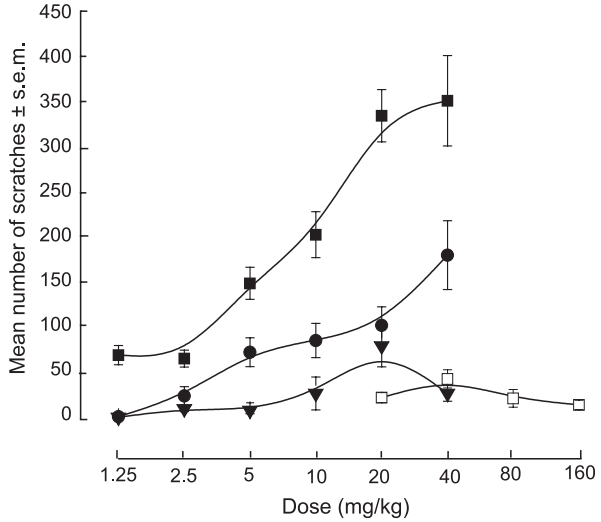

Fig. 1. Dose–response curves for chloroquine (CQ, s.c.; ■); ICI 204,448 (5 mg/kg, s.c. at −20 min)+CQ (s.c.; ·); nalfurafine (20 μg/kg, s.c. at −20 min)+CQ (s.c.; ▼); and CQ (p.o.; □) in inducing scratching behavior in mice (n=10).

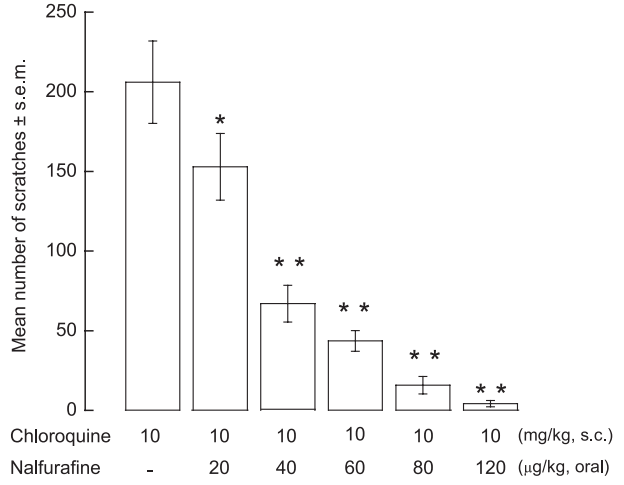

Fig. 2. Effect of pretreatment with nalfurafine (p.o. at −45 min) on scratching behavior induced in mice (n=10) by chloroquine (CQ; 10 mg/kg, s.c.). *P<0.05 and **P<0.01 vs. CQ alone (ANOVA and Newman–Keuls).

When given orally, chloroquine (20–160 mg/kg) was only minimally active, and the incidence of scratching was not dose-related. The maximum effect occurred at the 40 mg/kg dose level (i.e., 44±10 scratches). This number of scratches is just two to three times greater than our historical oral saline mean value.

3.2. Effect of ICI 204,448 on excessive scratching behavior

When mice were pretreated with a fixed dose of ICI 204,448 (5 mg/kg, s.c.), the chloroquine dose–response curve was shifted to the right and displaced downwards. Scratching associated with the top dose of chloroquine (40 mg/kg) was more than halved (Fig. 1).

3.3. Effects of nalfurafine on excessive scratching behavior

The incidence of scratching was impressively decreased when a fixed dose of nalfurafine (0.02 mg/kg, s.c.) was given 20 min before chloroquine (1.25–40 mg/kg). The chloroquine dose–response curve was essentially flattened to the abscissa with the exception of the 20 mg/kg dose level (81±23 scratches; Fig. 1). The mice displayed no obvious behavioral depression.

When mice were pretreated orally with increasingly higher doses of nalfurafine (0.02–0.12 mg/kg), 45 min before challenge with a fixed, submaximal dose of chloroquine (10 mg/kg, s.c.), the incidence of scratching was correspondingly decreased in a smooth and regular fashion to essentially zero (Fig. 2).

4. Discussion

We have shown that the mouse is very sensitive to the scratch-inducing action of chloroquine. Whereas an injection of 20 mg/kg of chloroquine s.c. behind the neck of mice elicits around 350 hind leg-to-neck scratches in 30 min, this dose given i.p. to rats provokes only 30 head/body scratches in the same time (Onigbogi et al., 2000). Furthermore, the chloroquine dose–response curve across 1.25–40 mg/kg in mice is sigmoidal (and therefore suitable for studies with antagonists), rather than shaped like an inverted U as has been reported for this antimalarial compound in rats (Onigbogi et al., 2000).

The pharmacology of nalfurafine has been examined extensively (e.g., Endoh et al., 2000; Tsuji et al., 2000; Mori et al., 2002; Mizoguchi et al., 2003; Suzuki et al., 2004). It is characterized as a chemically novel, centrally penetrating, selective agonist at a subtype of kappa opioid receptor. Of great interest to skin pharmacologists were reports describing the ability of nonsedating doses of nalfurafine to antagonize scratching elicited in mice by histamine, substance P (Togashi et al., 2002) or morphine (Umeuchi et al., 2003). The antipruritic activities of nalfurafine could be antagonized if the mice were pretreated with norbinaltorphimine, the selective kappa antagonist (Togashi et al., 2002; Umeuchi et al., 2003). It is therefore reasonable to conclude that kappa opioid receptors are involved in mediating the antipruritic effects of nalfurafine. Such laboratory findings led to the clinical testing of oral nalfurafine in hemodialysis patients (Kumagai et al., 2004) and its designation as an orphan drug for uremic pruritus by the European Organization for Rare Diseases.

In view of these results, we decided to evaluate nalfurafine against chloroquine-induced scratching in mice. Our previous experience with another opioid, naloxone, in this model had been disappointing. At 0.3, 1 and 3 mg/kg s.c., naloxone only partially (and to the same extent) antagonized the scratching associated with a submaximal dose of chloroquine (10 mg/kg; unpublished data). With nalfurafine, essentially complete antagonism of chloroquine-induced scratching was achieved. In the first instance, a fixed s.c. dose of nalfurafine (0.02 mg/kg) almost flattened the chloroquine dose–response curve. In the second case, increasingly higher doses of nalfurafine (0.02–0.12 mg/kg) were delivered by the *oral* route to mice, and, importantly, from a possible human use perspective, they increasingly antagonized the scratching provoked by a fixed dose of chloroquine (10 mg/kg). In line with these findings, Umeuchi et al. (2003) reported that nalfurafine (0.01 mg/kg, s.c.) significantly inhibited scratching induced by substance P in mice, while Togashi et al. (2002) found that *oral* administration of nalfurafine (0.003–0.10 mg/kg) increasingly antagonized scratching elicited by histamine in this species.

Despite the evolution of chloroquine-resistant strains of malarial parasites and the enduring political debate over newer costly drugs that work vs. cheaper, older ones that may not, this historically famous antimalarial agent continues to enjoy widespread use, particularly in Africa (McNeil, 2003; Yamey, 2003). Drugs in combination have long represented standard therapy against the parasitic

disease. Such "drug cocktails" have included, for example, chloroquine along with proguanil, a biguanide derivative. Another example (currently undergoing Phase III clinical trials) is chloroquine along with azithromycin, an antibiotic. Additionally, artemisinin-based combination therapy (ACT) is undergoing rigorous testing (Nosten and Brasseur, 2002; Duffy and Mutabingwa, 2004). The point being made is that a pharmaceutical product containing chloroquine in combination with another compound would not be unusual. We propose that chloroquine, in combination with nalfurafine, could lead to better treatment compliance because of a reduced incidence of pruritus. Furthermore, in anticipation of likely concern over possible kappa receptor-mediated psychotoxicity in humans (Pfeiffer et al., 1986; Barber and Gottschlich, 1997), our present promising antipruritic data with the peripherally selective ICI 204,448 in mice encourage the expanded clinical testing of peripherally restricted kappa agonists, such as ADL 10-0116, an arylacetamide (Kehner et al., 2000), against the phenomenon of chloroquine-induced itching.

Acknowledgements

It is a pleasure to thank Alex V. Rilling for technical assistance. The study was supported by DA13429 and T32DA07237 from NIDA.

References

Abila, B., Ikueze, R., 1989. Effects of clemastine, ketotifen and prednisolone on chloroquine-induced pruritus. J. Trop. Med. Hyg. 92, 356–359.

Adebayo, R.A., Sofowora, G.G., Onayemi, O., Udoh, S.J., Ajayi, A.A., 1997. Chloroquine-induced pruritus in malaria fever: contribution of malaria parasitaemia and the effects of prednisolone, niacin, and their combination, compared with antihistamine. Br. J. Clin. Pharmacol. 44, 157–161.

Ajayi, A.A., Akinleye, A.O., Udoh, S.J., Ajayi, O.O., Oyelese, O., Ijaware, C.O., 1991. The effects of prednisolone and niacin on chloroquine-induced pruritus in malaria. Eur. J. Clin. Pharmacol. 41, 383–385.

Barber, A., Gottschlich, R., 1997. Novel developments with selective, non-peptide kappa-opioid receptor agonists. Expert Opin. Investig. Drugs 6, 1351–1368.

Cowan, A., Kehner, G.B., 1997. Antagonism by opioids of compound 48/80-induced scratching in mice. Br. J. Pharmacol. 122, 169. (Suppl.).

Cowan, A., Ryan, R.R., Wheeler-Aceto, H., 1995. Defining the antipruritic potential of kappa opioid agonists. FASEB J. 9, A98.

Duffy, P.E., Mutabingwa, T.K., 2004. Drug combinations for malaria: time to ACT? Lancet 363, 3–4.

Endoh, T., Matsuura, H., Tajima, A., Izumimoto, N., Tajima, C., Suzuki, T., Saitoh, A., Suzuki, T., Narita, M., Tseng, L., Nagase, H., 1999. Potent antinociceptive effects of TRK-820, a novel κ-opioid receptor agonist. Life Sci. 65, 1685–1694.

Endoh, T., Tajima, A., Suzuki, T., Kamei, J., Suzuki, T., Narita, M., Tseng, L., Nagase, H., 2000. Characterization of the antinociceptive effects of TRK-820 in the rat. Eur. J. Pharmacol. 387, 133–140.

Gmerek, D.E., Cowan, A., 1984. In vivo evidence for benzomorphan-selective receptors in rats. J. Pharmacol. Exp. Ther. 230, 110–115.

Inan, S., Rilling, A.V., Cowan, A., 2003. TRK-820 and ICI 204,448 inhibit chloroquine-induced scratching in mice. Soc. Neurosci. (33rd Ann. Meeting, New Orleans, LA), Abstr. 585.14.

Kehner, G.B., DeHaven-Hudkins, D.L., Little, P.J., Koblish, M., Kumar, V., Marella, M., Zhang, W.Y., Cowan, A., 2000. Antinociceptive and antipruritic potencies of peripherally selective kappa agonists in mice. NIDA Res. Monogr. 180, 246.

Ko, M.C.H., Lee, H., Song, M.S., Sobczyk-Kojiro, K., Mosberg, H.I., Kishroka, S., Woods, J.H., Naughton, N.N., 2003. Activation of κ-opioid receptors inhibits pruritus evoked by subcutaneous or intrathecal administration of morphine in monkeys. J. Pharmacol. Exp. Ther. 305, 173–179.

Kumagai, H., Saruta, T., Matsukawa, S., Utsumi, J., 2004. Prospects for a novel κ-opioid receptor agonist, TRK-820, in uremic pruritus. In: Yosipovitch, G., Greaves, M.W., Fleischer, A.B., McGlone, F. (Eds.), Itch–Basic Mechanisms and Therapy. Marcel Dekker, New York, NY, pp. 279–286.

Lee, H., Naughton, N.N., Woods, J.H., Ko, M.C.H., 2003. Characterization of scratching responses in rats following centrally administered morphine or bombesin. Behav. Pharmacol. 14, 501–508.

McNeil, D.G., 2003. Ethiopia's Malaria War, Weapons are the Issue. New York Times. (9 Dec).

Mizoguchi, H., Hung, K.-C., Leitermann, R., Narita, M., Nagase, H., Suzuki, T., Tseng, L., 2003. Blockade of μ-opioid receptor-mediated G-protein activation and antinociception by TRK-820 in mice. Eur. J. Pharmacol. 461, 35–39.

Mori, T., Nomura, M., Nagase, H., Narita, M., Suzuki, T., 2002. Effects of a newly synthesized κ-opioid receptor agonist, TRK-820, on the discriminative stimulus and rewarding effects of cocaine in rats. Psychopharmacology 161, 17–22.

Nojima, H., Carstens, E., 2003. 5-Hydroxytryptamine-2 receptor involvement in acute 5-HT-evoked scratching but not in allergic pruritus induced by dinitrofluorobenzene in rats. J. Pharmacol. Exp. Ther. 306, 245–252.

Nosten, F., Brasseur, P., 2002. Combination therapy for malaria—the way forward? Drugs 62, 1315–1329.

Onigbogi, O., Ajayi, A.A., Ukponmwan, O.E., 2000. Mechanisms of chloroquine-induced body-scratching behavior in rats: evidence of involvement of endogenous opioid peptides. Pharmacol. Biochem. Behav. 65, 333–337.

Pfeiffer, A., Brantl, V., Herz, A., Emrich, H.M., 1986. Psychotomimesis mediated by κ opiate receptors. Science 233, 774–776.

Shaw, J.S., Carroll, J.A., Alcock, P., Main, B.G., 1989. ICI 204448: a κ-opioid agonist with limited access to the CNS. Br. J. Pharmacol. 96, 986–992.

Sorbera, L.A., Castaner, J., Leeson, P.A., 2003. Nalfurafine hydrochloride. Drugs Future 28, 237–242.

Sowunmi, A., Walker, O., Salako, L.A., 1989. Pruritus and antimalarial drugs in Africans. Lancet 2, 213.

Suzuki, T., Izumimoto, N., Takezawa, Y., Fujimura, M., Togashi, Y., Nagase, H., Tanaka, T., Endoh, T., 2004. Effects of repeated administration of TRK-820, a κ-opioid receptor agonist, on tolerance to its antinociceptive and sedative actions. Brain Res. 995, 167–175.

Togashi, Y., Umeuchi, H., Okano, K., Ando, N., Yoshizawa, Y., Honda, T., Kawamura, K., Endoh, T., Utsumi, J., Kamei, J., Tanaka, T., Nagase, H., 2002. Antipruritic activity of the κ-opioid receptor agonist TRK-820. Eur. J. Pharmacol. 435, 259–264.

Tsuji, M., Yamazaki, M., Takeda, H., Matsumiya, T., Nagase, H., Tseng, L.F., Narita, M., Suzuki, T., 2000. The novel κ-opioid receptor agonist TRK-820 has no effect on the development of antinociceptive tolerance to morphine in mice. Eur. J. Pharmacol. 394, 91–95.

Twycross, R., Greaves, M.W., Handwerker, H., Jones, E.A., Libretto, S.E., Szepietowski, J.C., Zylicz, Z., 2003. Itch: scratching more than the surface. Q. J. Med. 96, 7–26.

Umeuchi, H., Togashi, Y., Honda, T., Nakao, K., Okano, K., Tanaka, T., Nagase, H., 2003. Involvement of central μ-opioid system in the

scratching behavior in mice, and the suppression of it by the activation of κ-opioid system. Eur. J. Pharmacol. 477, 29–35.

Utsumi, J., Togashi, Y., Umeuchi, H., Okano, K., Tanaka, T., Nagase, H., 2004. Antipruritic activity of a novel κ-opioid receptor agonist, TRK-820. In: Yosipovitch, G., Greaves, M.W., Fleischer, A.B., McGlone, F. (Eds.), Itch—Basic Mechanisms and Therapy. Marcel Dekker, New York, NY, pp. 107–114.

Woster, P.M., 2003. New therapies for malaria. Ann. Rep. Med. Chem. 38, 203–211.

Yamaguchi, T., Nagasawa, T., Satoh, M., Kuraishi, Y., 1999. Itch-associated response induced by intradermal serotonin through 5-HT_2 receptors in mice. Neurosci. Res. 35, 77–83.

Yamey, G., 2003. Malaria researchers say global fund is buying "useless drug". Br. Med. J. 327, 1188.