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Antinociceptive activity of the S1P-receptor agonist FTY720

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Abstract

FTY720 is a novel immunosuppressive drug that inhibits the egress of lymphocytes from secondary lymphoid tissues and thymus. In its phosphorylated form FTY720 is a potent S1P receptor agonist. Recently it was also shown that FTY720 can reduce prostaglandin synthesis through the direct inhibition of the cytosolic phospholipase A2 (cPLA2). Since prostaglandins are important mediators of nociception, we studied the effects of FTY720 in different models of nociception. We found that intraperitoneal administration of FTY720 reduced dose-dependently the nociceptive behaviour of rats in the formalin assay. Although the antinociceptive doses of FTY720 were too low to alter the lymphocyte count, prostanoid concentrations in the plasma were dramatically reduced. Surprisingly, intrathecally administered FTY720 reduced the nociceptive behaviour in the formalin assay without altering spinal prostaglandin synthesis, indicating that additional antinociceptive mechanisms beside the inhibition of prostaglandin synthesis are involved. Accordingly, FTY720 reduced also the nociceptive behaviour in the spared nerve injury model for neuropathic pain which does not depend on prostaglandin synthesis. In this model the antinociceptive effect of FTY720 was similar to gabapentin, a commonly used drug to treat neuropathic pain. Taken together we show for the first time that FTY720 possesses antinociceptive properties and that FTY720 reduces nociceptive behaviour during neuropathic pain.

Keywords: FTY720 • sphingosine-1-phosphate • neuropathic pain • spinal cord • prostaglandin

Introduction

FTY720 is a recently discovered immunosuppressive drug that is based on the fungal substance myriocin. FTY720 is phosphorylated by sphingosine kinase 2 and is in its phosphorylated form a potent agonist for all known S1P receptors, except $S1P_2$ [1–3]. FTY720 potently inhibits a variety of experimental autoimmune disorders and was tested in phase II clinical trials for prevention of renal graft rejection. Currently it is in a phase III clinical trial for the treatment of multiple sclerosis [4–6]. The immunosuppressive properties of FTY720 are mainly based on its ability to prevent the egress of T cells from secondary lymphoid organs, sequestering them from the peripheral blood. Although, the many aspects of the mechanisms of action of FTY720 are still discussed, it is now clear that the S1P receptor S1P1 plays a major role in mediating the FTY720 effects towards lymphocyte migration and trafficking [1, 7–9]. However, since S1P is also an important chemoattractant in the immune system and modulates T cell responses to chemokines, the immunosuppressive properties of FTY720 are likely to be based on several mechanisms [10].

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Only recently it was demonstrated that FTY720 reduced the release of prostaglandin D_2 and cysteinyl-leukotrienes from mast cells, independently of its phosphorylation status and of S1P receptors. In this cell system FTY720 directly inhibited cPLA2 activity, thus decreasing the release of arachidonic acid and preventing the subsequent synthesis of prostaglandins [11]. Therefore it was speculated that FTY720 may reduce inflammatory responses that depend on eicosanoid synthesis, such as asthma [11].

However, several reports show that activation of S1P receptors enhances the spontaneous neurotransmitter release at neuromuscular junctions [12] and facilitates neuronal growth factor (NGF)-induced increases in the excitability of sensory neurons from dorsal root ganglia (DRG) [13, 14]. More recently, it was shown that S1P facilitates also glutamate secretion in hippocampal neurons [15]. Thus, since the activation of S1P receptors enhances the glutamate release in several neuronal systems, FTY720 may promote nociceptive processing not only by enhancing glutamate release of the sensory DRG neurons but as well by facilitating glutamate secretion of excitatory glutamatergic neurons within the spinal cord. On the other hand FTY720 may decrease nociception by reducing prostaglandin synthesis through inhibition of cPLA2.

Here we investigated whether FTY720 influences nociception in animal models that depend on the synthesis of prostaglandins and in animal models that do not depend on prostaglandin synthesis. Therefore we investigated which net effect FTY720 has on nociceptive processing. We found that systemic as well as intrathecally (i.th.) administered FTY720 decreased nociceptive behaviour in adult rats in the formalin assay. Additionally, FTY720 decreased the nociceptive behaviour in the spinal nerve injury (SNI) model for neuropathic pain similar to gabapentin, a commonly described drug for the treatment of neuropathic pain that appears to modulate nociceptive processing by binding to the $\alpha 2\delta$-subunit of voltage-gated calcium channels [16].

Materials and methods

Animals

Sprague Dawley rats (250–300 g) were supplied by Charles River Wiga GmbH (Sulzfeld, Germany). The animals had free access to food and water and were maintained in climate- (24±0.5°C) and light-controlled rooms. In all experiments the ethics guidelines for investigations in conscious animals were obeyed and the procedures were approved by the local Ethics Committee.

Implantation of lumbar catheters

Implantation of lumbar catheters was performed as described previously [17] with the exception that polyethylene catheters (ID 0.28 mm, OD 0.61 mm, Neolab, Heidelberg, Germany) were used.

Behavioural assays

The observer was unaware of the treatments in all tests. The formalin assay [17], hot-plate test [18] and the assessment of motor co-ordination using the pole test [19] were performed as described previously.

Neuropathic pain

Rats were anaesthetized with ketamine and midazolam (0.5–1 mg/kg i.p.) and the sciatic nerve (N.ischiadicus) with its three branches was exposed by blunt dissection. The common pereonal and the tibial branches were tightly ligated with 5-0 silk thread and cut distally from the ligature [20]. For the sham-operated animals the same procedure was applied but the nerve was not ligated and cut. Behavioural testing started before surgery and two baseline values for each group were recorded on separate days. Two time points (day 3 and 7) were recorded after surgery to confirm the neuropathic pain state. Animals were placed on an elevated wire grid and the lateral side of the plantar surface of the paw was stimulated using a Dynamic Plantar Aesthesiometer (Ugo Basile, Comerio VA, Italy). For cold plate testing the rats were placed on a cold steel plate (1±0.5°C) and the total number of flinches were counted in 5 min intervals. Von Frey hair testing [20] and the aceton test were performed as described previously [21].

Leukocyte count

Animals were anaesthetized with isoflurane (2%) and 200 µl of blood were collected by punction of the *v. jugularis* and unlabelled blood cells were counted using a Cell Dyne cytometer (Abbott Diagnostics, Wiesbaden, Germany) according to the manufacturer's instruction.

LC-MS/MS analysis

The following eicosanoids and their stable metabolites were monitored by LC-MS/MS: $PGF_{2\alpha}$, PGE_2, PGD_2, TxB_2, 11-dh-TxB_2, 6-keto-$PGF_{1\alpha}$, Δ^{12}-PGJ_2, 15d-PGJ_2, 13, 14-dh-15-keto-PGD_2, 13,14-dh-15-keto-PGE_2, PGA_2, PGB_2, 15d-PGD_2 and LTB_4. Lumbal spinal cords were prepared and directly frozen with liquid nitrogen. Then 200 µl PBS was added and the tissue quickly homogenized with a potter. 100 µl of the homogenate were mixed with 50 µl water, 20 µl methanol and 20 µl internal standard solution (25 ng/ml of [$^2H_4$]-PGE_2, [$^2H_4$]-PGD_2 and [$^2H_4$]-TxB_2 and 10 ng/ml of [$^2H_4$]-$PGF_{2\alpha}$ and [$^2H_4$]-6-keto-$PGF_{1\alpha}$ in methanol) and extracted twice with 800 µl ethyl acetate. The organic phase was removed at a temperature of 45°C under a gentle stream of nitrogen. The residues were reconstituted with 50 µl of acetonitrile/water/formic acid (20:80:0.0025, v/v, pH 4.0), and injected into the LC-MS/MS system.

Instrumentation

Sample analysis was performed by using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). The LC-MS/MS system consisted of an API 4000 triple-mass spectrometer (Applied Biosystems, Darmstadt, Germany) equipped with a Turbo-V-source operating in negative ESI mode, an Agilent 1100 binary HPLC pump and degasser (Agilent, Böblingen, Germany) and an HTC Pal autosampler (Chromtech, Idstein) fitted with a 100 µl LEAP syringe (Axel Semrau GmbH, Sprockhövel, Germany). A cooling stack was used to store the samples at 4°C in the autosampler. An inlet valve was used to truncate non-relevant signals (10-port, VICI Valco Instruments, Houston, USA). High-purity nitrogen for the mass spectrometer was produced by a NGM 22-LC/MS nitrogen generator (CMC Instruments, Eschborn, Germany).

LC-MS/MS conditions

For the chromatographic separation a Synergi Hydro-RP column and pre-column were used (150 x 2 mm I.D., 4-µm particle size and 80 Å pore size from Phenomenex, Aschaffenburg, Germany). A linear gradient was employed at a flow rate of 300 µl/min. Mobile phase A was water/formic acid (100:0.0025, v/v, pH 4.0) and mobile phase B was acetonitrile/formic acid (100:0.0025, v/v). Sample solvent was acetonitrile/water/formic acid (20:80:0.0025, v/v, pH 4.0). Total run time was 16 min and injection volume of samples was 45 µl. Retention times of the eicosanoids which were detectable in the spinal cord extracts were 6-keto-$PGF_{1\alpha}$, TxB_2, $PGF_{2\alpha}$, PGE_2 and PGD_2 were 7.4 min, 8.0 min, 8.2 min, 8.7 min and 9.2 min, respectively.

The mass spectrometer was operated in the negative ion mode with an electrospray voltage of (3300 V at 450°C. Multiple reaction monitoring (MRM) was used for quantification. The mass transitions used were *m/z* 351.1 → *m/z* 271.2 for PGE_2, *m/z* 351.1 → *m/z* 233.0 for PGD_2, *m/z* 353.1 → *m/z* 193.0 for $PGF_{2\alpha}$, *m/z* 369.1 → *m/z* 162.9 for 6-keto-$PGF_{1\alpha}$, *m/z* 369.1 → *m/z* 168.9 for TxB_2, *m/z* 355.1 → *m/z* 275.1 for [$^2H_4$]-PGE_2, *m/z* 355.1 → *m/z* 237.0 for [$^2H_4$]-PGD_2, *m/z* 357.0 → *m/z* 197.1 for [$^2H_4$]-$PGF_{2\alpha}$, *m/z* 373.2 → *m/z* 167.1 for [$^2H_4$]-6-keto-$PGF_{1\alpha}$ and *m/z* 373.2 → *m/z* 173.0 for [$^2H_4$]-TxB_2 all with a dwell time of 50 ms.

All quadrupoles were working at unit resolution. Quantitation was performed with Analyst Software V1.4 (Applied Biosystems, Darmstadt, Germany) using the internal standard method (isotope-dilution mass spectrometry). Ratios of analyte peak area and internal standard peak area (y-axis) were plotted against concentration (x-axis) and calibration curves for each prostaglandin were calculated by least square regression with 1/concentration^2 weighting. Values that were under the detection limit were set as 0.

FTY720 and FTY720-phosphate (FTY-P) analysis

After liquid–liquid extraction concentrations of FTY720 and FTY720-P and the internal standards C17-sphingosine (C17-SPH) and C17-sphingosine-1-phosphate (C17-S1P) were determined by liquid chromatography coupled to mass spectrometry. HPLC analysis was done under gradient conditions using a Luna C18 column (150 cm L x 2 mm ID, 5 µm particle size and 100 Å pore size, Phenomenex, Aschaffenburg, Germany). MS/MS analyses were performed on a 4000 Q TRAP triple quadrupole mass spectrometer with a Turbo V source (Applied Biosystems, Darmstadt, Germany). Precursor-to-product ion transitions of *m/z* 308 → 255 for FTY720, *m/z* 388 → 290 for FTY720-P, *m/z* 286 → 238 for C17-SPH and 366 → 250 for C17-S1P were used for the MRM. Concentrations of the calibration standards, quality controls and unknowns were evaluated by Analyst software (version 1.4; Applied Biosystems, Darmstadt, Germany). Variations in accuracy and intraday and interday precision (n = 6 for each concentration, respectively) were <15% over the range of calibration. Concentrations under the detection level were set as 0.

Results

To study the effects of FTY720 on nociceptive behaviour we first employed the formalin assay since this assay depends strongly on prostaglandin synthesis and especially on the spinal PGE_2 synthesis [17, 22].

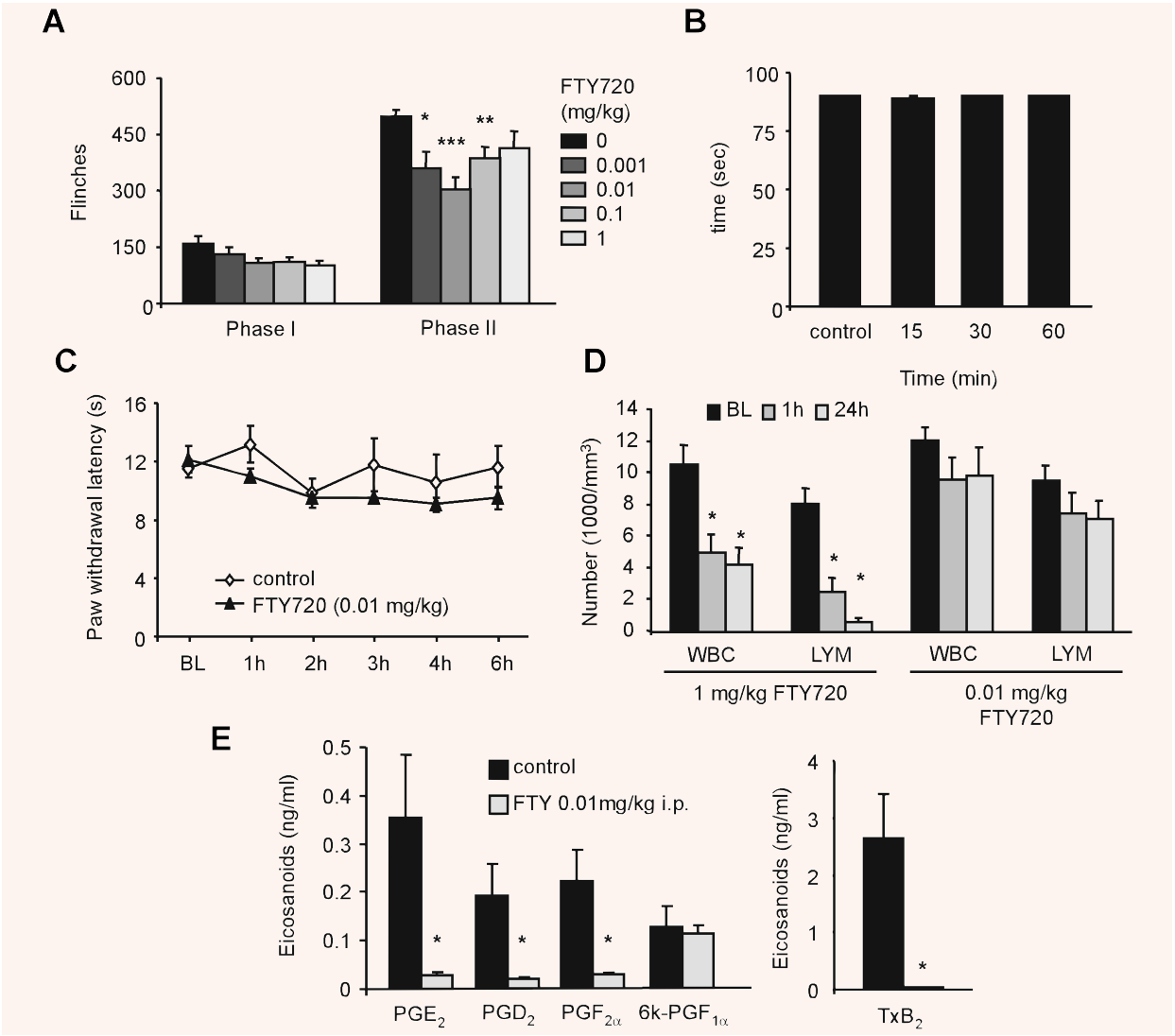

Fig. 1 FTY720 decreases the nociceptive behaviour of rats in the formalin assay. (**A**) Increasing concentrations of FTY720 or saline were given i.p. 15 min prior to the formalin injection in one hind paw. The number of flinches from 0–15 (phase I) min, 16–60 min (phase II) are shown. The data are expressed as the mean ± S.E.M. of six animals per group. Two-tailed Student's t-test: $*P \leqslant 0.025$, $**P \leqslant 0.01$, $***P \leqslant 0.001$ as compared to animals receiving only vehicle. (**B, C**) The effect of 0.01 mg/kg FTY720 i.p. on motor co-ordination was assessed using the pole test (B) and on pain thresholds using the hot-plate test (**C**). Data are presented as mean ± S.E.M. of 5–6 animals. (**D**) The number of white blood cells (WBC) and lymphocytes (LYM) were determined before (baseline, BL) and after a single i.p. administration of FTY720 to the indicated times. Data are presented as mean ± S.E.M. of 4–5 animals. Two-tailed Student's t-test: $*P \leqslant 0.02$ as compared to baseline. (**E**) Eicosanoid concentrations in the plasma 1 hr after i.p. injection of 0.01 mg/kg FTY720. Data are presented as mean ± S.E.M. (n = 8–9). Two-tailed Student's t-test: $*P \leqslant 0.03$ as compared to rats that received only vehicle.

Intraperitoneally (i.p.) injected FTY720 significantly reduced, at rather low doses (0.001 and 0.01 mg/kg), the nociceptive behaviour of adult rats (Fig. 1A). Surprisingly the antinociceptive effect disappeared when the doses were further increased (0.1–1 mg/kg; Fig. 1A). The FTY720 dose with the greatest antinociceptive potency (0.01 mg/kg) did not alter motor abilities of treated rats as determined by the pole assay (Fig. 1B) or the basal pain threshold as determined by the hot plate assay (Fig. 1C). Notably,

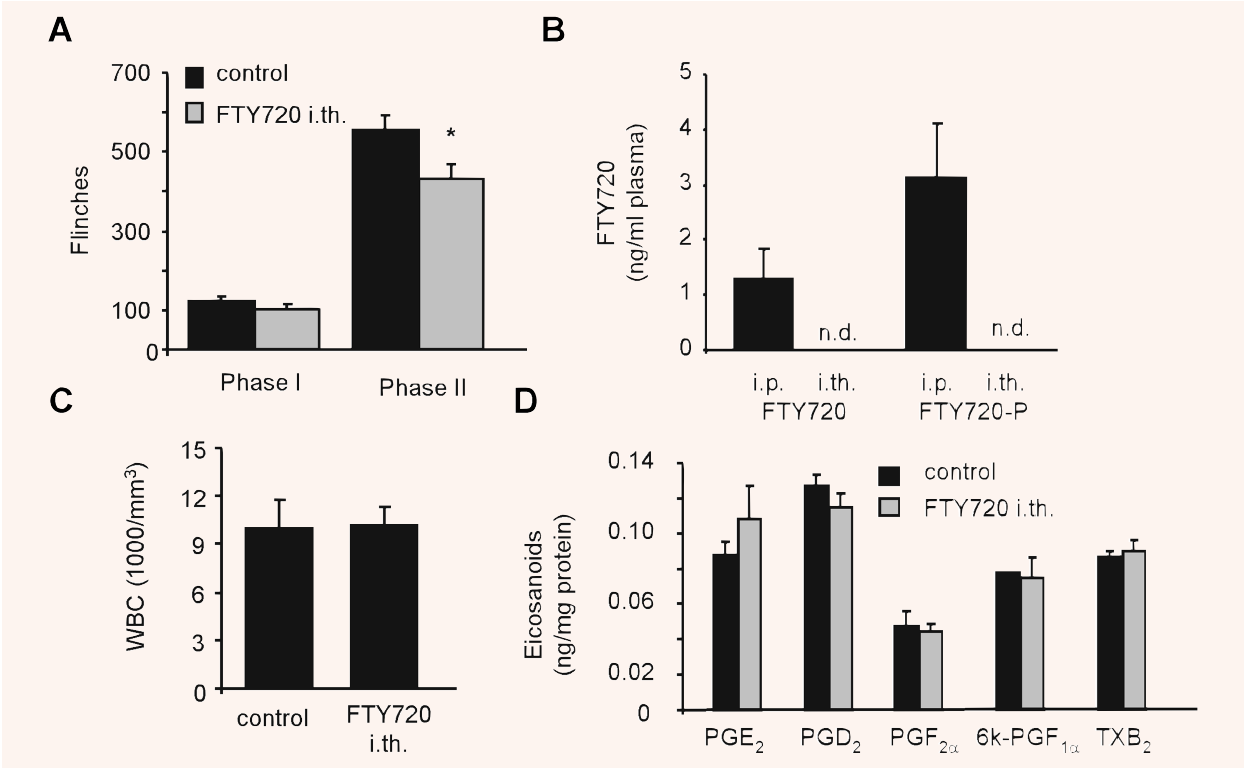

Fig. 2 FTY720 (i.th.) decreases the nociceptive behaviour in the formalin assay without altering the prostanoid synthesis. (**A**) FTY720 (3 μM) or saline were given i.th. 15 min prior to the formalin injection in one hind paw. The data are expressed as the mean ± S.E.M. of 7–10 animals per group. Two-tailed Student's t-test: *$P \leqslant 0.035$ as compared to control animals. (**B**) FTY720 and FTY720-phosphate concentrations were determined in the plasma of animals 1 hr after administering either 0.01 mg/kg i.p. or 3 μM i.th. FTY720. Data are expressed as the mean ± S.E.M. of 4–5 animals per group (nd = not detectable). (**C**) The number of white blood cells (WBC) were determined before and 1 hr after a single i.th. administration of 3 μM FTY720. Data are presented as mean ± S.E.M. of 4–5 animals. (**D**) Eicosanoids were extracted 1 hr after formalin injection from spinal cord segments L4–L5. Where indicated FTY720 (3 μM) was given i.th.. Data are presented as mean ± S.E.M. (n = 7–10).

doses from 0.1–10 mg/kg were previously reported to induce the immunosuppressive actions of FTY720 in rats [9]. Accordingly, at the highest dose (1 mg/kg) a significant reduction in the lymphocyte count occurred (Fig. 1D). At the dose with the strongest antinociceptive effect (0.01 mg/kg) a tendency towards a decreased lymphocyte count was seen, but did not reach statistical significance (Fig. 1D). This gives a first indication that the immunosuppressive properties of FTY720 may not play a major role in mediating its antinociceptive effects. In contrast, 1 hr after i.p. administration of 0.01 mg/kg FTY720, the plasma concentrations of PGE_2, PGD_2, $PGF_{2\alpha}$ and thromboxane A_2 (TxA_2), as determined by its stable metabolite TxB_2, were dramatically decreased. Interestingly, not all eicosanoid concentrations were altered by FTY720, since the plasma concentrations of PGI_2, as determined by its stable metabolite 6-keto$PGF_{1\alpha}$, were unchanged. The dramatic decrease of the eicosanoid level in the plasma, and here especially the decreased PGE_2 concentrations, could serve to explain the antinociceptive effect of FTY720, since inhibition of PGE_2 synthesis generally reduces the nociceptive behaviour in the formalin assay [17, 22, 23].

To investigate whether the antinociceptive effect of FTY720 has also a central component we administered FTY720 i.th. Rats that received FTY720 i.th. exhibited a significant decrease in their nociceptive behaviour in the formalin assay as compared to rats that received only saline (Fig. 2A). Neither FTY720 nor its phosphorylated form was detected in the plasma after i.th. administration (Fig. 2B) and accordingly, even with the highest dose of FTY720 (80 μM,

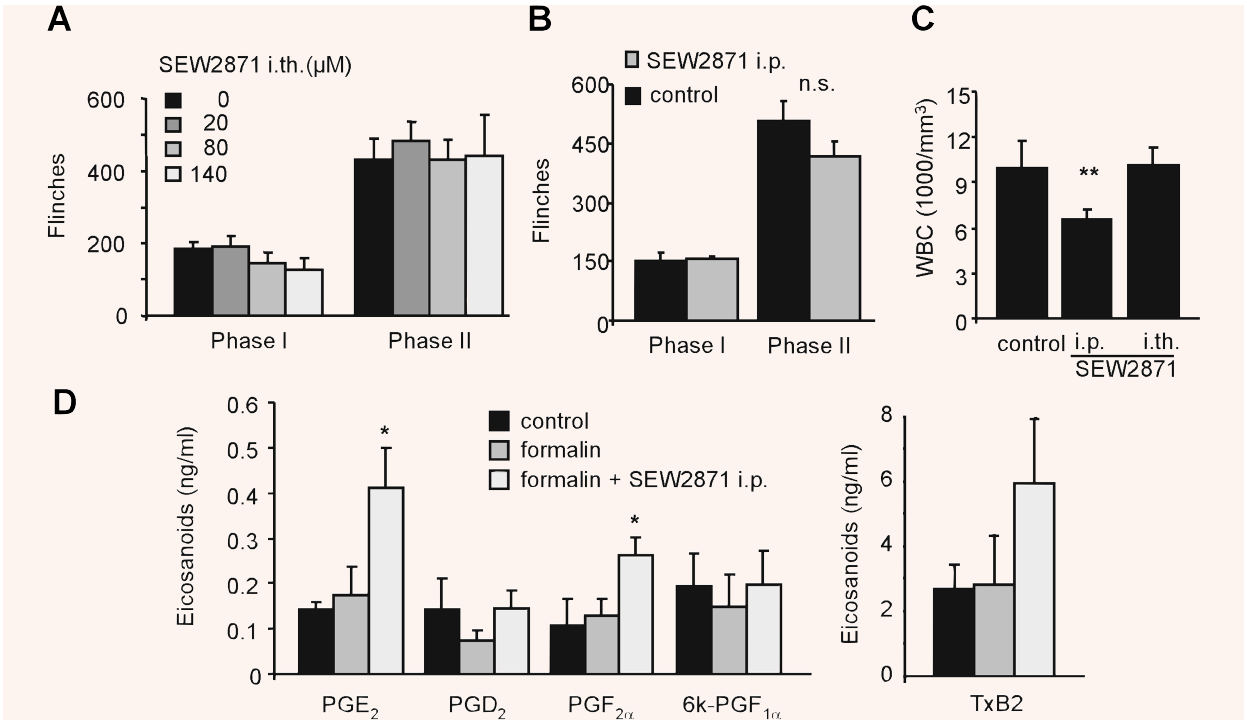

Fig. 3 SEW2871 does not alter nociceptive behaviour in the formalin assay. (**A**) Increasing concentrations of SEW2871 or saline were given i.th. 15 min prior to the formalin injection in one hind paw. The number of flinches from 0–15 (phase I) min, 16–60 min (phase II) are shown. The data are expressed as the mean ± S.E.M. of six animals per group. (**B**) Same as panel A except that SEW2871 (4 mg/kg, i.p.) or saline were given 15 min prior to the formalin injection in one hind paw. The data are expressed as the mean ± S.E.M. of six animals per group (n.s.; not significant). (**C**) The number of white blood cells (WBC) were determined before and 1 hr after a single administration of 10μl SEW2871 (80 μM) i.th. or 4 mg/kg SEW2871 i.p.. Data are presented as mean + S.E.M. of at least seven animals. Two-tailed Student's t-test: $*P \leqslant 0.003$ as compared to controls. (**D**) Eicosanoids were determined in the plasma in untreated animals and 1 hr after formalin injection. Where indicated SEW2871 (4 mg/kg) was given i.p.. Data are presented as mean ± S.E.M. (n = 7–9). Two-tailed Student's t-test: $*P \leqslant 0.003$ as compared to animals treated only with formalin.

i.th.) no effect on the number of white blood cells was observed (Fig. 2C). Since i.p. administration of FTY720 decreases the prostaglandin concentrations in the plasma, we compared the prostanoid synthesis in the spinal cord of animals that received FTY720 i.th. with control animals 1 hr after the formalin injection. Out of 14 eicosanoids that were monitored only five eicosanoids were above the detection limit in the spinal cord extracts and for none of these five eicosanoids significant differences between both animal groups were observed (Fig. 2D). This suggests that the antinociceptive effects of FTY720 in the spinal cord are not based on $cPLA_2$ inhibition.

Since the immunosuppressive effects of FTY720 are mediated by $S1P_1$ [24] we tested which role this receptor plays in mediating the spinal antinociceptive effects of FTY720. Interestingly, neither i.th. or i.p. administration of SEW2871, a selective $S1P_1$ agonist, caused significant effects on the nociceptive behaviour of rats in the formalin assay (Fig. 3A and B). In accordance with the above described findings with FTY720, i.th. administered SEW2871 did not alter the white blood cell count even at the highest dose of (80 μM) (Fig. 3C), while as expected i.p. administered SEW2871 significantly decreased the white blood cell count in the peripheral blood. These findings support the notion that the immunosuppressive property of FTY720 seems not to play a major role in mediating its antinociceptive effect. Moreover, i.p. administered SEW2871 increased significantly the plasma concentrations of PGE_2 as well as $PGF_{2\alpha}$ (Fig. 3D). Owing to the fact that increased PGE_2 levels are connected with increased nociception [17, 22, 23], this finding further speaks against a role of S1P1 in mediating the antinociceptive effects of FTY720.

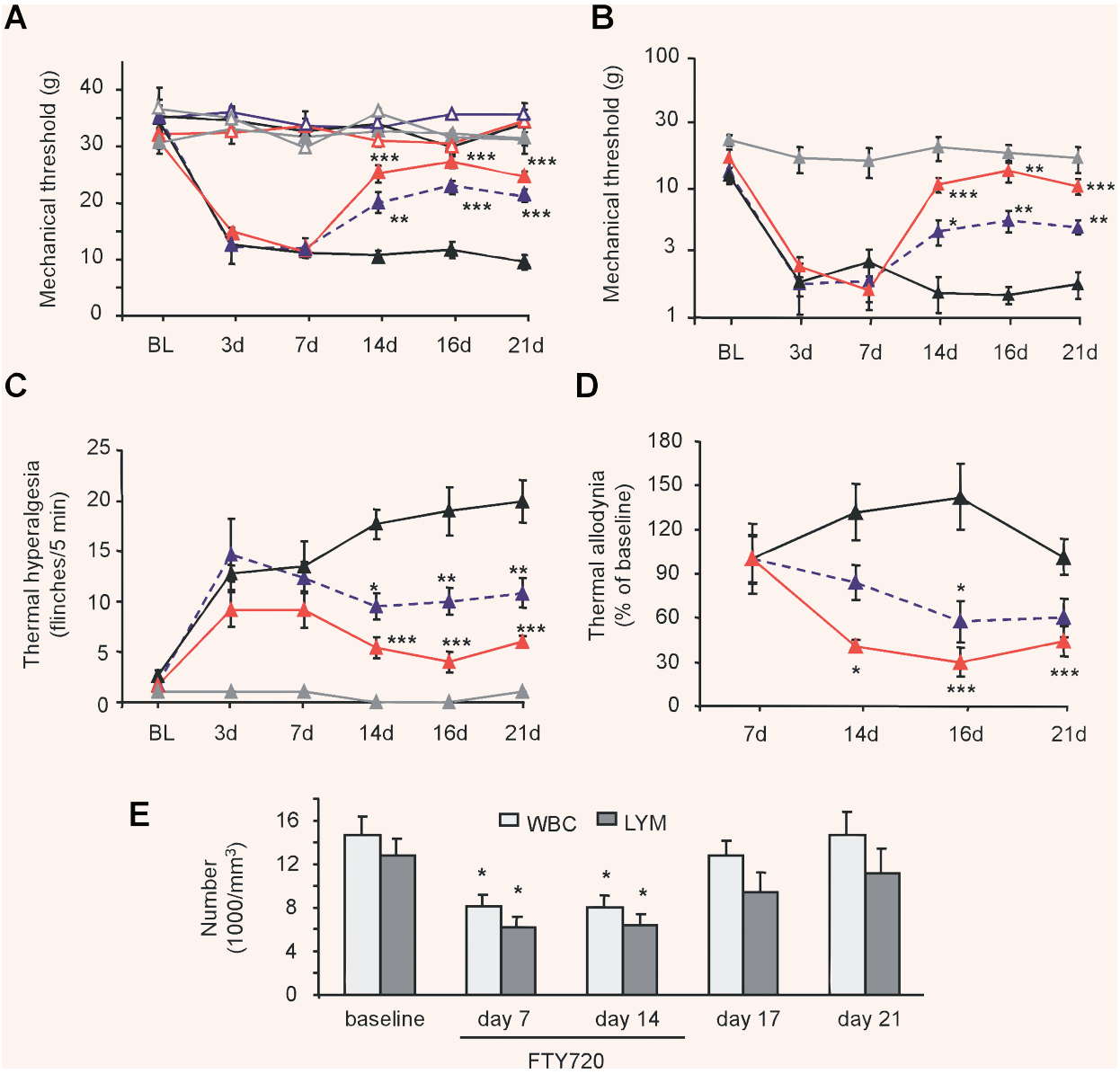

Fig. 4 FTY720 decreases nociceptive behaviour in the SNI model for neuropathic pain. The nociceptive behaviour in the SNI model for neuropathic pain was determined ipsilateral (full symbols) and contralateral (empty symbols) before the operation (BL) and at the indicated times after the operation. The animals received gabapentin (red), FTY720 (blue, dotted line) or vehicle (black). Sham operated animals are shown in grey. FTY720 (1x daily, 0.01 mg/kg i.p.) and gabapentin (2x daily 25 mg/kg) were given starting at day 7 after the operation. The mechanical threshold was determined using a plantar aesthesiometer (**A**) or von Frey hairs (**B**). Thermal allodynia was determined using the cold plate test (**C**) or the acetone test (**D**). Data are presented as mean + S.E.M. of six animals per group. (**E**) FTY720 was applied to adult rats for 14 days (1x daily, 0.01 mg/kg i.p.) and the number of white blood cells (WBC) and lymphocytes (LYM) was determined at the indicated times. After day 14 administration of FTY720 was discontinued. Data are presented as mean + S.E.M. of five animals. Two-tailed Student's t-test: *$P \leqslant 0.5$, **$P \leqslant 0.01$, ***$P \leqslant 0.001$ as compared to animals not receiving FTY720 or gabapentin.

Next, we tested the antinociceptive actions of FTY720 in the spared nerve injury (SNI) model for neuropathic pain, a pain model that has a different pathophysiological mechanism as compared to the formalin assay and is known to be independent of prostaglandin synthesis [19, 20]. Mechanical and thermal allodynia were allowed to develop over 7 days after surgery before the animals received intraperitoneally FTY720, gabapentin (positive control) or phosphate-buffered saline over 2 weeks. Mechanical allodynia as determined by a plantar aesthesiometer (Fig. 4A) and von Frey hair testing (Fig. 4B) as well as thermal allodynia as determined by cold plate (Fig. 4C) and acetone test (Fig. 4D), were significantly decreased after the treatment with FTY720. Interestingly, the antinociceptive effect of FTY720 was similar to that of gabapentin, a commonly prescribed drug for the treatment of neuropathic pain (Fig. 4A–D) [25]. The chronic daily administration of the low doses of FTY720 caused a statistically significant decrease in the number of peripheral blood lymphocytes (Fig. 4E). However, the number of lymphocytes was still within the range that is found in normal, healthy rats [26].

Discussion

Since it has been described that FTY720 can directly inhibit cPLA2 [11], which plays a major role in nociceptive processing, we speculated that FTY720 may reduce nociception in prostaglandin-dependent animal models for inflammatory pain. We present here data which show that FTY720 indeed decreases the nociceptive behaviour in the formalin assay, an animal model for acute pain that depends on prostaglandin synthesis [17, 22]. However, the underlying mechanism(s) that allow FTY720 to exhibit its antinociceptive property remain unclear.

According to the previous report showing direct inhibition of cPLA2 by FTY720, i.p. administered FTY720 decreased the plasma concentrations of PGE_2, PGD_2, $PGF_{2\alpha}$ and TxB_2. The decreased eicosanoid concentrations could serve to explain the antinociceptive effects of FTY720 in the formalin assay. However, i.th. administered FTY720 reduced significantly the nociceptive behaviour in the formalin assay without causing any significant changes in the spinal eicosanoid synthesis. Moreover, FTY720 decreased very effectively the nociceptive behaviour in the SNI model for neuropathic pain, although this model does not depend on prostaglandin synthesis [19, 20]. Taken together these findings make it seem unlikely that the inhibition of cPLA2 is the sole reason for the observed antinociceptive effects. However, it is also obvious that the observed effect of FTY720 on the peripheral prostaglandin synthesis will significantly add to the antinociceptive effect of FTY720 due to the role of prostaglandins in nociceptor sensitization.

Owing to the fact that the antinociceptive active dose of FTY720 (0.01 mg/kg) did not induce a significant decrease in the number of lymphocytes in the peripheral blood and since intrathecal administration of FTY720 had an antinociceptive effect without altering the lymphocyte count, it is also doubtful that the immunosuppressive properties of FTY720 are the main cause for its antinociceptive effect. However, since the repeated daily administration of FTY720 decreases the lymphocyte count in the peripheral blood by about 50% during the treatment in the SNI model, it is possible that the inflammatory component in this model is reduced and therefore might add to the antinociceptive effect of FTY720.

Since $S1P_1$ mediates the immunosuppressive properties of FTY720, we employed the $S1P_1$ agonist SEW2871, to investigate more closely the role of lymphocyte depletion for the antinociceptive activity of FTY720. I.p. administered SEW2871 decreased the white blood cell number in the peripheral blood and raised at the same time the plasma concentrations of PGE_2, PGD_2, $PGF_{2\alpha}$ and TxB_2 which would suggest a pronociceptive effect of $S1P_1$ activation. The increased prostaglandin levels are in accordance with previous reports which describe that S1P treatment as well as $S1P_1$ activation upregulate cyclooxygenase-2 and prostaglandin synthesis in several cell types [27–29]. Since SEW2871 treatment did not enhance the nociceptive behaviour in the formalin assay, either the increased plasma concentrations of the prostaglandins are insufficient to influence nociception in the formalin assay or SEW2871 exerts an unknown antinociceptive effect which compensates the pronociceptive effect of the raised prostaglandin levels. However, taken together the data show that $S1P_1$ activation alone is not to sufficient to execute an antinociceptive effect. Though we cannot rule out that at least in the SNI

model for neuropathic pain the $S1P_1$-mediated immunosuppressive actions of FTY720 might participate in the antinociceptive effects.

Another mechanism for the antinociceptive properties of FTY720 could be based on the activation of S1P receptors in the central nervous system or in the periphery. It is currently discussed that its immunosuppressive actions might be based on FTY720 acting as a 'functional $S1P_1$ receptor antagonist', inducing internalization and degradation of the receptor in lymphocytes to block S1P-directed migration out of lymphnodes [7, 24, 30, 31]. Thus, the reduced antinociceptive effect at higher doses in the formalin assay and in the SNI model may similarly be based on the downregulation of S1P receptors on cells that participate in nociceptive processing.

Neuropathic pain states often occur after peripheral or central nerve damage due to injuries, metabolic damages, viral infections or tumour growth. The pharmacological therapy of most neuropathic pain forms is to date unsatisfactory, since the available drugs are either not very effective or are limited in their usefulness by unwanted side effects [32, 33]. Since the reported side effects for FTY720 are relative moderate [5, 34, 35] and the most effective antinociceptive doses are lower than the doses used for immunosuppression, our data suggest that S1P-receptor agonists, such as FTY720, may turn out to be useful analgesic drugs for the treatment of neuropathic pain. If $S1P_1$ does not mediate the central antinociceptive effect of FTY720 and since FTY720 is not an agonist for $S1P_2$, then $S1P_3$, $S1P_4$ or $S1P_5$ are the most likely candidates to mediate the central antinociceptive effect. As so far no selective agonists or antagonists for these three receptors are available, our hypothesis that these receptors are of importance for the antinociceptive mode of action of FTY720 remains speculative. Thus, the future development of selective agonists for S1P receptors $S1P_{3–5}$ could open the perspective to circumvent the immunosuppression, the main side effect of FTY720, while keeping the analgesic potency.

Acknowledgements

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