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SPECIAL REPORT

Nitroparacetamol exhibits anti-inflammatory and anti-nociceptive activity

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Nitroparacetamol (NCX-701) is a newly synthesized nitric oxide-releasing derivative of paracetamol. Following i.p. administration, nitroparacetamol inhibits carrageenan-induced hindpaw oedema formation (ED_{50}, 169.4 μmol kg^{-1}) and mechanical hyperalgesia (ED_{50}, 156 μmol kg^{-1}) in the rat. In contrast, the parent compound, paracetamol, exhibits no significant anti-oedema activity in this model ($ED_{50} > 1986$ μmol kg^{-1}, i.p.) and is markedly less potent than nitroparacetamol as an inhibitor of carrageenan-mediated hyperalgesia (ED_{50}, 411.6 μmol kg^{-1}, i.p.). In a second model of nociception (inhibition of acetic acid induced abdominal constrictions in the mouse), nitroparacetamol administered orally (ED_{50}, 24.8 μmol kg^{-1}), was again considerably more potent than paracetamol (ED_{50}, 506 μmol kg^{-1}, p.o.). Thus, compared with paracetamol, nitroparacetamol not only exhibits augmented antinociceptive activity in both rat and mouse but, intriguingly, is also anti-inflammatory over a similar dose range.

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Keywords: Nitroparacetamol; paracetamol; carrageenan; carboxymethylcellulose; hyperalgesia; oedema; acetic acid

Abbreviations: CMC, carboxymethylcellulose; COX, cyclo-oxygenase; NO-NSAID, nitro non-steroidal anti-inflammatory drug; NSAID, non-steroidal anti-inflammatory drug

Introduction Paracetamol exhibits both analgesic and antipyretic activity and as such is frequently classified alongside the nonsteroidal anti-inflammatory group of drugs (NSAID). However, paracetamol differs from NSAID in a number of important ways. For example, unlike NSAID, paracetamol exhibits little or no anti-inflammatory activity in animals (Glenn *et al*., 1977; Seegers *et al*., 1981; Bianchi & Penerai, 1996) and very limited, if any, anti-inflammatory activity in man (Lokken & Skjelkbred, 1980). Furthermore, unlike NSAID, paracetamol usage is associated with minimal incidence of gastrointestinal damage (Stern *et al*., 1984).

We (al-Swayeh *et al*., 2000), and others (Elliot *et al*., 1995; Wallace *et al*., 1995), have reported that nitric oxide (NO) releasing derivatives of NSAID (so-called NO-NSAID) exhibit greater anti-inflammatory, antinociceptive and anti-thrombotic activity than the parent NSAID. Evidence has been presented that the augmented anti-inflammatory effect of NO-NSAID relates to the slow release of NO from these compounds although the precise mechanism(s) involved remain unclear (for review, see del Soldato *et al*., 1999).

With these observations in mind we have now evaluated the anti-oedema and anti-nociceptive activity of nitroparacetamol (NOP) which is a newly synthesized NO-releasing derivative of paracetamol.

Methods Rats (male, Wistar, 100–150 g, Tucks Ltd., U.K.) and mice (male, LACA, 22–35 g, Tucks Ltd., U.K.) were purchased from accredited suppliers and maintained in the Biological Services Unit of this College until required. All experiments were carried out 'blind' in that the observer was not aware of the identity or dose of drugs administered to individual animals and were conducted under the conditions of the Animals Scientific Procedures Act, U.K. (1986).

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Measurement of carrageenan-induced hindpaw oedema formation in the rat Experiments were conducted as described previously (al-Swayeh *et al*., 2000). Briefly, hindpaw volume (ml) was determined by plethysmography before, and at timed intervals (30 min–6 h), after intraplantar carrageenan injection (100 μl, 2% w v^{-1}). Nitroparacetamol (25–100 mg kg^{-1}), paracetamol (2.5–300 mg kg^{-1}) or vehicle (0.5% w v^{-1} carboxymethylcellulose, CMC, 1 ml kg^{-1}) were administered i.p. 15 min prior to carrageenan injection. At the end of the experiment, animals were killed by cervical dislocation and exsanguination. Results show mean ± s.e.mean, $n = 5–24$.

Measurement of carrageenan-induced hindpaw hyperalgesia Experiments were conducted as described previously (al-Swayeh *et al*., 2000). 'Baseline' measurements of hindpaw (injected and contralateral i.e. non-injected) mechanical nociceptive threshold were determined using a commercially available algesiometer (Ugo Basile Ltd.). Thereafter, hindpaw inflammation was induced by intraplantar injection of carrageenan (150 μl, 2% w v^{-1}) into either hindpaw chosen at random. Nitroparacetamol or paracetamol (25–100 mg kg^{-1}, i.p.) or an appropriate volume (5 ml kg^{-1}, i.p.) of vehicle (0.5% w v^{-1} CMC) were administered 2.5 h after carrageenan injection and the mechanical nociceptive threshold determined 30 min thereafter. Results (mean ± s.e.mean, $n = 10–15$) are shown as the difference in nociceptive threshold (arbitrary units) before ('pre-') and 3 h after ('post-') intraplantar injection of carrageenan. Statistical comparisons between groups was determined as the difference in 'post-pre' values.

Acetic acid-induced abdominal constriction assay Experiments were conducted as described previously (Moore *et al*., 1991). Mice were administered nitroparacetamol, paracetamol (2.5–100 mg kg^{-1}, p.o.) or vehicle (0.5% w v^{-1} CMC; 10 ml kg^{-1}, p.o.) 15 min prior to i.p. acetic acid (2% w v^{-1} in saline;

pH 2.7; 10 ml kg^{-1}). Animals were transferred immediately to individual observation cages and the number of abdominal constrictions monitored over the following 30 min. At the end of the observation period animals were killed by cervical dislocation and exsanguination Results show number of abdominal constrictions per 30 min test period and are mean ± s.e. mean ($n=10$).

Statistics The statistical significance of differences between groups was determined by ANOVA followed by *post hoc* Dunnett's test. A probability (P) value of 0.05 or less was taken to indicate statistical significance.

Materials Nitroparacetamol (NCX-701; 4-(nitroxy)butanoic acid 4-acetylaminophenyl ester) was obtained from NiCOX Ltd. (Sophia-Antipolis, France). Paracetamol and carrageenan were purchased from Sigma Ltd. Paracetamol and nitroparacetamol were suspended in 0.5% w v^{-1} carboxymethylcellulose (CMC). Carrageenan was suspended in saline. A reasonable suspension of all drugs was achieved after warming (45°C) and with constant sonication and agitation.

Results *Anti-inflammatory activity* Pretreatment with paracetamol (up to 300 mg kg^{-1}, i.p.) did not affect carrageenan-induced hindpaw oedema ($P>0.05$, ANOVA plus Dunnett's test) at any time point up to 6 h after carrageenan injection (Figure 1A). In marked contrast, nitroparacetamol administration resulted in a pronounced, dose- and time-related inhibition of carrageenan-induced hindpaw oedema (Figure 1B). The ED_{50} value for nitroparacetamol (calculated at 180 min) was 47.8 mg kg^{-1} (equivalent to 169.4 μmol kg^{-1}). For comparison, paracetamol (300 mg kg^{-1}, equivalent to 1986 μmol kg^{-1}) reduced oedema by 31.8% ($P>0.05$) at 180 min after intraplantar carrageenan injection.

Antinociceptive activity In control, vehicle-injected mice, i.p. administration of acetic acid caused 52.1 ± 2.7 ($n=10$) constrictions over the 30 min observation period. Pretreatment of animals (30 min before acetic acid injection) with paracetamol resulted in dose-related inhibition of abdominal constrictions (Figure 2A). The dose required for significant antinociceptive activity was 100 mg kg^{-1} (equivalent to 720 μmol kg^{-1}). Thus, it may be concluded that paracetamol exhibits modest antinociceptive activity in this model. Again, in marked contrast, nitroparacetamol caused dose related inhibition of acetic acid-induced abdominal constrictions (Figure 2B). In this case, the dose required for threshold antinociceptive activity was 2.5 mg kg^{-1} (equivalent to 8.9 μmol kg^{-1}) whilst the ED_{50} was 7 mg kg^{-1} (equivalent to 24.8 μmol kg^{-1}). Thus, the potency ratio (nitroparacetamol vs paracetamol) is calculated to be 20.3 on a mol for mol basis.

In separate experiments, intraplantar administration of carrageenan (2% w v^{-1}, 150 μl) in the rat caused significant hindpaw hyperalgesia as evidenced by a reduction in the threshold for noxious mechanical stimulation from 9.92 ± 1.0 units to 3.38 ± 0.37 units ($n=6$, $P<0.05$). Pre-treatment of animals (150 min after intraplantar carrageenan injection; i.e. 30 min before test) with paracetamol or nitroparacetamol (25–100 mg kg^{-1}, i.p.) inhibited carrageenan-induced hyperalgesia in the injected hindpaw (Figure 3). The calculated ED_{50} values for paracetamol and nitroparacetamol as inhibitors of mechanical hyperalgesia are 62.2 and 44.0 mg kg^{-1} (equivalent to 411.6 and 156 μmol kg^{-1}), respectively.

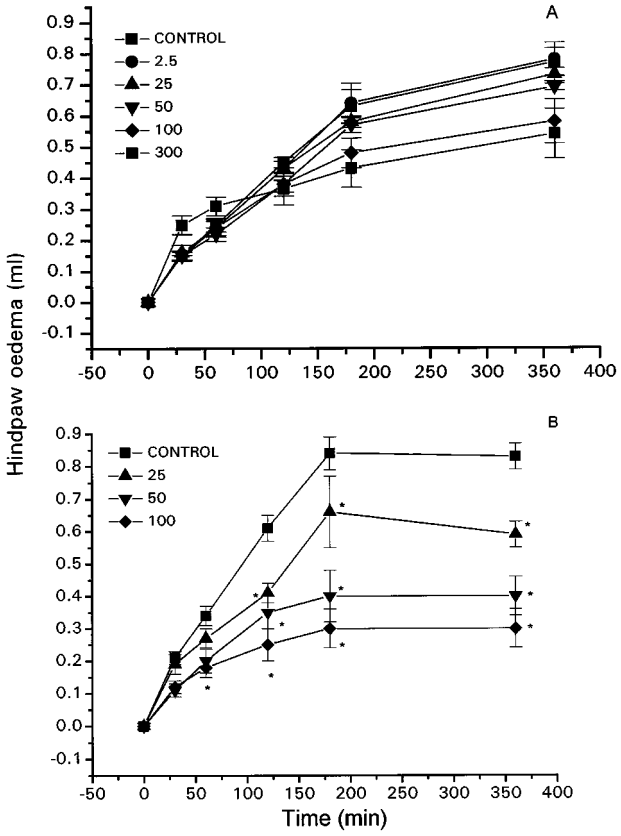

Figure 1 Rats were administered paracetamol (A) or nitroparacetamol (B) 15 min before intraplantar carrageenan. Doses shown are mg kg^{-1} (i.p.). Hindpaw oedema (ml) was measured by plethysmography. Results shown mean ± s.e.mean, $n=5-24$, $^*P<0.05$.

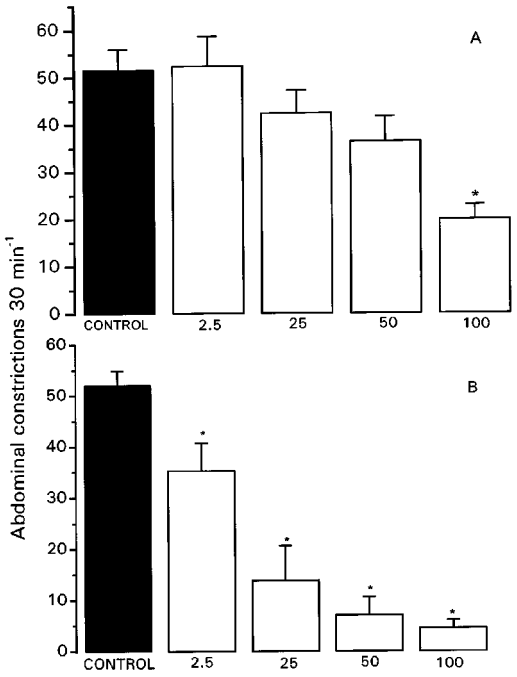

Figure 2 Inhibition of acetic acid induced abdominal constrictions in the mouse by paracetamol (A) and nitroparacetamol (B). Doses shown are mg kg^{-1} (i.p.). Control animals received an appropriate volume of vehicle. Results shown mean ± s.e.mean, $n=10$, $^*P<0.05$.

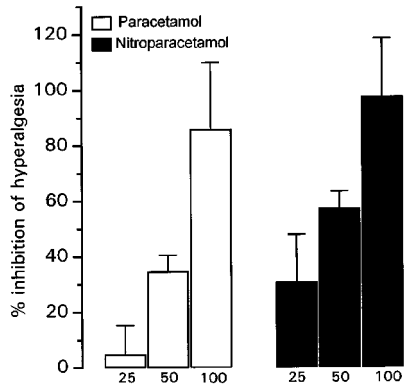

Figure 3 Inhibition of mechanical hyperalgesia by paracetamol and nitroparacetamol. Drugs (mg kg^{-1}, i.p.) were administered 2.5 h after intraplantar carrageenan injection. Results show mean ± s.e.mean, $n = 8$.

Discussion In the present study, pretreatment of animals with paracetamol caused dose related antinociceptive activity following either i.p. (rat) or p.o. (mouse) administration employing either carrageenan or acetic acid respectively as the nociceptive stimulus. The antinociceptive effect of paracetamol was apparent only at relatively high doses which lack of potency agrees with previous published reports on this compound. For example, Vinegar and colleagues (1976) noted that pretreatment with paracetamol inhibited acetic acid-induced abdominal constrictions in the mouse at a threshold dose of 200 mg kg^{-1}. Similarly high doses of paracetamol were also effective in other animal models e.g. zymosan-induced writhing (Doherty *et al*., 1990; threshold for antinociceptive activity, 200 mg kg^{-1}) as well as formalin-induced hindpaw licking in the mouse (Hunskaar & Hole, 1987) and rat (Kanui *et al*., 1993).

In separate experiments, paracetamol was devoid of significant anti-inflammatory activity (doses up to 300 mg kg^{-1}, i.p.). A similar lack of anti-inflammatory activity of paracetamol has also been noted by other researchers using similar animal models (e.g. Bianchi & Penerai, 1996; Glenn *et al*., 1977; Seegers *et al*., 1981).

With these observations in mind it is clearly of interest that nitroparacetamol not only exhibits greater antinociceptive activity than paracetamol with potency ratios (mol for mol basis) ranging from three (carrageenan-induced hindpaw hyperalgesia) to 20 (acetic acid induced abdominal constrictions) but also reduces carrageenan-induced hindpaw oedema formation at doses as low as 25 mg kg^{-1} (i.e. at least 20 times more potent than paracetamol).

The mechanism(s) underlying the enhanced anti-inflammatory and antinociceptive activity of nitroparacetamol have not been investigated in the present experiments. Nevertheless, some comment appears warranted.

The limited antinociceptive as well as the almost complete lack of anti-inflammatory activity of paracetamol has frequently been ascribed to the (at best) very weak cyclo-oxygenase (COX) inhibitory activity of this compound (Abdel-Halim *et al*., 1978; Bippi & Frolich, 1990; Seppala *et al*., 1983).

Over the last several years there has been considerable debate in the literature concerning the possibility of a 'cross talk' between COX, nitric oxide synthase (NOS) and their respective products. In the present experiments, it may reasonably be assumed that NO release from nitroparacetamol accounts for, or at least makes a major contribution to, the augmented biological activity of this compound. Thus, an inhibitory effect of NO on COX activity may be considered as a possible rationale for the increased biological activity of nitroparacetamol. In this context, NO donors such as S-nitroso-acetylpenicillamine have previously been reported to inhibit COX enzyme activity *in vitro* (e.g. Kosonen *et al*., 1998) whilst peroxynitrite inactivates prostacyclin synthase by nitration of strategically positioned tyrosine residues in the enzyme (Zou & Ullrich, 1996). However, it should also be noted that, under some conditions, NO may activate (not inhibit) COX enzyme activity (Salvemini *et al*., 1993). Thus, the precise interaction between NO (released from nitroparacetamol) and COX activity remains unclear. Other possible mechanisms of the anti-inflammatory effect of nitroparacetamol-derived NO include, (i) inhibition of caspase-1 (interleukin converting enzyme) and thus reduced formation of pro-inflammatory interleukin-1β (Dimmeler *et al*., 1997) and, (ii) inhibition of cellular transduction mechanisms underlying inflammation (e.g. NFκ-B cascade) (Minto *et al*., 1997). In this context, it is of interest that pretreatment of endotoxin-treated animals with nitroflurbiprofen has been reported to reduce iNOS induction in the stomach (Mariotto *et al*., 1995). Clearly, further experiments to examine the mechanism of the anti-inflammatory effect of nitroparacetamol are required.

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References

ABDEL-HALIM, M.S., SJOQUIST, B. & ANGGARD, E. (1978). Inhibition of prostaglandin synthesis in rat brain. *Acta Pharmacol. Toxicol.,* **43,** 266 – 272.

AL-SWAYEH, O.A., CLIFFORD, R.H., del SOLDATO, P. & MOORE, P.K. (2000). A comparison of the anti-inflammatory and anti-nociceptive activity of nitroaspirin and aspirin. *Br. J. Pharmacol.,* **129,** 343 – 350.

BIPPI, H. & FROLICH, J.C. (1990). Effects of acetylsalicylic acid and paracetamol alone and in combination on prostanoid synthesis in man. *Br. J. Clin. Pharmacol.,* **29,** 305 – 310.

DEL SOLDATO, P., SORRENTINO, R. & PINTO, A. (1999). NO-aspirins: a class of new anti-inflammatory and antithrombotic agents. *Trends in Pharmacological Sciences,* **20,** 319 – 323.

DIMMELER, S., HAENDELAR, J., NEHLS, M. & ANDREAS, M. (1997). Suppression of apoptosis by nitric oxide via inhibition of interleukin-1beta-converting enzyme (ICE)-like and cysteine protease protein (CPP)-32-like proteases. *J. Exp. Med.,* **185,** 601 – 607.

DOHERTY, N.S., BEAVER, T.H., CHAN, K.Y., DINERSTEIN, R.J. & DIEKEMA, K.A. (1990). The antinociceptive activity of paracetamol in zymosan-induced peritonitis in mice: the role of prostacyclin and reactive oxygen species. *Br. J. Pharmacol.,* **101,** 869 – 874.

ELLIOT, S.N., MCKNIGHT, W., CIRINO, G. & WALLACE, J.L. (1995). A nitric oxide releasing non-steroid anti-inflammatory drug accelerates gastric ulcer healing in rats. *Gastroenterology,* **109,** 524 – 530.

GLENN, E.M., BOWMAN, B.J. & ROHLOFF, N.A. (1977). Anti-inflammatory and PG inhibitory effects of phenacetin and acetaminophen. *Agents and Actions,* **7,** 513 – 516.

HUNSKAAR, S. & HOLE, K. (1987). The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain,* **30,** 103 – 114.

KANUI, T.I., KARIM, F. & TOWETT, P.K. (1993). The formalin test in the naked mole-rat (Heterocephalus glaber): analgesic effects of morphine, nefopam and paracetamol. *Brain Res.,* **600,** 123 – 126.

KOSONEN, O., KANKAANRANTA, H., MALO-RANTA, U., RISTIMAKI, A. & MOILANEN, E. (1998). Inhibition by nitric oxide-releasing compounds of prostacyclin production in human endothelial cells. *Br. J. Pharmacol.,* **125,** 247 – 254.

LOKKEN, P. & SKJELBRAND, P. (1980). Analgesic and anti-inflammatory effects of paracetamol evaluated by bilateral oral surgery. *Br. J. Clin. Pharmacol.,* **10,** 253S – 260S.

MARIOTTO, S., MENEGAZZI, M., CARCERERI DE PRATI, A., CUZZOLIN, L., ADAMI, A., SUZUKI, H. & BENONI, G. (1995). Effect of a new non-steroidal anti-inflammatory drug, nitroflurbiprofen, on the expression of inducible nitric oxide synthase in rat neutrophils. *Br. J. Pharmacol.,* **116,** 1713 – 1714.

MINTO, M., DEL SOLDATO, P., GHEZZI, P. & FRATELLI, M. (1997). A novel acetylsalicylic acid nitroderivative inhibits NF-κB activation and is antiproliferative to L929 cells. *Inflamm. Res.,* **46,** S3.

MOORE, P.K., OLUYOMI, A.O., BABBEDGE, R.C., WALLACE, P. & HART, S.L. (1991). L-NG-nitro arginine methyl ester exhibits antinociceptive activity in the mouse. *Br. J. Pharmacol.,* **102,** 198 – 202.

SALVEMINI, D., MISKO, T.P., MASFERRER, J.L., SEIBERT, K., CURRIE, M.G. & NEEDLEMAN, P. (1993). Nitric oxide activates cyclooxygenase enzymes. *Proc. Natl. Acad. Scu., U.S.A.,* **90,** 7240 – 7244.

SEEGERS, A.J., JAGER, L.P., ZANDBERG, P. & VAN NOORDIWJK, J. (1981). The anti-inflammatory, analgesic and antipyretic activities of non-narcotic analgesic drug mixtures in rats. *Arch. Int. Pharmacodyn. Ther.,* **251,** 1265 – 1275.

STERN, A.I., HOGEN, D.L., KAHN, L.H. & ISENBERG, J.I. (1984). Protective effect of acetaminophen against aspirin- and ethanol-induced damage to the human gastric mucosa. *Gastroenterology,* **86,** 728 – 733.

VINEGAR, R., TRUAX, J.F. & SELPH, J.L. (1976). Quantitative comparison of the analgesic and anti-inflammatory activities of aspirin, phenacetin and acetaminophen in rodents. *Eur. J. Pharmacol.,* **37,** 23 – 30.

ZOU, M.H. & ULLRICH, V. (1996). Peroxynitrite formed by simultaneous generation of nitric oxide and superoxide selectively inhibits bovine aortic prostacyclin synthase. *FEBS Letts.,* **382,** 101 – 104.

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