The antinociceptive activity of paracetamol in zymosan-induced peritonitis in mice: the role of prostacyclin and reactive oxygen species

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- 1 Oral administration of high doses of paracetamol (600 mg kg⁻¹ or more) resulted in inhibition of the writhing and reduced the levels of prostacyclin (PGI₂, measured as 6-keto-PGF_{1α}) induced by intraperitoneal administration of zymosan in mice. The high oral doses of paracetamol required were accompanied by behavioural toxicity which may have contributed to the inhibition of writhing.
- 2 The number of writhes per mouse and the proportion of mice writhing at least once correlated significantly with the levels of 6-keto-PGF_{1α}. However, inhibition of writhing by paracetamol occurred at higher levels of 6-keto-PGF_{1α} than was previously observed with acidic non-steroidal anti-inflammatory agents.
- 3 When injected i.p., PGI₂, carbacyclin and iloprost (agonists at the PGI₂ receptor) induced writhing. Intraperitoneal injection of PGI₂ reversed the inhibition of writhing induced by indomethacin (1 mg kg⁻¹, p.o.) but not that induced by oral administration of paracetamol.
- 4 Paracetamol at 800 mg kg⁻¹, p.o., inhibited carbacyclin-induced writhing but indomethacin at 1 mg kg⁻¹ p.o. did not. Paracetamol administered i.p. at 100 mg kg⁻¹ reduced the peritoneal levels of 6-keto-PGF_{1α} and inhibited zymosan-induced but not carbacyclin-induced writhing and did not produce behavioural toxicity.
- 5 The *in vitro* potency of paracetamol as a prostaglandin synthesis inhibitor is known to be reduced by the presence of lipid peroxides. However, no lipid peroxides, measured as thiobarbituric acid reactive material, were detected in the peritoneal lavage fluid of zymosan-injected mice.
- 6 Intraperitoneal administration of a mixture of superoxide dismutase and catalase reduced detectable superoxide anion by 98% without inhibiting the writhing response to zymosan or the antinociceptive potency of paracetamol.
- 7 The data are consistent with the suggestion that inhibition of PGI₂ synthesis in the peritoneal cavity by paracetamol is responsible for only a part of its antinociceptive activity in this test. However, extremely high oral doses of paracetamol were required which produced behavioural toxicity which clearly contributed to the inhibition of writhing. The low potency of paracetamol in this model cannot be attributed to the generation of lipid peroxides via the oxidative burst.

Introduction

Prostaglandins have been shown to be important mediators of inflammatory pain (Moncada *et al.*, 1978; Ferreira, 1981; Brune & Lanz, 1984). Non-steroidal, acidic, anti-inflammatory drugs (NSAIDs e.g. aspirin, indomethacin, ibuprofen) exert their analgesic effects in inflammatory pain largely by inhibiting the synthesis of prostaglandins in the inflammatory lesion (Vane, 1971; Nakamura & Shimizu, 1981; Nakamura *et al.*, 1983; Doherty *et al.*, 1987; Doherty, 1987). N-(4-hydroxyphenyl) acetamide (acetaminophen, paracetamol) also inhibits inflammatory pain but, despite extensive study, its mechanism of action remains obscure. For example, the effect of paracetamol on prostaglandin synthesis in the most widely used cell-free system (ram seminal vesicle microsomes) is highly dependent on experimental conditions: in the presence of high levels of lipid peroxides which inactivate the enzyme, paracetamol enhances prostaglandin synthesis; in the presence of low levels of lipid peroxides, paracetamol inhibits prostaglandin synthesis (Lands *et al.*, 1976; Robak *et al.*, 1978; Hanel & Lands, 1982; Lands & Hanel, 1982; Hertz & Cloarec, 1984). These results make it difficult to predict the *in vivo* effects of paracetamol on prostaglandin synthesis in a particular *in vivo* situation. Few whole animal studies on the effect of paracetamol

on prostaglandin synthesis at the site of an inflammatory reaction have been reported (Flower *et al.*, 1972).

In contrast to the above, paracetamol was shown to be a relatively potent inhibitor of cell-free prostaglandin synthetase from neural tissue (Flower & Vane, 1972) and to inhibit the increased synthesis of prostaglandins which occurs in the central nervous system (CNS) of animals with fever (Feldberg & Gupta, 1973; Feldberg & Milton, 1978). These observations, plus evidence that increased levels of prostaglandins in the CNS play a role in inflammatory pain (Okuyama & Aihara, 1985; Taiwo & Levine, 1986; Horiguchi *et al.*, 1986; Okuyama & Aihara, 1986) have led to the suggestion that paracetamol exerts its analgesic activity largely or exclusively by inhibiting CNS prostaglandin synthesis, rather than prostaglandin synthesis in inflamed peripheral tissues (Flower & Vane, 1972; Tolman *et al.*, 1983; Hertz & Cloarec, 1984). There is also evidence that inhibition of CNS prostaglandin synthesis may contribute to the analgesic activity of NSAIDs under some circumstances (Ferreira *et al.*, 1978; Bhattacharya & Das, 1984; reviewed in Doherty, 1987).

In the present report, the effect of paracetamol on the levels of prostaglandin in an inflammatory lesion (zymosan-induced peritonitis in mice) has been examined and related to the nociceptive response (writhing) that also occurs in these animals. The role of superoxide radicals, generated by the zymosan-stimulated peritoneal macrophages and a potential source of lipid peroxides, in determining the activity of paracetamol was also examined. A preliminary account of this work was presented at the 4th International Conference of the Inflammation Research Association (October 23–27, 1988, White Haven, PA, U.S.A.).

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Methods

Zymosan-induced peritonitis

The methods used have been comprehensively described previously (Doherty *et al.*, 1985; 1987). Briefly, inflammation (oedema and leukocyte accumulation) and a nociceptive response (writhing) were induced by the intraperitoneal injection of zymosan (1 mg/mouse in 0.5 ml saline) into male CD1 mice (Charles River Breeding Labs, Wilmington, MA, U.S.A.). The number of writhes was counted over a 15 min period, 5–20 min after injection of zymosan, after which the mice were killed and the peritoneal cavity lavaged with saline. The levels of 6-keto prostaglandin $\text{PGF}_{1\alpha}$ (6-keto $\text{PGF}_{1\alpha}$, a measure of prostaglandin I_2 (PGI_2) synthesis) and prostaglandin E_2 (PGE_2) were measured with commercially available radioimmunoassay kits (New England Nuclear, Boston, MA, U.S.A.). Prostaglandins were also determined by gas chromatograph/mass spectrometry (GC/MS), as described previously (Doherty *et al.*, 1987).

Determination of lipid peroxides

The levels of thiobarbituric acid reactive material, indicative of lipid peroxides, in the peritoneal lavage fluids were determined by the method described by Uchiyama & Mihara (1978). Malonyldialdehyde was used to construct the calibration curve.

Chemiluminescence measurements

Zymosan-induced lucigenin-dependent chemiluminescence by resident peritoneal macrophages was measured by a method based on those described by Allen (1986). For *in vitro* chemiluminescence studies, resident peritoneal macrophages were obtained from CD-1 mice by lavage with 8 ml of RPMI 1640 buffer containing 25 mM HEPES and sodium heparin 136 mg l^{-1} . The cells were centrifuged ($400g$ for 15 min) and resuspended in RPMI buffer. Cells pooled from 25 mice (final concentration of $1 \times 10^6 \text{ ml}^{-1}$) were suspended in RPMI buffer containing $50 \mu\text{M}$ lucigenin (Sigma Chemical Co., St. Louis, Mo, U.S.A.) and varying concentrations of inhibitors or enzymes. Aliquots of cells (1 ml) were placed in a Picolite chemiluminometer (Los Alamos Diagnostics, Los Alamos, NM, U.S.A.) and allowed to incubate in the dark for 30 min. The cells were then stimulated with unopsonized zymosan (0.4 mg ml^{-1} , final concentration) and the chemiluminescence measured over 4 h. The inhibitory effects of the treatments of the macrophage were determined by comparing the integrals of the chemiluminescence curves.

To assess the *in vivo* effect of treatments, mice were injected i.p. with 0.5 ml of saline or saline containing superoxide dismutase plus catalase. After 20 min, each animal was lavaged with 5 ml of Hanks' balanced salt solution and the cells obtained counted and chemiluminescence measured as above in aliquots from individual mice. Results were corrected for differences in cell number and expressed as counts per 1×10^6 cells. This procedure avoids washing the cells free of

the enzymes administered *in vivo* but results in at least a 5 fold dilution of the compounds or enzymes present in the peritoneal cavity at the time of collection.

Drug administration

For oral administration, compounds were prepared as suspensions in water containing 2 drops of Tween 80 (Fisher Scientific, Fairlawn, NJ, U.S.A.) per 10 ml and were homogenized in a teflon-in-glass homogenizer to reduce particle size. The dose volume used was 10 ml kg^{-1} . For subcutaneous administration, compounds were prepared in saline. For intraperitoneal injection, PGI_2 sodium salt was dissolved in ice cold pH 10 carbonate buffer immediately before use, in order to minimize spontaneous breakdown (Cho & Allen, 1978). The PGI_2 analogues, carbacyclin and iloprost, were prepared similarly.

Indomethacin was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.; paracetamol from S.B. Penick & Co., Lyndhurst, NJ, U.S.A.; PGI_2 (sodium salt) from Chemical Dynamics Corp., South Plainfield, NJ, U.S.A.; superoxide dismutase (SOD) from DDI Pharmaceuticals, Inc., Mountain View, CA, U.S.A.; catalase from Sigma. Carbacyclin and iloprost were gifts from Upjohn (Kalamazoo, MI, U.S.A.) and Schering AG (Berlin), respectively.

Animals were allocated to treatments randomly. Treatment groups were compared with controls by analysis of variance followed by Dunnett's test (Steel & Torrie, 1960), or, when homogeneity of variance problems made analysis of variance inappropriate, by multiple *t* tests with Bonferroni's adjustment (Neter & Wasserman, 1974). For comparison of proportions (e.g. % of mice writhing), Fisher's exact test was used and for assessment of the significance of the linear relationship between parameters, least squares regression was performed (Steel & Torrie, 1960).

Results

Paracetamol administered orally proved to be a very weak inhibitor of zymosan-induced writhing: significant inhibition required doses of 600 mg kg^{-1} , p.o., or above (Table 1). The doses which inhibited writhing also produced behavioural changes (decreased spontaneous motor activity, piloerection). Despite this behavioural toxicity, inhibition of writhing was obtained at non-lethal doses: over a 7-day observation period, no deaths occurred in groups of 5 mice given paracetamol at doses up to 800 mg kg^{-1} , p.o., but 3 out of 5 died after 1600 mg kg^{-1} , p.o.

High doses of paracetamol also reduced the levels of 6-keto- $\text{PGF}_{1\alpha}$ in the peritoneal lavage fluid (Table 1). Pooling data from several experiments showed that as the levels of 6-keto- $\text{PGF}_{1\alpha}$ were reduced, the number of writhes per mouse (Figure 1a) and the proportion of mice which showed a writhing response (regardless of the number of writhes) (Figure 1b) decreased. Although there was a significant ($P = 0.0006$) relationship between levels of 6-keto- $\text{PGF}_{1\alpha}$ and the number of writhes, the correlation was poor ($r = 0.4638$). However,

Table 1 Effect of oral administration of paracetamol (PA) on zymosan-induced writhing and levels of 6-keto- $\text{PGF}_{1\alpha}$ and PGE_2

PA (mg kg^{-1} , p.o.)	No. of writhes	Responders/ Total	6-keto $\text{PGF}_{1\alpha}$ (ng/mouse)	PGE_2 (ng/mouse)
0	8.6 ± 2.2	8/10	79.2 ± 10.8	0.96 ± 0.08
200	4.3 ± 1.6	6/6	58.4 ± 10.6	0.79 ± 0.17
400	4.0 ± 1.7	5/6	$41.4 \pm 4.8^*$	$0.41 \pm 0.08^*$
600	0*	0/6*	$27.7 \pm 3.5^*$	$0.41 \pm 0.09^*$

Values are mean $\pm$ s.e.mean.

Paracetamol or vehicle (water) was administered 1 h before intraperitoneal administration of zymosan (1 mg in 0.5 ml). The number of writhes per mouse was counted over the 5–20 min period following zymosan injection. The mean number of writhes per mouse (including those which failed to writhe) and the number of mice which writhed at least once (responders) are shown. Twenty minutes after zymosan injection the mice were killed and the total amount of 6-keto- $\text{PGF}_{1\alpha}$ and PGE_2 in the peritoneal lavage fluid was determined by RIA.

* $P < 0.05$ compared with vehicle-treated control.

Figure 1 6-Keto-PGF_{1α} levels and the writhing response to zymosan. (a) The total number of writhes (counted over the period 5–20 min after zymosan administration) and the levels of 6-keto-PGF_{1α} (ng/mouse) are shown for each mouse: (○) vehicle-treated; (●) paracetamol-treated (100–800 mg kg⁻¹, p.o.) (b) The proportion of mice writhing at least once, grouped according to whether peritoneal levels of 6-keto-PGF_{1α} were above or below 33 ng/mouse. The numbers at the top of the columns indicate the number of mice within that range of 6-keto-PGF_{1α} levels.

even the highest doses of paracetamol used did not lower levels of 6-keto-PGF_{1α} to the extent achieved with acidic non-steroidal anti-inflammatory agents (Doherty *et al.*, 1987). The levels of 6-keto-PGF_{1α} below which the percentage of mice writhing at least once was reduced (below 33 ng/mouse compared to above 33 ng/mouse, $P = 0.00006$, Fisher's exact test) was higher than was found in previous studies with acidic non-steroidal anti-inflammatory agents (10 ng/mouse, Doherty *et al.*, 1987). In fact, even the highest doses of paracetamol did not reduce 6-keto-PGF_{1α} levels below 10 ng/mouse. By use of GC/MS, the inhibition of 6-keto-PGF_{1α} synthesis was confirmed and no increases in the very low levels of PGE₂, PGF_{2α} or thromboxane B₂ (TxB₂) were detected (data not shown). By use of a sensitive radioimmunoassay, the levels of PGE₂ were found to fall in parallel with the levels of 6-keto-PGF_{1α} (Table 1).

In several experiments (not shown), paracetamol at 100 mg kg⁻¹ administered orally had no effect on writhing or prostaglandin levels. When paracetamol was administered locally (intraperitoneally, mixed with the zymosan), 100 mg kg⁻¹ was the lowest dose which consistently inhibited writhing (not shown and Table 2a). This dose also reduced levels of 6-keto-PGF_{1α} (Table 2a) without exhibiting behavioural toxicity. No increase in LDH levels in the lavage fluid was detected (data not shown), indicating the absence of a

direct cytotoxic effect on peritoneal cells under these conditions.

In previous studies, it was found that intraperitoneal administration of PGI₂ was able to induce writhing and to reverse completely the inhibition of writhing produced by indomethacin and other acidic prostaglandin synthesis inhibitors (Doherty *et al.*, 1987). These data have been confirmed for indomethacin in the present series of experiments (Table 3) and two PGI₂ analogues, carbacyclin and iloprost, have been shown to induce writhing when injected intraperitoneally (Table 4). Paracetamol (800 mg kg⁻¹, s.c.) was found to block partially the PGI₂ reversal of inhibition of writhing produced by indomethacin (Table 3). Different routes of administration were used for paracetamol and indomethacin to avoid possible interference of one compound on the absorption of the other. In addition, PGI₂ produced only partial reversal of the ability of paracetamol (800 mg kg⁻¹, p.o.) to inhibit writhing (Table 3).

Paracetamol administered orally at 800 mg kg⁻¹ did block carbacyclin-induced writhing while indomethacin at 1 mg kg⁻¹ p.o. did not (Table 2c). In contrast, paracetamol administered i.p. at 100 mg kg⁻¹ did not block carbacyclin-induced writhing (Table 2c).

No increase in thiobarbituric acid reactive material was detectable in lavage fluids collected immediately, 10 to 20 min after injection of zymosan, as compared to saline injected controls (not shown). The lower limit of detection in this assay is 400 ng of malonyldialdehyde equivalents/mouse. In other experiments, the zymosan was administered in a saline solution of malonyldialdehyde (400 μM) and mice were killed and lavaged either immediately or 20 min after injection. Analysis of the malonyldialdehyde levels in the lavage fluid showed 23% was recovered immediately after injection and 6% 20 min after injection (not shown).

When macrophages were removed from the peritoneal cavity of untreated mice and then stimulated with zymosan *in vitro*, a burst of lucigenin-dependent chemiluminescence was generated which peaked at about 30 min and disappeared over a 4 h period, as reported by others (Allen, 1986). The area under the curve for the 0–4 h period was used to quantify the response in subsequent studies. When superoxide dismutase (SOD) was present in the *in vitro* incubation medium, this response was inhibited by 95% (not shown). Intraperitoneal administration of SOD plus catalase (0.5 ml of saline containing 2 mg ml⁻¹ each of SOD and catalase), produced a marked reduction of *ex vivo* zymosan induced chemiluminescence measured 20 min after i.p. administration (Table 5) indicating that effective levels of the enzymes remained in the peritoneal cavity over this period of time. This dose of SOD plus catalase did not induce writhing, did not inhibit zymosan induced

Table 2 Effect of paracetamol (PA) and indomethacin on zymosan-induced writhing and 6-keto-PGF_{1α} levels and carbacyclin-induced writhing

Treatment	No. of writhes (% inhibition)	Responders/ Total	6-keto-PGF _{1α} (ng/mouse) (% inhibition)
(a) Zymosan-induced writhing			
Vehicle	16.3 ± 3.0	14/14	63.8 ± 8.8
PA 100 mg kg ⁻¹ , i.p.	0.7 ± 0.5 (96*)	2/7	23.7 ± 4.3 (63*)
(b) Carbacyclin-induced writhing			
Vehicle	6.0 ± 1.8	10/10	—
PA 100 mg kg ⁻¹ , i.p.	5.3 ± 1.4 (12)	7/10	—
(c) Carbacyclin-induced writhing			
Vehicle	4.1 ± 0.7	9/10	—
PA 800 mg kg ⁻¹ , p.o.	0.2 ± 0.2 (95*)	1/10	—
Indomethacin 1 mg kg ⁻¹ , p.o.	6.1 ± 1.5 (–49)	8/10	—

Values are mean ± s.e.mean.

* $P < 0.05$ cf. vehicle control. Percentage inhibition shown in parentheses. In (a) and (b), the zymosan (1 mg) and PA, or carbacyclin (2 μg) and PA, were administered i.p., simultaneously. In (c), the PA and indomethacin were administered one hour prior to i.p. injection of carbacyclin (2 μg).

Table 3 Effect of paracetamol (PA) on (a,b,c) the ability of PGI₂ to reverse the inhibition of zymosan-induced writhing produced by indomethacin; (d) zymosan-induced writhing in the presence and absence of PGI₂

PA (mg kg ⁻¹)	Indomethacin (mg kg ⁻¹)	PGI ₂ (i.p.)	No. of writhes	Responders/ Total
(a)				
— (Control)	—	—	3.0 ± 1.5	5/7
—	1 s.c.	—	0.3 ± 0.2*	2/7
—	1 s.c.	+	4.6 ± 1.0	6/7
800 p.o.	1 s.c.	—	0.1 ± 0.1*	1/7
800 p.o.	1 s.c.	+	0.9 ± 0.7	2/7
(b)				
— (Control)	—	—	3.3 ± 1.6	7/10
—	1 p.o.	—	0 ± 0*	0/10
—	1 p.o.	+	3.6 ± 1.4	7/10
800 s.c.	—	—	0.1 ± 0.1*	1/10
800 s.c.	1 p.o.	+	0.5 ± 0.2*	4/10
(c)				
— (Control)	1 p.o.	+	4.8 ± 2.1	7/10
800 s.c.	1 p.o.	+	1.3 ± 0.6*	5/10
(d)				
— (Control)	—	—	6.4 ± 1.1	10/10
800 p.o.	—	—	0 ± 0*	0/10
800 p.o.	—	+	1.2 ± 1.0*	2/10

Values are mean ± s.e.mean.

Zymosan was administered i.p. at 1 mg/mouse in 0.5 ml saline to all mice. PGI₂ (2 µg/mouse, i.p.) was administered 10 min after the zymosan.

* $P < 0.05$ cf. control.

(a) PA and indomethacin were administered 1 h before zymosan.

(b)(c) PA and indomethacin were administered 0.5 h and 1 h before zymosan, respectively.

(d) PA was administered 1 h before zymosan.

Table 4 Induction of writhing by intraperitoneal administration of two prostacyclin analogues, carbacyclin and iloprost

Compound	Dose (µg/mouse)	Expt. 1		Expt. 2	
		No. of Writhes	Responders/ Total	No. of Writhes	Responders/ Total
Vehicle	—	0.3 ± 0.2	3/10	0 ± 0	0/10
Iloprost	0.02	0.3 ± 0.2	2/10	0 ± 0	0/10
Iloprost	0.22	0.8 ± 1.3*	6/10	4.5 ± 1.8*	8/10
Iloprost	2	4.9 ± 0.8*	10/10	6.6 ± 1.9*	10/10
Carbacyclin	0.02	0 ± 0	0/10	—	—
Carbacyclin	0.21	0.1 ± 1.1	1/10	—	—
Carbacyclin	2	4.7 ± 1.1*	9/10	—	—

* $P < 0.05$ cf. vehicle-treated group.

Groups of 10 mice were injected intraperitoneally with 0.5 ml of carbonate buffer (pH 10), or buffer plus PGI₂ analogue. The numbers of writhes over the subsequent 30 min were counted.

Table 5 Effect of *in vivo* treatment of mouse peritoneal macrophages on *in vitro* zymosan-induced superoxide anion generation

Treatment	Dose	Superoxide generation (Mean counts ¹ (× 10 ⁴))	% inhibition
Vehicle	—	46.6 ± 12.2 (10)	—
Superoxide dismutase + Catalase	4 mg ml ⁻¹ + 4 mg ml ⁻¹	0.9* ± 0.4 (7)	98.1

Values are mean ± s.e.mean.

Groups of mice were injected intraperitoneally with 0.5 ml saline containing the indicated concentrations of enzymes. After 20 min peritoneal macrophages were collected from individual mice without washing, stimulated with zymosan (0.4 mg ml⁻¹) and superoxide generation measured by chemiluminescence. Number in parentheses = *n*.

* $P < 0.05$ cf. vehicle control.

¹ Total chemiluminescence counts.

writhing and did not alter the anti-writhing potency of paracetamol (not shown).

Discussion

It is immediately apparent that paracetamol, although effective, has very low potency in inhibiting zymosan-induced writhing. The ED₅₀ was between 400 and 600 mg kg⁻¹, p.o. while therapeutic doses in man are 5–15 mg kg⁻¹, p.o. (Flower

et al., 1985). In contrast, the ED₅₀ for indomethacin in this test (0.3 mg kg⁻¹, p.o., unpublished observations) is within the range of therapeutic doses used in man (0.3–1.5 mg kg⁻¹, p.o.) (Flower *et al.*, 1985). The high doses required in this test also produced behavioural side effects which may have indirectly interfered with the animals ability to writhe, in addition to exerting a specific antinociceptive action. Since the doses required to inhibit zymosan-induced writhing were higher than those reported for paracetamol in most animal models of inflammatory pain (Vinegar *et al.*, 1976; Winter *et al.*, 1979; Pruss *et al.*, 1980; Foote *et al.*, 1988), it seems possible that

there is some aspect of this model that makes it particularly unresponsive to paracetamol.

The doses of paracetamol which inhibited writhing also reduced the synthesis of PGI_2 , as indicated by the reduced levels of immunoreactive 6-keto- $\text{PGF}_{1\alpha}$. GC/MS analysis confirmed the RIA data and indicated that arachidonic acid, the precursor of PGI_2 , had not been shunted into the other cyclo-oxygenase products examined. This indicates that paracetamol inhibited cyclo-oxygenase itself rather than one of the endoperoxide isomerases. As with the NSAIDs (Doherty *et al.*, 1987), there was a significant correlation between the levels of 6-keto- $\text{PGF}_{1\alpha}$ and both the proportion of mice that writhed at least once, and with the number of writhes per mouse. However, the level of 6-keto- $\text{PGF}_{1\alpha}$ below which the proportion of responders decreased was higher than that noted previously for the NSAIDs. In fact, even the highest doses of paracetamol used did not lower the levels of 6-keto- $\text{PGF}_{1\alpha}$ to the extent found to be associated with inhibition of writhing by acidic non-steroidal anti-inflammatory agents. This difference in threshold could be due to the ability of the behavioural toxicity of paracetamol to suppress writhing, independently of 6-keto- $\text{PGF}_{1\alpha}$ levels.

As has been reported previously (Doherty *et al.*, 1987), PGI_2 , injected i.p., can both induce writhing and reverse the ability of indomethacin to inhibit zymosan-induced writhing. Carbacyclin (as already reported by Smith *et al.*, 1985) and iloprost, two stable agonists at the PGI_2 receptor, also induced writhing when injected i.p., supporting the suggestion that PGI_2 is involved in inflammatory pain in the mouse peritoneal cavity.

Several other lines of evidence support the suggestion that oral doses of paracetamol inhibit zymosan-induced writhing by a mechanism in addition to lowering PGI_2 levels: (1) Oral administration of paracetamol, in contrast to the NSAID tested previously (Doherty *et al.*, 1987), partially blocked the ability of PGI_2 to reverse inhibition of zymosan-induced writhing produced by indomethacin. (2) Intraperitoneal administration of PGI_2 failed to reverse the ability of paracetamol to inhibit zymosan-induced writhing. (3) Oral administration of paracetamol (800 mg kg^{-1}), but not indomethacin (1 mg kg^{-1} , p.o.), inhibited carbacyclin-induced writhing.

It is, however, noteworthy that 100 mg kg^{-1} paracetamol administered i.p. inhibited writhing and reduced 6-keto- $\text{PGF}_{1\alpha}$ levels without producing behavioural toxicity. This activity cannot be attributed to antagonism at the prostacyclin receptor since the same dose of paracetamol administered i.p. failed to block writhing induced by i.p. injection of carbacyclin. Therefore, local inhibition of PGI_2 synthesis in the

peritoneal cavity by paracetamol can clearly inhibit writhing without involving behavioural toxic effects.

Since the *in vitro* potency of paracetamol, as an inhibitor of prostaglandin synthesis, is regulated by levels of peroxides in the reaction medium (Lands *et al.*, 1976; Robak *et al.*, 1978; Lands & Hanel, 1982; Hertz & Cloarec, 1984), attempts were made to measure levels of lipid peroxides in the peritoneal cavity. However, a standard assay method failed to detect lipid peroxides, possibly because of rapid elimination as was demonstrated here and has been discussed by Salaris & Babbs (1988). An alternative approach was, therefore, investigated. Zymosan activates the oxidative burst in leukocytes and the superoxide anion generated can be converted to highly reactive species which may generate lipid peroxides (reviewed in Chow, 1988; Marshall & Lands, 1986). It therefore seems reasonable to suppose that the oxidative burst could be a major source of lipid peroxides in zymosan-induced peritonitis and attempts were made to regulate this phenomenon.

One means of regulating the consequences of the oxidative burst is to eliminate the superoxide anion generated. This was achieved by local *in vivo* administration of a mixture of SOD and catalase. However, despite 98% reduction in detectable superoxide, no inhibition of writhing or modification of the potency of paracetamol was seen. Therefore, there is no evidence to implicate the oxidative burst in determining the antinociceptive potency of paracetamol in this model.

Overall, the data presented here are consistent with the suggestion that inhibition by paracetamol of PGI_2 synthesis in the peritoneal cavity is responsible for only a part of its antinociceptive activity in this test. However, the extremely high oral doses required produce behavioural toxicity which clearly contributes to the inhibition of writhing. The large difference in potency between the mouse and man suggests that in man, paracetamol may have additional mechanisms of action, not represented in this mouse model. One possible mechanism is inhibition of CNS prostaglandin synthesis triggered by release of interleukin-1 or other cytokines from the inflammatory lesion, as previously suggested (Doherty, 1987). In the acute model used here (20 min duration) there is insufficient time for synthesis and release of cytokines, consistent with the absence of a febrile response (data not shown). It seems unlikely that the low potency of paracetamol in this model is due to high levels of lipid peroxide generated from the oxidative burst, although other sources of lipid peroxides may be present. In particular, paracetamol itself has been shown to generate lipid peroxides when it is metabolized (Van De Straat *et al.*, 1988) and these could be a factor at the high doses used here.

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