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Analgesic effect of thalidomide on inflammatory pain

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Abstract

Tumor necrosis factor α (TNF-α) may have a pivotal role in the genesis of mechanical inflammatory hyperalgesia in rats and in the nociceptive writhing response in mice. Thalidomide has been shown to selectively inhibit TNF-α production. We therefore investigated the effect of thalidomide on these responses as well as on the hot plate response in mice. Hyperalgesic responses to intraplantar (i.pl.) injections of carrageenin or bradykinin, which act by stimulating TNF-α release, but not responses to TNF-α or prostaglandin E_2, were inhibited in a dose-dependent manner by pretreatment of the animals with thalidomide. The nociceptive writhing responses induced by intraperitoneal (i.p.) injections of zymosan or acetic acid were also inhibited in a dose-dependent manner by pretreatment of mice with thalidomide. Moreover, the thalidomide pretreatment also reduced the TNF-α mRNA levels in the peritoneal cells induced by injection of zymosan in mice. The analgesic effect of thalidomide is not due to a central effect, since the drug had no effect in the hot plate test. The demonstration that thalidomide is able to inhibit inflammatory hyperalgesia in rats and the writhing nociceptive response in mice suggests that these analgesic effects seem to be a consequence of the inhibition of TNF-α production, and indicates the need for investigations on the possibility of the use of thalidomide for the treatment of pain refractory to classical non-narcotic analgesics. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Thalidomide; TNF-α (tumor necrosis factor-α); Hyperalgesia, inflammatory; Pain

1. Introduction

Cytokines constitute a link between cellular injury, recognition of non-self and the development of local and systemic signs and symptoms of inflammation, such as cell migration, oedema, fever and hyperalgesia (Dinarello, 1996; Dinarello et al., 1986; Faccioli et al., 1990). Using a model of mechanical hyperalgesia, it was shown that carrageenin-evoked hyperalgesia results from the combined effects of the release of cyclo-oxygenase products and of sympathomimetic amines (Nakamura and Ferreira, 1987), and that a cascade of cytokine release preceded the generation of these mediators (Cunha et al., 1992). Carrageenin and *Escherichia coli* endotoxin cause release of bradykinin, which stimulates the release of tumor necrosis factor-α (TNF-α), which in turn, induces the release of interleukin 1β and interleukin 6, which stimulated the production of cyclo-oxygenase products, as well as interleukin 8, a stimulant of the production of sympathomimetic mediators (Ferreira et al., 1988, 1993; Cunha et al., 1991). Using a different model, the writhing test in mice, (Ribeiro et al., 1999) showed that TNF-α is also involved in the writhing nociceptive response induced by acetic acid or zymosan. TNF-α has also been suggested to be a mediator of inflammatory pain in man since a monoclonal antibody, which neutralizes human TNF-α, diminished the pain associated with rheumatoid arthritis (Rankin et al., 1995).

The development of therapies designed to inhibit TNF-α production could be an important aim in the management of pain. Drugs like pentoxifylline and chlorpromazine have been shown to inhibit TNF-α production in vitro and in vivo (Ohtsuka et al., 1997) and to reduce pain in humans (Dubost et al., 1997) and animals (Gorizontova and Mironova, 1995). It is important in this context to note that pentoxifylline also inhibits the production of other cytokines like interleukin 1 (Weinberg et al., 1992).

Thalidomide (α-*N*-phthalylglutamic-acid-imine) has been shown to selectively inhibit TNF-α production by human monocytes stimulated by *E. coli* endotoxin or *Mycobacterium leprae* products in vitro (Sampaio et al.,

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1991; Moreira et al., 1993), and in vivo stimulated by *E. coli* endotoxin or Bacille Calmette-Guérin (BCG) administration (Aarestrup et al., 1995; Moreira et al., 1997). The ability of thalidomide to inhibit TNF-α production, has been associated with its clinical benefits in the treatment of many immune inflammatory diseases, like rheumatoid arthritis (Gutierrez-Rodriguez et al., 1989), graft vs. host disease (Vogelsang et al., 1992), discoid lupus erythematous (Randall, 1990) and oral aphthous ulceration in patients with HIV infection (Jacobson et al., 1997). Thalidomide has furthermore shown a beneficial effect in the therapy of erithema nodosum leprosum, an acute inflammatory state occurring in lepromatous leprosy, which is associated with the production of TNF-α. Thalidomine treatment reduces TNF-α concentrations in serum of these patients (Mohr, 1971; Sarno et al., 1991), and was shown to be able to reduce TNF-α production by human alveolar macrophages from patients with active inflammatory lung disease (Tavares et al., 1997). Recently, Sommer et al. (1998) demonstrated that thalidomide reduces pain due to chronic constriction injury of the sciatic nerve in the rat. The authors correlated this analgesic effect with the reduction of TNF-α expression in the sciatic endoneurial area (Sommer et al., 1998).

In the present study, the rat paw pressure test, a procedure in which TNF-α has a pivotal role in the development of hyperalgesia, was used to investigate the nature of the effect of thalidomide on inflammatory hyperalgesia. Effects of thalidomide on the writhing response in mice induced by acetic acid or zymosan on the hot plate response induced in mice and in the TNF-α mRNA levels in the peritoneal cells of mice injected with zymosan were also investigated.

2. Materials and methods

2.1. Animals

Male Wistar rats (150–180 g) and Male Swiss mice (20–25 g) were housed in temperature-controlled rooms (22–25°C), with water and food ad libitum until use. The ethical guidelines described in the NIH Guide for Care and Use of Laboratory Animals were followed throughout the experiments.

2.2. Drugs and reagents

2.2.1. Drugs

Thalidomide (RBI, USA), zymosan A (Sigma, St. Louis, USA), glacial acetic acid (Merck, Brazil), carrageenin (FMC, Philadelphia, USA), bradykinin (Sigma), prostaglandin E_2 (Sigma). Trizol Reagent (GibcoBRL, MD, USA), dNTP (Pharmacia, Uppsala, Sweden), Superscript Reverse Transcriptase (GibcoBRL), First strand buffer (GibcoBRL), Taq DNA Polymerase (GibcoBRL), dNTP mix (Pharmacia), DNA ladder (GibcoBRL). National Institute for Biological Standards and Control (NIBSC, England, UK) preparations of rat TNF-α, rat interleukin 1β and human interleukin 8 were kindly provided by Dr. S. Poole (NIBSC). The monoclonal IgG antibodies to murine interleukin 4 (BVDG), and to murine interleukin 10 (JEA.5.1) were kindly provided by Prof. F. Liew (University of Glasgow, UK).

2.3. Nociceptive tests

2.3.1. Mechanical hyperalgesia

A constant pressure of 20 mmHg measured with a sphygmomanometer was applied via a syringe piston moved by compressed air to an area of 15 mm^2 of the dorsal surface of the hindpaws of rats, and discontinued when they presented a typical 'freezing reaction', signaled by brief apnoea, concomitantly with head and forepaw retraction and a reduction in the movements that animals frequently make to escape from the position imposed by the experimental situation. Usually, the apnoea was associated with successive waves of muscular tremor. For each animal, the latency from the moment of the first application of pressure to the onset of the freezing reaction, was measured at zero time, prior to and again at different times following administration of a hyperalgesic agent. The intensity of hyperalgesia was quantified as the reaction time reduction, calculated by subtracting the value of the second measurement from of first (Ferreira et al., 1978; Poole et al., 1999b). Typically, reaction times were 32–34 s (standard errors of the mean [S.E.M.] of 0.5–1.0 s), prior to injection of the hyperalgesic agents. Multiple paw treatments did not alter basal reaction times. Solutions to be injected, injections and measurements of reaction times, respectively, were made by different individuals.

2.3.2. Writhing test

The writhing model (Collier et al., 1968) was tested in mice. The test animals were injected into the peritoneal cavities with the nociception stimulating substances and placed in a large glass cylinder and the intensity of nociception was quantified by counting the cumulative number of writhes occurring between 0 and 30 min after stimulus injection. The writhing response consists of a contraction of the abdominal muscles together with a stretching of hindlimbs.

2.3.3. Hot plate test

Reaction times were measured by the low temperature (51.5 ± 2°C) hot plate method of Eddy and Leimbach (1953), each mouse receiving two trials on the hot plate, separated by a 30-min interval from each other. The first trial familiarized the animal with the test procedure and the second trial served as the control reaction time (licking of hindfeet or jumping) for the animal. Male Swiss mice were pre-selected, those showing a reaction time greater than 10

s not being used. The reaction time for each mouse was determined on the hot plate surface at 30-min intervals after drug administration for a total of 90 min. To avoid possible injury, a cut-off period of 40 s was made during reaction time measurements.

2.4. Paw oedema measurements

Rats were given an intraplantar (i.pl.) injection of 100 μg of carrageenin diluted in 100 μl of saline. Oedema was measured plethysmographically 3 h after carregeenin injection, using a 7150 Ugo Basile (Italy) plethysmometer. Increases in paw volume (delta volume) were obtained by subtracting paw volume measured prior to carrageenin injection from volumes obtained 3 h after injection.

2.5. Reverse transcriptase polymerase chain reaction (RT-PCR)

The peritoneal cells were taken from mice previously (30 min) injected with zymosan (1 mg/mouse), counted in an cell counter (COULTER® A^{C}T, Miami, USA) and 0.5 million were homogenized in 1.0 ml of Trizol reagent. Then 0.2 ml of chloroform was added to the samples, which were shaken vigorously for 30 s. The suspension was centrifuged for 13,000 × *g* at 4°C for 15 min. The aqueous phase was transferred to a fresh tube to which an equal volume of isopropanol was added. After mixing, the samples were incubated for 15 min at −20°C. Samples were centrifuged for 15 min at 13,000 × *g* (4°C). The RNA precipitate was washed with 0.5 ml of ethanol the preparation was suspended in 50 μl of diethylpyrocarbonate-treated water containing 1 mM EDTA.

Reverse transcription involved incubation of 10 μl of total RNA with dNTP, 200 U of the superscript reverse transcriptase, first strand buffer, and diethyl pyrocarbonate-treated water ($_{DEPc}H_20$) for 1 h at 37°C. The reaction was terminated by heating at 90°C for 5 min and cooling at 4°C for 5 min.

PCR amplification utilized primers to mTNF-α (Fwd-GATCTCAAAGACAACCAACTAGTG; RVS-CTC-CAGCTGGAAGACTCCTCCCAG) and β-actin (FWd-TGGAATCCTGTGGCATCCATGAAAC; RVS-TAAAACGCAGCTCAGTAACAGTCCG) and was performed in the presence of Taq DNA Polymerase, PCR buffer, and 1.5 mM $MgCl_2$, dNTP mix, and sterile water. The PCR was conducted in a Perkin-Elmer Cetus GeneAmp PCR system 9600 starting with a 3-min incubation at 95°C, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 65°C for 60 s, and extension at 72°C for 2 min. The final extension was at 72°C for 7 min. The PCR products were separated on a 6% polyacrilamide gel. The 100-bp DNA ladder was used as a molecular size marker. Gel was run at 120 V for 45 min and stained with silver nitrate. Finally, the gel was scanned with PC PhotoShop program.

2.6. Experimental protocols

2.6.1. Effect of pretreatment of animals with thalidomide on hyperalgesia induced by carrageenin, bradykinin, TNF-α or prostaglandin E_2

Hyperalgesia was measured 2, 3 and 4 h following injection of carrageenin (100 μg), and 3 h following injections of respectively, bradykinin (1 μg), TNF-α (2.5 pg) or prostagladin E_2 (100 ng), each injected in a volume of 100 μl into the hindpaws (i.pl.) of rats. Thalidomide (5, 15 and 45 mg/kg) was diluted in 50 μl of DMSO (dimethyl sulfoxide) and 450 μl of saline and injected intraperitonealy (i.p.) 30 min before carrageenin administration. Thalidomide (45 mg/kg) was also administrated 30 min prior to bradykinin, TNF-α, or prostagladin E_2 injections. In other sets of experiments, thalidomide (45 mg/kg) was administrated 1 h or 15 min prior to, or 1 h after i.pl. administration of carrageenin (100 μg).

2.6.2. Effect of pretreatment with thalidomide on paw oedema induced by carrageenin

Animals were pretreated with thalidomide (5, 15 or 45 mg/kg) and after 30 min, 100 μg carrageenin diluted in 100 μl were injected i.pl. and oedema was determined after 3 h, as described above.

2.6.3. Effect of antibodies against interleukin 4 and interleukin 10 on the anti-hyperalgesic effect of thalidomide

Monoclonal antibodies to interleukin 4 (BVDG, 50 μg), or to interleukin 10 (JEA-5, 50 μg), or a control imunoglobulin G (50 μg), diluted in 50 μl, were injected into hindpaws; 30 min later animals were given thalidomide (45 mg/kg) or saline (controls). After a further 30-min period, 100 μg carrageenin in 100 μl of saline, were injected into the same paw that had received the antibodies. To confirm that the antibodies were active, animals injected with BVDG or with JEA-5 were also injected into the same paw with recombinant interleukin 4 (10 ng in 50 μl or interleukin 10 (100 ng in 50 μl), respectively, and after a further 30 min, carrageenin (100 μg in 100 μl) were injected i.pl. Hyperalgesia was assessed after another 3-h period.

2.6.4. Effect of pretreatment with thalidomide on the writhing response induced by zymosan or acetic acid

Mice were treated with, respectively, 5, 15 or 45 mg/kg thalidomide; after 30 min, zymosan (1 mg/mouse), or a 0.6% v/v solution of acetic acid were injected i.p. in a volume of 0.2 ml/mouse (Ribeiro et al., 1999). The number of writhes was counted as described above.

2.6.5. Effect of pretreatment with thalidomide on the hot plate test

Immediately after the second trial (control reaction time, see above), groups of six mice were treated with saline, thalidomide (45 mg/kg, i.p.), morphine (5 mg/kg,

i.p.) or indomethacin (2 mg/kg, i.p.) in a volume of 10 ml/kg. Reaction times were measured as described above.

2.6.6. Effect of pretreatment of mice with thalidomide on TNF-α mRNA levels

Mice were pretreated with saline or thalidomide (45 mg/kg) and after 30 min, zymosan (1 mg/mouse) was injected i.p., in a volume of 0.2 ml/mouse. The total resident peritoneal cells were harvested 30 min after and total RNA was isolated and TNF-α and β-actin mRNA levels were analyzed as described above.

2.7. Statistical analysis

Results are presented as means ± S.E.M. of measurements made on at least five animals in each group. Differences between responses were evaluated by analysis of variance (ANOVA), followed by Bonferroni's *t*-test. Results with $P < 0.05$ were considered significant.

3. Results

3.1. Effect of thalidomide on hyperalgesia induced by carrageenin, bradykinin, TNF-α or prostagladin E_2

The injection of carrageenin into the hindpaw of rats evoked a hyperalgesic effect, measured 2, 3 and 4 h later. Thalidomide injected i.p. 30 min prior to carrageenin inhibited this effect in a dose-dependent manner (Fig. 1, panel A). Thalidomide given i.p., at a dose of 45 mg/kg, only inhibited carrageenin-evoked hyperalgesia when injected 1 h or 15 min prior to the injection of carrageenin (Fig. 2). Treatment of the animals with thalidomide 1 h after carrageenin administration did not affect hyperalgesia (Fig. 2).

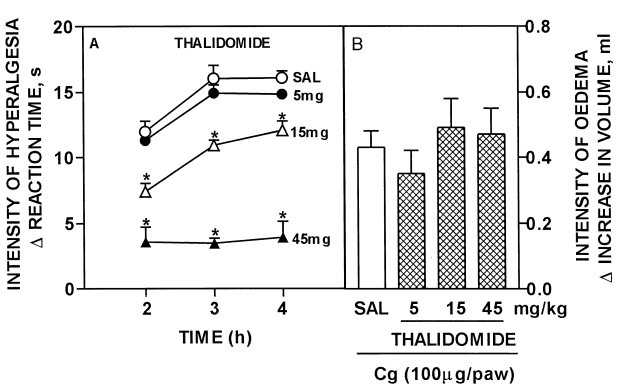

Fig. 1. Effect of systemic administration of thalidomide on the hyperalgesia (panel A) and paw oedema (panel B) induced by carrageenin. Hyperalgesic responses were measured 2, 3 and 4 h after injection of carrageenin (100 μg/paw, i.pl.) and paw oedemas were measured in the same animals 3 h after carrageenin. Thalidomide (5, 15 and 45 mg/kg, i.p.) or saline were given 30 min prior to carrageenin. Results are expressed as means ± S.E.M. of groups of five rats. * Statistically significant differences ($P < 0.05$) from untreated animals.

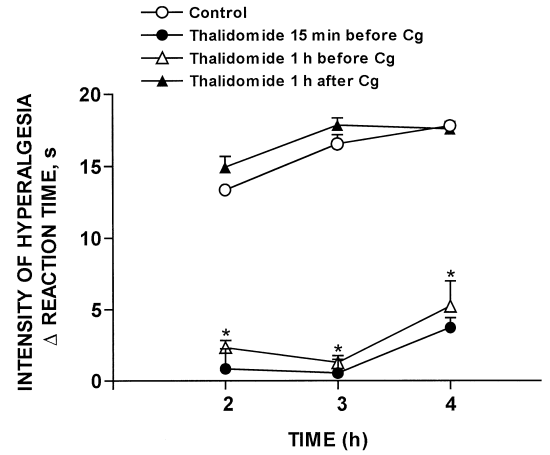

Fig. 2. Effect of systemic administration of thalidomide 1 h and 15 min prior to and 1 h after carrageenin. Hyperalgesic responses were measured 3 h after injection of carrageenin. Thalidomide (45 mg/kg, i.p.) was given 15 min (●), 1 h (△) before and 1 h after (▲) carrageenin. Controls were pretreated with saline 1 h before (○). Results are expressed as means ± S.E.M. of groups of five rats. * Statistically significant differences ($P < 0.05$) from untreated animals.

Injection of bradykinin (1 μg), TNF-α (2.5 pg) or prostaglandin E_2 (100 ng) into the hindpaw of rats evokes hyperalgesic effects, measurable 3 h later. Thalidomide (45 mg/kg) injected i.p. 30 min prior to bradykinin inhibited its hyperalgesic effect. In contrast, hyperalgesias evoked by TNF-α or by prostaglandin E_2 were not affected by pretreatment of the animals with thalidomide (Fig. 3).

3.2. Effect of thalidomide on paw oedema induced by carrageenin

To investigate whether the anti-hyperalgesic action of thalidomide was a specific effect, or a consequence of a

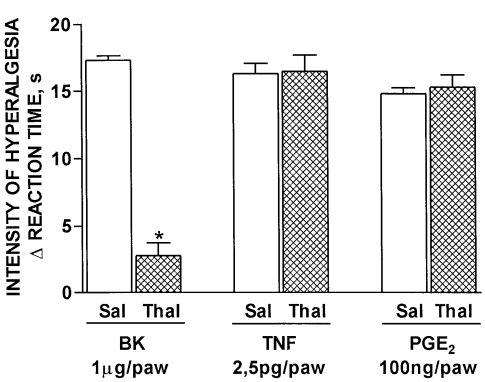

Fig. 3. Effect of systemic administration of thalidomide on the hyperalgesia induced by bradykinin, tumour necrosis factor or prostaglandin. Hyperalgesic responses were measured 3 h after i.pl. injection of bradykinin (BK, 1 μg/paw), tumor necrosis factor (TNF, 2.5 pg/paw) or prostaglandin (PGE_2 100 ng/paw). Saline (open columns) or thalidomide (45 mg/kg, hatched columns), were given 30 min prior to the hyperalgesic stimuli. Results are expressed as means ± S.E.M. of groups of five rats. * Statistically significant differences ($P < 0.05$) from untreated animals.

reduction of inflammation, the effect of the drug on paw oedema evoked by carrageenin, measured after 3 h, was investigated. Thalidomide (5, 15 and 45 mg/kg), injected i.p. 30 min prior to carrageenin did not affect oedema formation (Fig. 1, panel B).

3.3. Effect of antibodies against interleukin 4 and interleukin 10 on the anti-hyperalgesic effect of thalidomide

It had been demonstrated that interleukin 4 or interleukin 10 inhibit hyperalgesia induced by carrageenin, bradykinin, TNF-α, but not hyperalgesia induced by interleukin 8, dopamine or prostaglandin E_2 (Poole et al., 1995; Cunha et al., 1999). In order to investigate whether the anti-hyperalgesic effect of thalidomide is mediated by the release of endogenous interleukin 4 or interleukin 10, the effect of the drug was studied in animals pretreated with antibodies against interleukin 4 (BVDG) or interleukin 10 (JEA-5), 30 min prior to its administration. Fig. 4 shows that the i.pl. administration of antibodies to interleukin 4 or to interleukin 10, in a dose (50 μg), that inhibited the anti-hyperalgesic effect of the respective cytokines, did not affect the anti-hyperalgesic effect of thalidomide on carrageenin-evoked hyperalgesia.

3.4. Effect of thalidomide on writhing responses induced by acetic acid or zymosan

I.p. injection 0.2 ml of a solution of 0.6% (v/v) of acetic acid, or of zymosan (1 mg/animal) in mice, induced a writhing response, determined after 0 to 30 min. Thalidomide injected i.p. 30 min prior to these nociceptive stimulants inhibited their effects in a dose-dependent manner (Fig. 5).

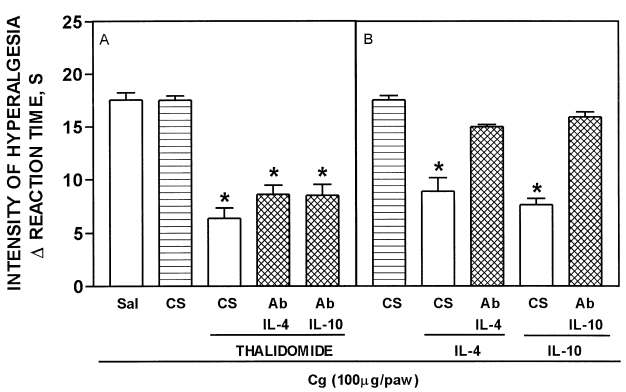

Fig. 4. Effect of antibodies to interleukin 4 or interleukin 10 on the anti-hyperalgesic effect of thalidomide. Rats were injected i.pl. with 50 μg in 50 μl of antibodies to interleukin 4 (Ab IL-4) or to interleukin 10 (Ab IL-10), or with a control antibody (50 μg, CS); 30 min later they received an i.p. injection of saline (Sal), or of thalidomide (45 mg/kg, i.p., panel A). I.pl. interleukin 4 (IL-4, 10 ng/50 μl) or interleukin 10 (IL-10, 100 ng/50 μl), were administered to the same paw which had received the corresponding antibodies (panel B). After a further 30 min, carrageenin (100 μg/100 μl)/paw was injected, and hyperalgesia determined after 3 h. Results are expressed as means ± S.E.M. of groups of five rats. * Statistically significant differences ($P < 0.05$).

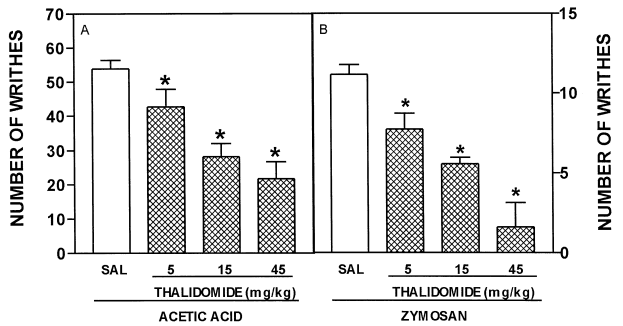

Fig. 5. Effect of systemic administration of thalidomide on the writhing response induced by acetic acid or zymosan in mice. The number of writhings was determined between 0 and 30 min, after i.p. injection of acetic acid at a concentration of 0.6% (v/v), 0.1 ml/10 g of animal (panel A), or zymosan (1 mg/mouse, panel B). Thalidomide (5, 15 and 45 mg/kg, i.p.) or saline were given 30 min before acetic acid or zymosan. Results are expressed as means ± S.E.M. of groups of five rats. * Statistically significant differences ($P < 0.05$).

3.5. Effect of thalidomide on the hot plate response

Thalidomide (45 mg/kg), or indomethacin (2 mg/kg), administrated i.p. to mice, produced no change in 'reaction time during 90 min of observation. In contrast, morphine (5 mg/kg, i.p.), used as a positive control, caused a significant elevation of the reaction time of the animals during this period (Fig. 6).

3.6. Effect of thalidomide on in vivo TNF-α mRNA levels induced by zymosan in mice

I.p. injection of zymosan (1 mg/animal) in mice, induced an increase in the mTNF-α mRNA levels deter-

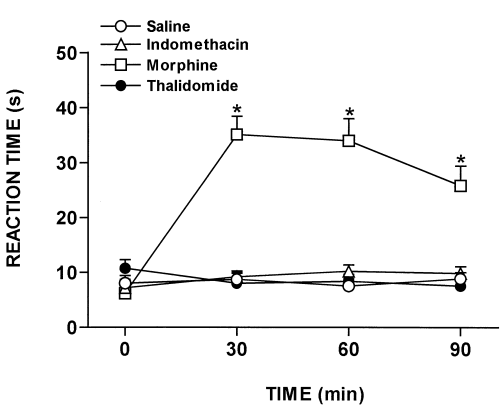

Fig. 6. Effect of thalidomide, morphine or indomethacin on the course of the reaction times to thermal stimuli (hot plate), induced in mice. Mice were treated i.p. with saline, morphine (5 mg/kg), indomethacin (2 mg/kg) or thalidomide (45 mg/kg). Reaction times were measured prior to drug injections (control time) and 30, 60 and 90 min afterwards. Results are expressed as means ± S.E.M. of groups of five rats. * Statistically significant differences ($P < 0.05$).

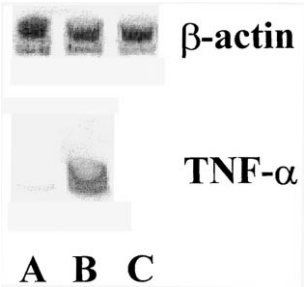

Fig. 7. Effect of systemic administration of thalidomide on the in vivo TNF-α mRNA levels in peritoneal cells harvested from mice injected with zymosan. The TNF-α and β-actin mRNA levels in peritoneal cells harvested from mice injected 30 min before with saline (lane A), zymosan (lane B) or thalidomide (45 mg/kg) and zymosan (lane C). RT-PCR was performed as described in Material and methods. Results are similar to those in two additional experiments.

mined by RT-PCR in the peritoneal cells harvested 30 min after zymosan injection. Thalidomide injected i.p. 30 min prior to zymosan reduced the TNF-α mRNA levels but did not affect of the β-actin mRNA levels (Fig. 7).

4. Discussion

Using the rat paw hyperalgesia test, we had shown that inflammatory stimuli such as carrageenin and endotoxin release a cascade of hyperalgesic cytokines, initiated locally by TNF-α (Cunha et al., 1992). This cytokine activates two distinct pathways: the first is responsible for the induction of cyclo-oxygenase 2 and subsequent synthesis of eicosanoids via the release of interleukin 1β; the second induces sympathomimetic amine production via interleukin 8. Depending on the intensity and nature of the stimuli, the release of TNF-α is preceded by the formation of bradykinin (Cunha et al., 1992; Ferreira et al., 1993; Poole et al., 1999b). In the present study, we used this model to investigate the analgesic effect of thalidomide, a recognized in vivo and in vitro inhibitor of TNF-α synthesis (Sampaio et al., 1991; Moreira et al., 1993, 1997; Aarestrup et al., 1995). Thalidomide inhibited the hyperalgesia induced by carrageenin in a dose dependent manner. It did not, however, show a parallel anti-oedematous effect. Actually, TNF-α, interleukin 1β or interleukin 8, at doses which induced hyperalgesia, did not cause oedema (Poole et al., 1999a). It might therefore well be that in our present model, the initial event is the formation of kinins causing venular permeability increase by releasing prostacyclin from the endothelium, potentiating kinin's direct venular effects. On the other hand, sensitization of primary sensory neurons by eicosanoids or sympathetic amines depends on the release of cytokines by other resident cells due to inflammatory stimuli or bradykinin (Ferreira et al., 1993; Poole et al., 1999a,b). In line with this idea, we have shown that bradykinin stimulates membrane-associated and soluble TNF-α release by peritoneal macrophages (Crosara-Alberto et al., 1997).

Thalidomide seems to act in the cascade of hyperalgesic mediators by inhibiting TNF-α production, since besides being shown to inhibit hyperalgesia induced by carrageenin, it also inhibited hyperalgesia caused by bradykinin, but had no effect on the hyperalgesic action of TNF-α or prostaglandin E_2. The fact that post-treatment with thalidomide was ineffective on carrageenin-induced hyperalgesia is in agreement with an early release of TNF-α. Supporting this suggestion is the demonstration (Sommer et al., 1998) that thalidomide treatment reduces endoneurial TNF-immunoreactivity caused by chronic constriction injury in sciatic nerve sections. The reduction of TNF-immunoreactivity correlated with the reduction of pain due the constriction of the sciatic nerve (Sommer et al., 1998).

Thalidomide has been shown to stimulate the production of interleukin 10 in vivo (Moreira et al., 1997). We have also shown that cytokines interleukin 4 and interleukin 10 inhibit the inflammatory hyperalgesic responses to carrageenin and bradykinin, due to their capacity to inhibit the release of hyperalgesic cytokine and the expression of cyclo-oxygenase 2 (Poole et al., 1995; Cunha et al., 1999). These observations prompted us to investigate whether the anti-hyperalgesic effect of thalidomide is secondary to the enhancement of interleukin 10 or interleukin 4 production. Our results indicate this not to be so, since at doses that blocked the anti-hyperalgesic effect of their respective cytokine antigens, antibodies against interleukin 4 or interleukin 10 were unable to inhibit the anti-hyperalgesic effect of thalidomide.

Thalidomide caused a dose-dependent analgesia of the writhing response induced in mice by acetic acid or zymosan. TNF-α seems to play an important role in this nociceptive test, since specific antibodies against TNF-α reduced the writhing response induced by these stimuli by more than 50% (Ribeiro et al., 1999). Therefore, blockade of TNF-α production could explain the analgesic effect of thalidomide in this test. Confirming this suggestion, we demonstrated that thalidomide treatment reduced the TNF-α mRNA levels in the peritoneal cells of the mice injected with zymosan. This reduction of TNF-α mRNA levels is in accordance with the demonstration that the mechanism by which thalidomide inhibited TNF-α production is due its ability to enhance the degradation of TNF-α mRNA (Moreira et al., 1993).

To evaluate a possible central component of thalidomide's analgesic effect, the hot plate test was used. While morphine controls caused a significant elevation in these test reaction times, thalidomide had no effect, thus excluding the central nervous system as a relevant site of its action.

In conclusion, we demonstrated that thalidomide is able to inhibit inflammatory hyperalgesia in rats and the writhing nociceptive response in mice, seemingly as a consequence of the inhibition of TNF-α production. These results strengthen requirements for investigations on the possible

use of thalidomide for treatment of pain refractory to classical non-narcotic analgesics, and for the development of new drugs free of thalidomide undesirable toxic side-effects.

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