Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 511 (2005) 183–189

www.elsevier.com/locate/ejphar

Morphine modulates inducible nitric oxide synthase expression and reduces pulmonary oedema induced by α-naphthylthiourea

Mustafa Comert[a], Emine Yilmaz Sipahi[b,*], Huseyin Ustun[c], Fulden Isikdemir[b], Gamze Numanoglu[d], Figen Barut[d], Hanife Altunkaya[e], Yetkin Ozer[e], Ferruh Niyazi Ayoglu[f], Tunc Hakan Sipahi[g], Ishak Ozel Tekin[h], Z. Nur Banoglu[b]

[a]*Department of General Surgery, Faculty of Medicine, Zonguldak Karaelmas University, Zonguldak, Turkey*
[b]*Department of Pharmacology, Faculty of Medicine, Zonguldak Karaelmas University, Zonguldak, Turkey*
[c]*Department of Pathology, Ankara Hospital, Ankara, Turkey*
[d]*Department of Pathology, Faculty of Medicine, Zonguldak Karaelmas University, Zonguldak, Turkey*
[e]*Department of Anaesthesiology, Faculty of Medicine, Zonguldak Karaelmas University, Zonguldak, Turkey*
[f]*Department of Public Health, Faculty of Medicine, Zonguldak Karaelmas University, Zonguldak, Turkey*
[g]*Department of Family Physician, Ankara, Turkey*
[h]*Department of Immunology, Faculty of Medicine, Zonguldak Karaelmas University, Zonguldak, Turkey*

Received 24 January 2005; accepted 28 January 2005
Available online 17 March 2005

Abstract

This study was designed to investigate the possible participation of morphine in pulmonary oedema induced by α-naphthylthiourea (ANTU), which is a well-known noxious chemical agent in the lung. Injection of ANTU (15 mg/kg i.p.) produced pulmonary oedema as indicated by an increase in lung weight/body weight ratio and pleural effusion reaching a maximum within 4 h in rat. Administration of morphine prior to ANTU significantly inhibited to pulmonary oedema with a dose-dependent manner. The protective effect of morphine is prevented by peripheral opioid receptor antagonist, naloxone methiodide. ANTU-treated rats were shown positive by inducible nitric oxide synthase immunohistochemical staining. There was no staining in the control group. On the other hand, the degree of staining was markedly reduced in tissue sections by morphine. These results suggest that previous administration of subcutaneous morphine has preventive effect on ANTU-induced pulmonary inflammatory reaction and its effect mediated via peripheral opioid receptors. Application of naloxone with ANTU has no effect on the lung parameters indicating that endogenous opioids do not modulate ANTU-induced damage.

Keywords: α-Naphthylthiourea; Morphine; Naloxone methiodide; Pulmonary oedema; Inflammation

1. Introduction

Alpha-naphthylthiourea is a chemical agent largely used as a rodenticide which produces a dose and time-dependent inflammatory reaction characterized by pulmonary oedema secondary to permeability changes in the lung microvasculature (Richter, 1952). Morphological studies with light and electron microscopy indicate that the capillary endothelial cell of the lung is the primary cellular target of ANTU toxicity (Cunningham and Hurley, 1972; Meyrick et al., 1972). Injury to the endothelium appears as blebbing and scalloping of the cell surface with eventual loss of the endothelial barrier. This loss of endothelial barrier integrity results in increased capillary permeability and the production of an interstitial and alveolar oedema (Pine et al., 1976).

The exact mechanisms of ANTU on pulmonary tissue are not clearly demonstrated. It has been speculated that vasoactive substances originating from the pulmonary vascular bed and airways may contribute to the pulmonary

* Corresponding author. Zonguldak Karaelmas Universitesi, Tip Fakultesi Egitim Bloklari, Farmakoloji A.D., 67600 Kozlu-Zonguldak, Turkey. Tel.: +90 372 2610243; fax: +90 372 2610155.
E-mail address: dresipahi@yahoo.com (E. Yilmaz Sipahi).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.01.038

oedema induced by ANTU. This damage has been shown to be partially mediated through arachidonic acid metabolites (Pankhania and Bakhle, 1982). Following i.v. injection of radiolabeled ANTU, covalent binding to macromolecules is observed in the lung and liver and desulphuration of this toxic compound produces reactive molecules. ANTU is also partially metabolized by cytochrome P450 monooxygenase in both liver and lung microsomes to an intermediate which is also capable of covalent binding (Hardwick et al., 1991). We have recently shown the protective effects of endothelin peptides and nitric oxide synthase inhibitors on ANTU induced pulmonary oedema (Sipahi et al., 1996, 1997).

Many anesthetic agents modulate the lung inflammatory responses via affecting the vascular endothelium and muscle functions (Sipahi et al., 2002). Although the traditional view is that morphine is an anti-nociceptive by actions in the central nervous system, but it is now clear that morphine is effective peripherally in the presence of tissue injury and inflammation (Sengupta et al., 1999). Endogenous opioids have been shown to have anti-inflammatory properties in humans (Brix-Christensen et al., 1997). In the same way it has been shown that morphine treatment reduced paw inflammation by the carrageenan injection (Gyires et al., 1985) and adjuvant-induced arthritis in rats (Walker et al., 1996) and peritoneal inflammation in response to thioglycollate injection in fish (Chadzinska et al., 1997).

Opioid receptors have been identified in rat lung, and activation of these receptors may alter cell function and immune responsiveness in this tissue (Cabot et al., 1994, 1996). While there are numerous scientific publications on the influence of morphine on the motor aspects of respiratory function (Munjal et al., 1995; Chang and Voelkel, 1986), little work has examined the effects of opioids on pleural effusion and pulmonary oedema. The aims of the present study were to investigate the dose-dependent effects of systemic morphine in an acute inflammatory model by using ANTU-induced oedema in rat and evaluate the effects of systemic morphine on inducible nitric oxide synthase tissue activity.

2. Materials and methods

2.1. Assessment of pulmonary oedema

Experiment was carried out on albino rats of either sex weighing 190–230 g obtained from the animal laboratory of Zonguldak Karaelmas University. They were housed under standard laboratory conditions with 12 h light/12 h dark cycle and allowed free access to food and water. All procedures and protocols were in accord with our institutional guideline, which is similar to 'Guide for the Care and Use of Laboratory Animals (US National Institute of Health, revised 1985)'.

During the experimental procedure, the animals were placed in separate cages and kept at room temperature (22 °C). ANTU (suspended in olive oil 4 mg/kg) was injected intraperitoneally (i.p.) at the dose of 15 mg/kg. The control group received olive oil alone at the same volume. Four hours later, the animals were anesthetized with urethane (1.5 g/kg, S.C.) and were bled by cutting the carotid artery. The thorax was opened and pleural effusion was carefully collected by suction and measured volumetrically. Care was also taken to eliminate blood contamination with pleural effusion. The lungs were removed and all surrounding tissues were dissected and weighed with an analytical balance. The volume of pleural effusion (PE) (as millilitre), the lung weight/body weight (LW/BW) and pleural effusion/body weight (PE/BW) ratios were calculated and considered as an index of pulmonary oedema.

2.2. Experimental protocol

The animals were divided into 12 groups. All groups are listed in Table 1.

2.3. Histological examination

For histopathological examination, the lungs were immersed in 10% formalin and allowed for fixation during 3 days. All lobes of each lung were used for histological examination. 10 μm cross-sections were processed for standard haematoxylin and eosin staining (Sheehan and Hrapchak, 1980). These sections were examined via a light microscope and photographed.

2.4. Inducible nitric oxide synthase (iNOS) immunohistochemical staining method in lung

Biopsy materials were fixed in 10% buffered formaldehyde and 3–5 μm sections were prepared from paraffin-embedded tissues. All tissue sections transferred to slides. After deparaffinization, tissue sections were boiled in 10 nM

Table 1
Study groups are listed below

Groups	Chemicals	
1	Olive oil	i.p.
2	ANTU (15 mg/kg)	i.p.
3	ANTU+morphine (1 mg/kg)[a]	i.p./s.c.
4	ANTU+morphine (2 mg/kg)[a]	i.p./s.c.
5	ANTU+morphine (4 mg/kg)[a]	i.p./s.c.
6	Naloxone (1 mg/kg)+olive oil	s.c./i.p.
7	ANTU+naloxone (1 mg/kg)[a]	i.p./s.c.
8	ANTU+naloxone (1 mg/kg)[a]+morphine (2 mg/kg)[a]	i.p./s.c./s.c.
9	ANTU+naloxone (2 mg/kg)[a]+morphine (2 mg/kg)[a]	i.p./s.c.
10	Naloxone methiodide(10 mg/kg)+olive oil	s.c./i.p.
11	ANTU+naloxone methiodide(10 mg/kg)[a]	i.p./s.c.
12	ANTU+naloxone methiodide(10 mg/kg)[a]+morphine (2 mg/kg)[a]	i.p./s.c.

[a] All these treatments were made 30 min before ANTU injection.

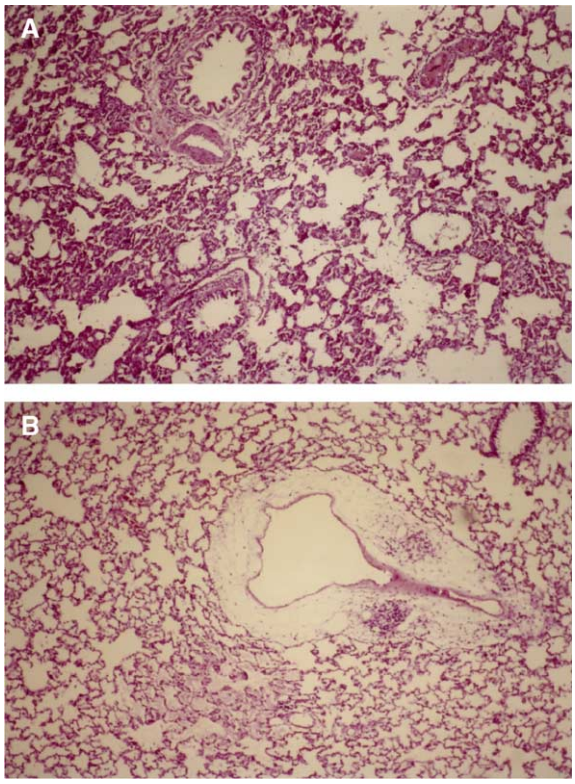

Fig. 1. (A) Normal histological appearance of olive oil-treated rat lungs. Section was taken from the middle lobe of the right lung. Haematoxylin–eosin (H–E) stain ×50. (B) Prominent perivascular, alveolar oedema, thickening of alveolar septa and eosinophilic oedema fluid deposition after ANTU treatment (H–E ×50). Section was taken from the same place.

citrate buffer pH 6.0, for 10–20 min followed by cooling at room temperature for 20 min. All slides were incubated with primary antibodies (nitric oxide synthase, inducible-iNOS Ab-1 Rabbit P Ab Neomarkers, Biogen Medical). The avidin–biotin complex method was used for immunohistochemically analysis.

2.5. Chemicals

The following chemicals were used in this study: α-naphthylthiourea (Interchim) was suspended in olive oil (4 mg/kg); a gift from Dr. E. Schillinger, Schering AG, Berlin, Germany. Olive oil and naloxone methiodide were purchased from Sigma (St. Louis, MO, USA). Naloxone was purchased from Abbott Laboratories (North Chicago, IL 60064, USA). Inducible nitric oxide synthase immunohistochemical staining kit was purchased from Neomarkers (Fremont, CA 94539 USA).

2.6. Statistical analysis of results

Results were expressed as mean±S.E.M. Comparisons between groups were made using a Kruskall–Wallis non-parametric analysis of variance, followed in case of significance by a Mann–Whitney *U*-test. $P<0.05$ was accepted as significant.

3. Results

3.1. Effect of α-naphthylthiourea on pulmonary vasculature

On histopathological examination, ANTU triggered severe pulmonary injury documented as perivascular, peribronchial and alveolar septal oedema and, loss or whole destruction of interstitial cellular elements (Fig. 1B). There were no differences on histopathology changes, LW/BW ratio, and PE parameters between control ($247.9\pm19.2\times10^{-4}$) and olive oil-treated rats ($P>0.05$) (Fig. 1A).

A significant lung oedema was developed after 4 h i.p. injection of ANTU. LW/BW ratio measured as $547.9\pm42.8\times10^{-4}$ for ANTU-treated rats while it was found to be $247.9\pm19.2\times10^{-4}$ for olive oil-treated rats ($P<0.001$) (Fig.

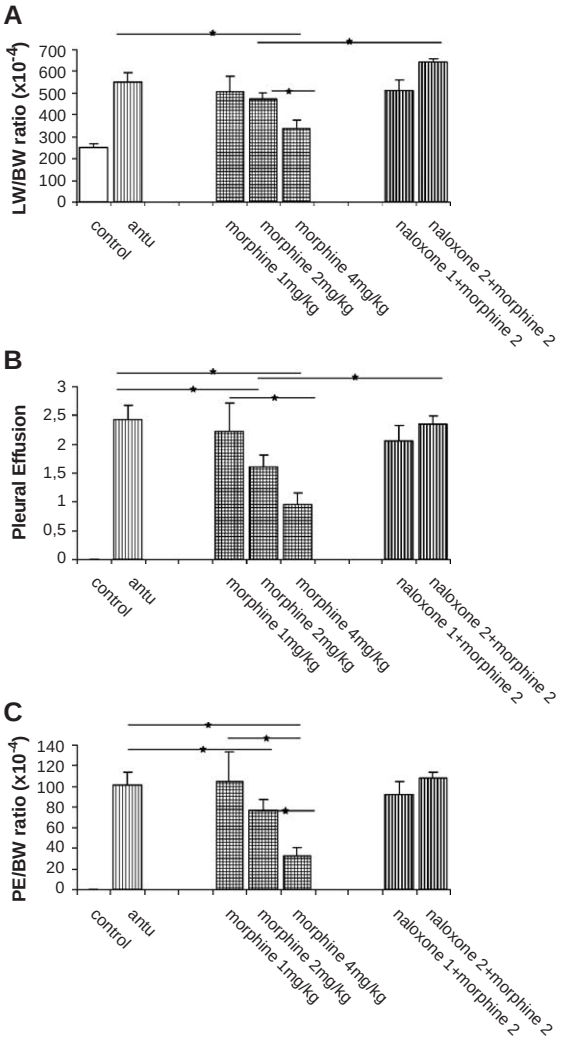

Fig. 2. Figure represents the calculated results of lung oedema induced by α-naphthylthiourea and alterations by morphine (1, 2, and 4 mg/kg), naloxone (1 mg/kg)+morphine (2 mg/kg) and naloxone (2 mg/kg)+morphine (2 mg/kg), as evaluated by the changes of lung weight/body weight (LW/BW) ratio (A), pleural effusion (PE) (ml) (B) and pleural effusion/body weight (PE/BW) ratio (C). Each column (except columns for olive oil control ($n=10$) and α-naphthylthiourea ($n=20$)) shows the mean value of 8 experiments, vertical bars on the columns represent S.E.M. *$P<0.05$.

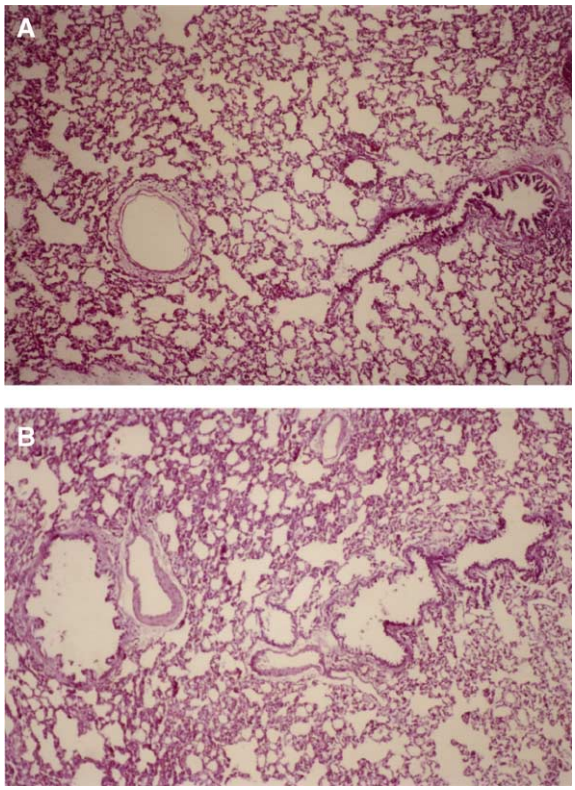

Fig. 3. (A) Reduced perivascular oedema and fluid deposition in alveoli after pre-treatment with morphine (2 mg/kg) of an α-naphthylthiourea-injected rat. Section was taken from the lower lobe of left lung (H–E ×50). (B) The reduction of oedema is more prominent and histopathological changes returned to control lung by 4 mg/kg morphine (H–E ×50). Section was taken from the same place.

2A). Although PE was measured as 2.43×0.26 ml in ANTU-treated rats, PE was not observed in olive oil-treated group (Fig. 2B).

3.2. The effect of morphine on α-naphthylthiourea-induced oedema

Morphine administration prior to ANTU obtained a dose-dependent amelioration in LW/BW ratio and PE. Morphine, at low dose (1 mg/kg), did not affect lung parameters significantly. 2 mg/kg morphine has shown to significantly decrease in PE and PE/BW ratio ($P<0.05$). This dose of morphine affect LW/BW ratio but this is not significantly different. Morphine at dose of 4 mg/kg significantly decreased the LW/BW ratio, PE and PE/BW ratio ($P<0.05$) (Fig. 2).

2 mg/kg morphine expressed prominent shrinkage in the perivascular and peribronchial oedema and alveolar fluid deposition (Fig. 3A). 4 mg/kg morphine completely prevented ANTU induced pulmonary oedema histopathologically (Fig. 3B).

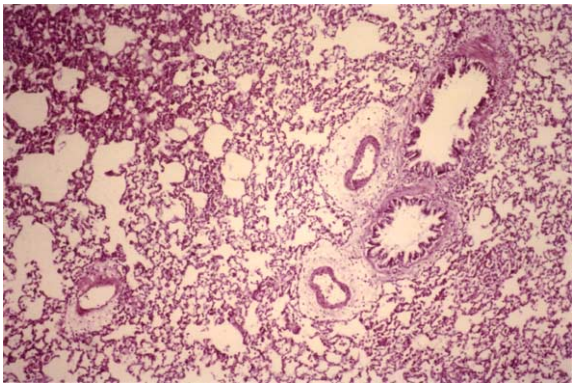

Fig. 4. The prophylactic effects of morphine in histopathological examination are prevented by naloxone and histopathological changes were observed like ANTU treated lungs (H–E ×50).

3.3. Effects of naloxone, naloxone methiodide and combinations with morphine on α-naphthylthiourea-induced oedema

Naloxone alone has no deleterious effect on healthy lung parameters. When injected 30 min before ANTU, it also did not have any changes in the parameters (LW/BW: 515.5±31.6; PE: 117.75±10.57 and histopathological examination). The prophylactic effect of morphine was significantly and dose-dependently abolished by naloxone at doses of 1 mg/kg and 2 mg/kg ($P<0.05$) (Figs. 2 and 4).

In order to discriminate the mechanism of action of naloxone, a peripheral opioid receptor antagonist naloxone

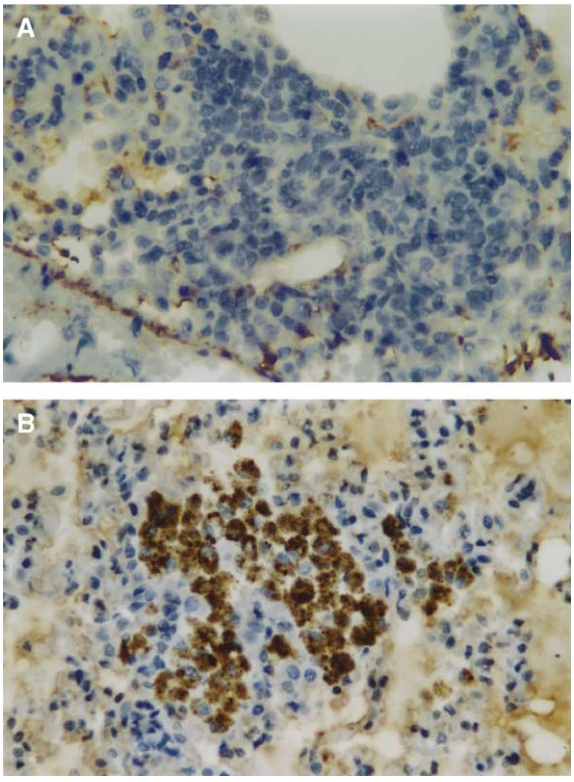

Fig. 5. (A) Immunohistochemical localisation of iNOS in the rat lung. Staining was shown minimal only in vascular wall in control tissue. (B) At 4 h following ANTU injection, positive staining (dense cytoplasmic granular staining in alveolar macrophage) for iNOS (nitric oxide synthase, inducible, Rabbit Pab, Biogen) was observed.

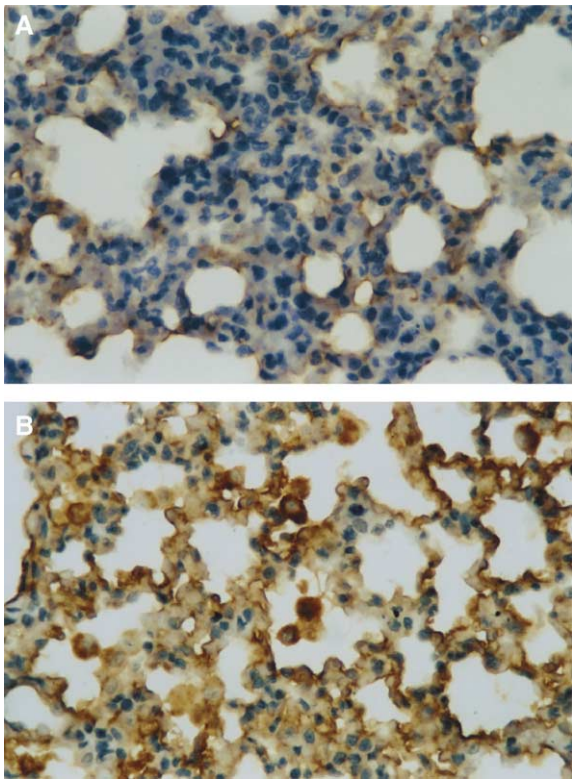

Fig. 6. (A) There was a marked reduction in the immunostaining in the lungs of ANTU-treated rats pre-treated with morphine. (B) The prophylactic effects of morphine in iNOS expression are significantly prevented by naloxone methiodide.

methiodide was also used. Naloxone methiodide alone has no influence on lung parameters (data not shown). The prophylactic effect of morphine was also significantly diminished (data not shown) by naloxone methiodide. The prophylactic effects of morphine in histopathological examination is also prevented by naloxone methiodide and histopathological changes were observed like ANTU treated lungs (data not shown).

3.4. Effects of ANTU, morphine and naloxone methiodide on iNOS expression

INOS expression in the lungs was increased 4 h after ANTU injection (Fig. 5). Pre-treatment with morphine prevented iNOS expression induced by ANTU. On the other hand, the prophylactic effects of morphine in iNOS expression returned by naloxone methiodide and immunohistochemical changes were observed like ANTU treated lungs (Fig. 6).

4. Discussion

Lung oedema characterized with the increase in LW/BW ratio and PE was observed at 4 h after ANTU injection. Pre-treatment with morphine decreased ANTU-induced lung inflammatory response. It also decreased ANTU-induced augmentation of inducible nitric oxide synthase expression. The prophylactic effects of morphine on lung oedema and iNOS expression were significantly inhibited by peripheral opioid receptor antagonist naloxone methiodide.

It is clear that administration of opioids can have marked effects on immunity, and studies have demonstrated the immunosuppressive effects of opioids in humans and animals (Louria et al., 1967; Brown et al., 1974; Briggs et al., 1967; Olsson and Romansky, 1962; Hussey and Katz, 1950). The mechanisms of morphine effects on immunomodulation and inflammation are not clearly demonstrated. Opioids can modulate immune cell proliferation, chemotaxis, superoxide and cytokine production, mast cell degranulation, plasma extravasation and oedema (Bryant and Holaday, 1993). Opioids may diminish the synthesis or release of cytokines from macrophages and mast cells (Bryant and Holaday, 1993) or suppress T-lymphocyte function (Thomas et al., 1995), downregulate the expression of adhesion molecules, and reduce the migration of immune cells into the injured tissue (Wilson et al., 1998).

Opioids, though widely used as analgesia, have not been seriously considered as therapy for inflammatory diseases. Anti-inflammatory effects of morphine have been the subject of several studies in recent years (Gyires et al., 1985; Planas et al., 1995; Joris et al., 1990; Binder et al., 2001; Perrot et al., 2001; Coussons-Read et al., 1999). Endogenous opioids have been shown to have anti-inflammatory properties in human (Brix-Christensen et al., 1997), and beta-endorphin reduces the cutaneous inflammatory response to yeast injection in rodents (Sacerdote et al., 1996). Our results concur with those obtained by other investigators which have demonstrated an inhibitory effect of exogenously administered opioids (morphine) on inflammation, probably related to a poorly understood action on inflammatory mediators. One of them, nitric oxide (NO) is an important mediator in acute and chronic inflammation. We have known that overproduction of NO due to the expression of the inducible isoform of nitric oxide synthase play an important role in various models of inflammation (Moncada et al., 1991; Nathan, 1996; Cuzzocrea et al., 1998). NO can react with superoxide anion to form peroxynitrite, a potent oxidizing molecule capable of eliciting lipid peroxidation and cellular damage (Lysle and Carrigan, 2001; Rubbo et al., 2002). It has been shown that large quantities of NO may contribute to carrageenan-induced pleurisy in rats and selective iNOS inhibitors inhibited carrageenan-induced paw oedema (Tracey et al., 1995; Cuzzocrea et al., 1998). In our previous study, we shown that nitric oxide synthase inhibitor L-NAME, N^G-nitro-L-arginine methyl ester, significantly inhibited ANTU-induced lung damage dose dependently (Sipahi et al., 1997). Consequently, we suggested that overproduction of NO plays a role in ANTU-induced lung damage. In the present study, we also found that morphine has a prophylactic effect in ANTU-induced lung damage via inhibition of inducible nitric oxide synthase expression. Recently the authors

demonstrated that the metabolite of morphine, morphine-6β-glucuronide, induces a pronounced reduction of iNOS expression in lung and endogenous opioids regulate the expression of inducible nitric oxide synthase (Lysle and Carrigan, 2001; Lysle and How, 1999, 2000).

In this study, we used naloxone, a non-receptor-selective opioid antagonist, in order to unmask the effects of endogenously released opioids peptides during inflammation. When injected 30 min before ANTU, naloxone did not have any changes in the parameters, indicating that endogenous opioids do not modulate lung oedema induced by ANTU.

The present study has demonstrated that opioids reduced ANTU-induced oedema and plasma extravasation. Naloxone administration blocked the anti-inflammatory effects of morphine. This result suggests that opioid receptors might be involved in the anti-oedematous action of exogenously administered opioid (morphine). Our results concur with those obtained by other investigators which have demonstrated blocking effect of naloxone on morphine induced anti-inflammatory influence in different inflammation models (Joris et al., 1990; Lysle et al., 1993; Lembeck and Donnerer, 1985). In order to clarify the receptor localisation of opioid's effect on ANTU-induced lung damage, we used specific peripheral opioid receptors antagonist, naloxone methiodide. The prophylactic effect of morphine was significantly blocked by naloxone methiodide. These results suggest that morphine pre-treatment have a prophylactic effect on ANTU-induced lung inflammation in rats via peripheral opioid receptors.

Some of prior works have shown that naloxone does not reverse the effect of morphine or block the endogenous opioids which is activated during inflammation (Gyires et al., 1985; Kayser and Guilbaud, 1990; Kayser et al., 1988). The controversy in literature regarding naloxone could partly be due to the use of different inflammation models, doses and animals. These findings clearly demonstrate the need for the use of different tests and different doses in the evaluation of the effects of opioids and naloxone in order to clarify their roles in pulmonary oedema and pleural effusion.

In conclusion, taken together with these data, the present findings support the anti-inflammatory properties of morphine. Our study demonstrates that morphine pre-treatment has a prophylactic effect on ANTU-induced pulmonary oedema in rats and the effect is mediated via peripheral opioid receptors. However, further investigations are needed to improve our knowledge about the anti-inflammatory effects of morphine.

Acknowledgements

The authors would like to thank Dr. E. Schilliger, Schering AG, Berlin, Germany, for his gift of α-naphthylthiourea (Interchem). We thank Hasan Tahsin Yilmaz and Ramazan Temel for their assistance in research laboratory. This study was supported by intramural funding from Zonguldak Karaelmas University's Office of Research and Sponsored Programs.

References

Binder, W., Machelska, H., Mousa, S., Schmitt, T., Riviere, P.J.M., Junien, J.L., Stein, C., Schafer, M., 2001. Analgesic and antiinflammatory effects of two novel K-opioid peptides. Anesthesiology 94, 1034–1044.

Briggs, J.H., Mckerron, C.G., Souhami, R.L., Taylor, D.J., Andrews, H., 1967. Severe systemic infections complicating mainline heroin addiction. Lancet 2 (7528), 1227–1231.

Brix-Christensen, V., Tonnesen, E., Sanchez, R.G., Bilfinger, T.V., Stehano, G.B., 1997. Endogenous morphine levels increase following cardiac surgery as part of the antiinflammatory response. Int. J. Cardiol. 62 (3), 191–197.

Brown, S.M., Stimmel, B., Taub, R.N., Kochwa, S., Rosenfield, R.E., 1974. Immunologic dysfunction in heroin addicts. Arch. Intern. Med. 134 (6), 1001–1006.

Bryant, H.U., Holaday, J.W., 1993. Opioids in immunologic processes. In: Herz, A. (Ed.), Handbook of Experimental Pharmacology: Opioids II. SpringerVerlag, Berlin, pp. 361–392.

Cabot, P.J., Dodd, P.R., Crammond, T., Smith, M.T., 1994. Characterization of non-conventional opioid binding sites in rat and human lung. Eur. J. Pharmacol. 268 (2), 247–255.

Cabot, P.J., Crammond, T., Smith, M.T., 1996. Quantitative autoradiography of peripheral opioid binding sites in rat lung. Eur. J. Pharmacol. 310 (1), 47–53.

Chadzinska, M., Jozefowski, S., Bigaj, J., Plytycz, B., 1997. Morphine modulation of thioglycollate-elicited peritoneal inflammation in the goldfish, Carassius auratus. Arch. Immunol. Ther. Exp. (Warsz.) 45 (4), 321–327.

Chang, S.W., Voelkel, N.F., 1986. Actions of opiate agonists, naloxone, and parben preservatives in the rat lung circulation. Proc. Soc. Exp. Biol. Med. 181, 404–410.

Coussons-Read, M.E., Daniels, M., Gilmour, M.I., 1999. Morphine reduces pulmonary inflammation in response to influenza infection. Life Sci. 65 (11), 1141–1152.

Cunningham, A.L., Hurley, J.V., 1972. Alpha-naphthylthiourea-induced pulmonary oedema in the rat: a topographical and electron microscopy study. J. Pathol. 106, 25–35.

Cuzzocrea, S., Zingarelli, B., Gilard, E., Hake, P., Salzman, A.L., Szabo, C., 1998. Anti-inflammatory effects of mercaptoethylguanidine, a combined inhibitor of nitric oxide synthase and peroxynitrite scavenger, in carrageenan-induced models of inflammation. Free Radic. Biol. Med. 24, 450–459.

Gyires, K., Budavari, I., Furst, S., Molnar, I., 1985. Morphine inhibits the carrageenan-induced oedema and chemoluminescence of leukocytes stimulated by zymosan. J. Pharm. Pharmacol. 37 (2), 100–104.

Hardwick, S.J., Skamarauskas, J.T., Smith, L.L., Upshall, D.G., Cohen, G.M., 1991. Protection of rats against the effects of α-naphthylthiourea (ANTU) by elevation of non-protein sulphydryl levels. Biochem. Pharmacol. 42, 1203–1208.

Hussey, H.H., Katz, S., 1950. Infections resulting from narcotic addiction; report of 102 cases. Am. J. Med. 9 (2), 186–193.

Joris, J., Costello, A., Dubner, R., Hargreaves, K.M., 1990. Opiates suppress carrageenan-induced oedema and hyperthermia at doses that inhibit hyperalgesia. Pain 43, 95–103.

Kayser, V., Guilbaud, G., 1990. Differential effects of various doses of morphine and naloxone on two nociceptive test thresholds in arthritic and normal rats. Pain 41 (3), 353–363.

Kayser, V., Besson, J.M., Guilbaud, G., 1988. Paradoxical effects of low doses of naloxone in experimental models of inflammatory pain. Prog. Brain Res. 77, 301–312.

Lembeck, F., Donnerer, J., 1985. Opioid control of the function of primary afferent substance P fibres. Eur. J. Pharmacol. 114 (3), 241–246.
Louria, D.B., Hensle, T., Rose, J., 1967. The major medical complications of heroin addiction. Ann. Intern. Med. 67 (1), 1–22.
Lysle, D.T., Carrigan, K.A., 2001. Morphine-6β-glucuronide modulates the expression of inducible nitric oxide synthase. Inflammation 25 (4), 267–275.
Lysle, D.T., How, T., 1999. Endogenous opioids regulate the expression of inducible nitric oxide synthase by splenocytes. J. Pharmacol. Exp. Ther. 288, 502–508.
Lysle, D.T., How, T., 2000. Heroin modulates the expression of inducible nitric oxide synthase. Immunopharmacology 46, 181–192.
Lysle, D.T., Coussons, M.E., Watts, V.J., Bennett, E.H., Dykstra, L.A., 1993. Morphine-induced alterations of immune status: dose-dependency, compartment specificity and antagonism by naltrexone. J. Pharmacol. Exp. Ther. 265, 1071–1078.
Meyrick, B., Miller, J., Reid, L., 1972. Pulmonary edema induced by ANTU or by high or low oxygen concentrations in rat—an electron microscopic study. Br. J. Exp. Pathol. 53, 347–358.
Moncada, S., Palmer, R.M.J., Higgs, E.A., 1991. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol. Rev. 43, 109–142.
Munjal, I.D., Minna, J.D., Manneckjee, R., Bieck, P., Spector, S., 1995. Possible role of endogenous morphine and codeine on growth regulation of lung tissue. Life Sci. 57, 517–521.
Nathan, C., 1996. Nitric oxide as a secretory product of mammalian cells. FASEB J. 6, 3051–3064.
Olsson, R.A., Romansky, M.J., 1962. Staphylococcal tricuspid endocarditis in heroin addicts. Ann. Intern. Med. 57, 755–762.
Pankhania, J.J., Bakhle, Y.S., 1982. Effect of pulmonary oedema by alphanaphthylthiourea on synthesis of cyclooxygenase products in isolated rat lungs. Prostaglandins 30, 37–49.
Perrot, S., Guilbaud, G., Kayser, V., 2001. Differential behavioral effects of peripheral and systemic morphine and naloxone in a rat model of repeated acute inflammation. Anesthesiology 94, 870–875.
Pine, M.B., Beach, P.M., Cottrell, T.S., Scala, M., Turino, G.H., 1976. The relationship between right duct lymph flow and extravascular lung water in dogs given ANTU. J. Clin. Invest. 58, 482–492.
Planas, M.E., Rodriguez, L., Sanchez, S., Pol, O., Puig, M.M., 1995. Pharmacological evidence for the involvement of the endogenous opioid system in the response to local inflammation in the rat paw. Pain 60, 71–76.
Richter, C.P., 1952. The physiology and cytology of pulmonary edema and pleural effusion produced in rats by alphanaphthylthiourea (ANTU). J. Thorac. Surg. 23, 66–90.
Rubbo, H., Trostchansky, A., Botti, H., Batthyany, C., 2002. Interaction of nitric oxide and peroxynitrite with low-density lipoprotein. Biol. Chem. 383, 547–552.
Sacerdote, P., Bianchi, M., Panerai, A.E., 1996. Involvement of beta-endorphin in the modulation of paw inflammatory edema in the rat. Regul. Pept. 63 (2–3), 79–83.
Sengupta, J.N., Snider, A., Su, X., Gebhart, G.F., 1999. Effects of kappa opioids in the inflamed rat colon. Pain 79, 175–185.
Sheehan, D.C., Hrapchak, B.B., 1980. Theory and Practice of Histotechnology, 2nd ed. CV Mosby Co, St. Louis, MO.
Sipahi, E., Hodoglugil, U., Ercan, Z.S., Turker, R.K., 1996. Acute effect of endothelin-1 on lung oedema induced by alpha-naphthylthiourea (ANTU). Pharmacol. Res. 33, 375–378.
Sipahi, E., Hodoglugil, U., Ustun, H., Zengil, H., Turker, RK., Ercan, Z.S., 1997. An unexpected interaction between N^G-nitro-L-arginine methyl ester and L-arginine in α-naphthylthiourea-induced pulmonary oedema in rats. Eur. J. Pharmacol. 321, 45–51.
Sipahi, E., Ustun, H., Ayoglu, F.N., 2002. Acute effects of pentobarbital, thiopental and urethane on lung oedema induced by alpha-naphthylthiourea (ANTU). Pharm. Res. 45 (3), 235–239.
Thomas, P.T., Bhargava, H.N., House, R.V., 1995. Immunomodulatory effects of in vitro exposure to morphine and its metabolites. Pharmacology 50, 51–62.
Tracey, W.R., Nakane, M., Kuk, J., Budzik, G., Klinghofer, V., Haris, R., Carter, G., 1995. The nitric oxide synthase inhibitor, L-N^G-monomethyl arginine, reduces carrageenan-induced pleurisy in the rat. J. Pharmacol. Exp. Ther. 273, 1295.
Walker, J.S., Chandler, A.K., Wilson, J.L., Binder, W., Day, R.O., 1996. Effect of mu-opioids morphine and buprenorphine on the development of adjuvant arthritis in rats. Inflamm. Res. 45 (11), 557–563.
Wilson, J.L., Walker, J.S., Antoon, J.S., Perry, M.A., 1998. Intercellular adhesion molecule-1 expression in adjuvant arthritis in rats: inhibition by kappa-opioid agonist but not by NSAID. J. Rheumatol. 25 (3), 499–505.