

Role of prostaglandins in tumour necrosis factor induced weight loss

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Summary Administration of either tumour necrosis factor alpha (TNF- α) or 16,16-dimethylprostaglandin E₂ (PGE₂) to female NMRI mice caused a decrease in body weight accompanied by a reduction in both food and water intake and a decrease in carcass water content. A single injection of TNF- α caused an enhanced production of PGE₂ by spleen cells from treated animals, that was significant within 1 h of treatment, and persisted until at least 6 h. These results suggest that the anorectic effect of TNF- α may be mediated by a prostaglandin intermediate. Indomethacin (10 mg kg⁻¹) administered 2 h before TNF- α (7.5 × 10⁷ U kg⁻¹) caused a significant reduction in the extent of weight loss and inhibited PGE₂ production. Administration of indomethacin 0.5–1.5 h before the TNF- α had no significant effect on loss of body weight, but still inhibited PGE₂ production. Also PGE₂ production was still enhanced in response to TNF- α administered chronically, despite the inability of prolonged TNF- α administration to produce continued loss of body weight. These results suggest that prostaglandins are not involved in the anorectic effect of TNF- α .

The catabolic states associated with infection or endotoxemia have been attributed to the production by phagocytic cells of soluble proteins such as interleukin 1 and cachectin (Rouzer & Cerami, 1980; Moldawer *et al.*, 1987a; Cerami *et al.*, 1985). A high degree of homology has been shown to exist between the N-terminal sequence of mouse cachectin and the N-terminal sequence for human tumour necrosis factor-alpha (TNF- α) (Beutler *et al.*, 1985) and the catabolic states have been extended to include also cancer-associated cachexia (Beutler & Cerami, 1986). Severe weight loss and increased mortality have been observed in mice bearing transgenic tumours that persistently secrete human cachectin (Oliff *et al.*, 1987) and chronic administration of sublethal doses of TNF- α to rats caused anorexia, weight loss, depletion of body lipid and protein, a reduction of red blood cell mass, leukocytosis and tissue inflammation (Tracey *et al.*, 1988). However, in a number of studies (Stovroff *et al.*, 1988; Mahony & Tisdale, 1988) administration of TNF- α caused a loss in body weight accompanied by a drop in food and water intake, which was only apparent over the first 24 h, after which animals became resistant to subsequent dosing. In addition TNF- α has not been detectable in the serum of patients with clinical cancer cachexia (Socher *et al.*, 1988) and in clinical trials of recombinant human TNF- α there was no clinical evidence of accelerated cachexia, although anorexia was present during administration (Sherman *et al.*, 1988). Also the effects on host metabolism produced by TNF- α appear to differ from that produced by a cachexia-inducing tumour (Mahony *et al.*, 1988).

The toxic and metabolic effects of TNF- α can be blocked by a single injection of the cyclo-oxygenase inhibitors indomethacin or ibuprofen before the TNF- α treatment (Kettlehut *et al.*, 1987). This suggests that some of the effects of TNF- α may be mediated through a prostaglandin intermediate in analogy with septic shock where large increases in circulatory prostaglandins have been reported in a variety of experimental models (Cook *et al.*, 1980). In order to study the role of prostaglandins in the mechanism of weight loss induced by TNF- α we have used NMRI mice, a strain we have utilised to passage a colon adenocarcinoma which induces cachexia in recipient animals (Mahony *et al.*, 1988).

Materials and methods

Animals

Pure strain female NMRI mice (age 6–8 weeks) were purchased from Banting and Kingman (Hull, UK) and were

fed *ad lib* a rat and mouse breeding diet (Pilsbury's Birmingham, UK). All animals were given free access to food and water and both food and water intake were monitored daily.

TNF

Human recombinant TNF- α (6 × 10⁷ U mg⁻¹) was kindly donated by Boehringer Ingelheim Ltd (Bracknell, Berks, UK) and was stored at 4°C. The endotoxin content was less than 0.125 EU ml⁻¹. Fresh solutions of TNF- α were made up daily in 0.9% NaCl and 200 μ l of the appropriate concentration (7.5 × 10⁷ U kg⁻¹) was injected into the tail veins of female NMRI mice (19–22 g). Controls were injected with 200 μ l of 0.9% NaCl. Body weights and food and water intake were monitored at the same time each day.

Body water content

Carcasses were heated at 80°C until a constant weight was achieved. The carcasses were then reweighed and the water content was determined from the difference between the wet and dry weights.

Indomethacin administration

Fresh solutions of indomethacin (2 mg ml⁻¹) in arachis oil containing 10% DMSO were made up daily and 10 mg kg⁻¹ were injected i.p. into female NMRI mice (19–22 g) 0.5–2 h before TNF- α administration (7.5 × 10⁷ U kg⁻¹ i.v.). Controls were injected with arachis oil containing 10% DMSO 2 h before 0.9% NaCl administration (200 μ l, i.v.). Body weights and food and water intake were monitored over a 24 h period and body composition analysis was performed. Urine and faeces production was measured by placing animals in metabolic cages throughout the experiment.

Prostaglandin E₂ administration

Fresh solutions of 16,16-dimethyl PGE₂ (0.125 mg ml⁻¹) dissolved in triolein were made up daily in 0.9% NaCl and were administered i.p. (0.5 mg kg⁻¹) at 6 h intervals (11 a.m., 5 p.m., 11 p.m.) into female NMRI mice (19 ± 1 g). Controls were injected with 200 μ l of 0.9% NaCl containing triolein. Body weights and food and water intake were monitored over a 24 h period and body water content was determined. Mice were placed in metabolic cages throughout the experiment and the urine and faeces production was determined.

PgE₂ analysis

Male NMRI mice (20–26 g) were injected i.v. with 7.5×10^7 U kg⁻¹ TNF- α or 0.9% NaCl. At specified time points after injection animals were killed by cervical dislocation and the spleens rapidly removed and weighed. Spleens were sliced on filter paper moistened with cold 0.85% NaCl and then placed in a 25 ml flask containing 2 ml of Krebs-Ringer bicarbonate medium containing 1 mg ml⁻¹ each of glucose and bovine serum albumin. The slices were first incubated at 37°C for 20 min in a gas phase of 5% CO₂/95% N₂ and then transferred to flasks containing 5% CO₂/95% O₂ for a further 15 min. At the end of the incubation spleens were removed and the medium was immediately frozen at -196°C and only defrosted immediately before extraction.

For the determination of PgE₂ 1 ml of thawed medium was removed, adjusted to pH 3 to 3.5 with 2N HCl and extracted twice with 3 ml of ethyl acetate. The extract was evaporated to dryness under a stream of nitrogen and dissolved in 0.025 M phosphate buffer, pH 6.8, containing 0.01 M EDTA, 0.9% NaCl, 0.3% bovine γ -globulin, 0.005% triton \times 100 and 0.05% sodium azide and the concentration of PgE₂ was determined using a radioimmunoassay (NEN, Dreieich, FR Germany).

Statistical analysis

All results were analysed statistically using the analysis of variance or F ratio.

Results

We have utilised PgE₂ production by spleen cells rapidly removed from TNF- α treated animals as an indirect method of measuring prostaglandin production because of difficulties in measuring plasma levels of PgE₂ directly. The radioimmunoassay utilised for these measurements had not previously been tested on mouse plasma and our investigations revealed an inhibition of the binding of PgE₂ from mouse plasma with the antibody used in the assay. The results presented in Figure 1 show an enhanced production of PgE₂ by spleen cells after administration of TNF- α (7.5×10^7 U kg⁻¹), which was significantly greater than saline infused controls within 1 h after administration, and remained elevated up to 6 h after treatment. This suggests

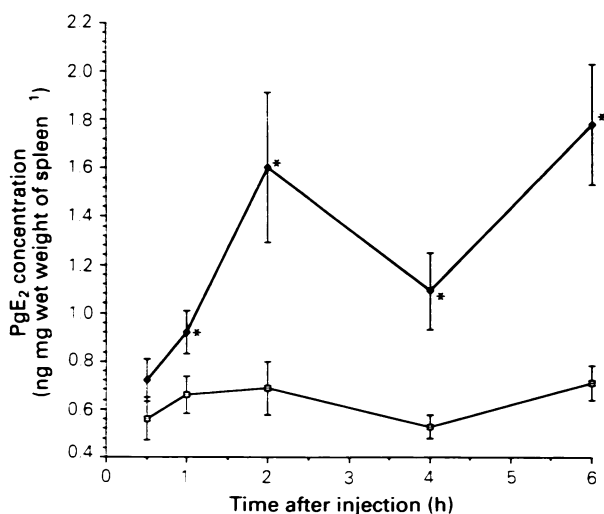


Figure 1 Effect of a single i.v. injection of TNF- α (7.5×10^7 U kg⁻¹) on PgE₂ production by spleen cells. Spleens were rapidly removed from control (□) and TNF- α (◆) treated animals and PgE₂ production *in vitro* was determined by a radioimmunoassay. The values represent means \pm s.e.m. for 5–6 animals. * $P \leq 0.001$ from controls.

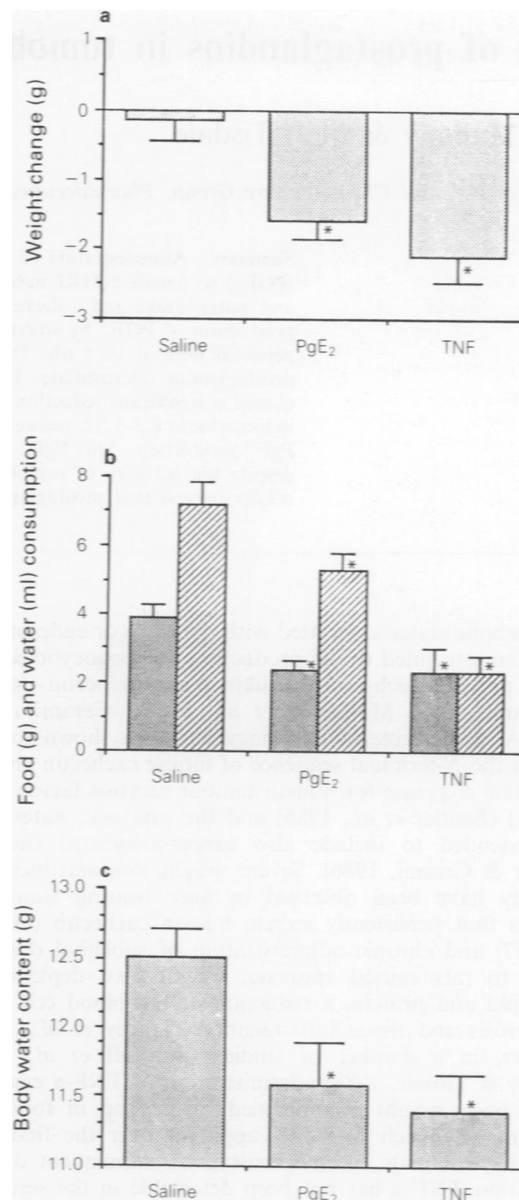


Figure 2 Effect of TNF- α and 16,16-dimethyl PgE₂ administration on body weight change (a), food (▨) and water (▩) consumption (b) and body water content (c). Female NMRI mice (19.0 ± 1.0 g) were given either a single i.v. injection of TNF- α (7.5×10^7 U kg⁻¹) or dimethyl PgE₂ (0.5 mg kg⁻¹) administered i.p. at three 6-hourly intervals and the parameters were measured as described in methods. The values represent means \pm s.e.m. for nine animals. * $P \leq 0.001$ from controls.

that some of the metabolic effects of TNF- α may be mediated via prostaglandin production.

Administration of TNF- α (7.5×10^7 U kg⁻¹) produced a decrease in body weight within the first 24 h after administration (Figure 2a), accompanied by a decrease in food and water intake (Figure 2b). Body composition analysis revealed a decrease in the body water content of TNF- α treated mice, when compared with saline infused controls (Figure 2c). Essentially similar results were obtained after administration of the stable PgE₂ analogue 16,16-dimethyl PgE₂ at a dose of 0.5 mg kg⁻¹ administered i.p. three times daily. No effect was seen after a dose of 1.25 mg kg⁻¹ administered as a single i.v. injection. Thus after 24 h PgE₂ treated animals showed a reduction in body weight (Figure 2a) which was similar to that produced by TNF- α in that it was accompanied by a reduction in both food and water intake (Figure 2b) and a decrease in the body water content (Figure

Table I Effect of TNF- α and indomethacin alone and in combination on body weight, food and water intake and PgE₂ production by spleen cells

Treatment	Body weight change (g)	Food intake (g)	Water intake (ml)	PgE ₂ (ng mg ⁻¹ wet weight)
Controls, i.v. saline	0.00 ± 0.17	4.39 ± 0.26	7.04 ± 0.46	0.69 ± 0.11
i.v. saline + 10 mg kg ⁻¹ indomethacin	+0.19 ± 0.13 ^d	4.89 ± 0.22	5.77 ± 0.36	0.17 ± 0.41
TNF- α (7.5 × 10 ⁷ U kg ⁻¹)	-1.35 ± 0.14 ^a	2.30 ± 0.16 ^a	3.40 ± 0.33 ^a	1.60 ± 0.31 ^a
TNF- α (7.5 × 10 ⁷ U kg ⁻¹) + 10 mg kg ⁻¹ indomethacin 30 min before TNF- α	-1.63 ± 0.19	1.63 ± 0.49	4.5 ± 0.33	0.44 ± 0.17 ^c
TNF- α (7.5 × 10 ⁷ U kg ⁻¹) + 10 mg kg ⁻¹ indomethacin 1.5 h before TNF- α	-1.23 ± 0.54	1.98 ± 0.46	5.75 ± 0.36	0.53 ± 0.47 ^c
TNF- α (7.5 × 10 ⁷ U kg ⁻¹) + 10 mg kg ⁻¹ indomethacin 2 h before TNF- α	-1.53 ± 0.11 ^{b,c}	2.76 ± 0.17 ^{e,f}	3.91 ± 0.41 ^f	0.43 ± 0.27 ^c

Results represent means ± s.e.m. for 5–16 animals for each group.

^a*P* ≤ 0.001 from controls; ^b*P* ≤ 0.005 from controls; ^c*P* ≤ 0.001 from TNF- α alone; ^d*P* ≤ 0.001 from TNF- α /indomethacin 2 h; ^e*P* ≤ 0.05 from TNF- α ; ^f*P* ≤ 0.001 from indomethacin 2 h.

2c). These results suggest that the effect of TNF- α on body weight may be mediated via a prostaglandin intermediate, and that it may be possible to reverse the effects by the inhibition of prostaglandin synthesis.

Administration of indomethacin (10 mg kg⁻¹) 2 h before a single injection of TNF- α (7.5 × 10⁷ U kg⁻¹) caused a significant reduction in the TNF- α induced weight loss (Table I). The time of administration of indomethacin

appeared to be critical since no weight reversal was observed 0.5 or 1.5 h before the TNF- α (Table I). When compared with indomethacin treated controls the decrease in water intake in the 2 h indomethacin/TNF- α treated mice (32%) was not as great as in the TNF- α treated mice compared with saline infused controls (52%), although the food intake was reduced to about the same extent in both cases (44% and 48% respectively, Table I) compared with the respective controls. There was a significant increase in food consumption of the TNF- α /indomethacin treated mice when compared with TNF- α treatment alone, and body composition analysis showed an increase in the total body water content of the TNF- α /indomethacin group when compared with indomethacin alone (Figure 3). This difference was not explained by decreased excretion of urine (Table II). Animals treated with TNF- α had a significant reduction in the excretion of both urine and faeces, indicating that the decrease in body water content did not arise from a diuretic effect of TNF- α . 16,16-Dimethyl PgE₂ also caused a reduction in urine and faeces production (Table II). Indomethacin had no effect on urine or faeces production in control animals, although there was a small increase in faeces production in TNF- α treated animals.

The effect of indomethacin on PgE₂ production by spleen cells from TNF- α treated animals is shown in Table I. All values were measured 2 h after TNF- α administration since PgE₂ production in response to TNF- α was significantly elevated at this point (Figure 1). Indomethacin inhibited PgE₂ production in response to PgE₂ irrespective of the time of administration with respect to TNF- α , although the reversal of body weight loss was highly dependent on the time of administration (Table I). This suggests that indomethacin did not reverse the TNF- α induced weight loss as a result of inhibition of prostaglandin synthesis and that prostaglandin production was not necessary for weight loss to occur.

This conclusion was also substantiated by measurement of

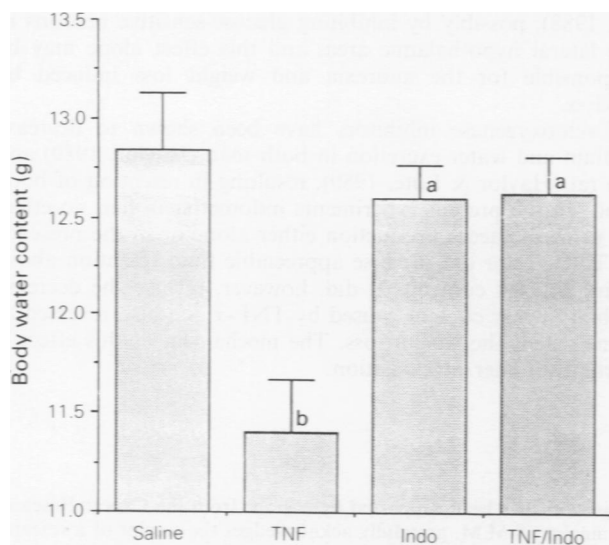


Figure 3 Effect of indomethacin on the body water content of TNF- α treated mice. Animals were given either 0.9% NaCl i.v., 7.5 × 10⁷ U kg⁻¹ TNF- α i.v., 10 mg kg⁻¹ indomethacin i.p. 2 h before 0.9% NaCl i.v., or 10 mg kg⁻¹ indomethacin i.p. 2 h before 7.5 × 10⁷ U kg⁻¹ TNF- α i.v. The values represent mean ± s.e.m. for seven animals. ^{*}*P* ≤ 0.001 from TNF- α treated animals. [†]*P* ≤ 0.001 from saline injected controls.

Table II Effect of TNF- α , indomethacin and 16,16-dimethyl PgE₂ on excretion of urine and faeces

Treatment	Urine volume (ml)	Wet faeces weight (g)	Dry faeces weight (g)	Total fluid excretion (ml)
Controls, i.v. saline	1.28 ± 0.3	2.13 ± 0.9	1.15 ± 0.4	2.05 ± 0.34
10 mg kg ⁻¹ indomethacin	0.97 ± 0.2	2.24 ± 0.3	1.18 ± 0.2	2.00 ± 0.30
TNF- α (7.5 × 10 ⁷ U kg ⁻¹)	0.44 ± 0.2 ^b	0.50 ± 0.08 ^a	0.32 ± 0.06 ^b	0.62 ± 0.16 ^c
TNF- α (7.5 × 10 ⁷ U kg ⁻¹) + 10 mg kg ⁻¹ indomethacin	0.66 ± 0.2	0.80 ± 0.13 ^{d,e}	0.51 ± 0.08 ^{d,e}	0.95 ± 0.26 ^d
Dimethyl PgE ₂ , 0.5 mg kg ⁻¹ 3 × daily	0.75 ± 0.2	0.97 ± 0.2 ^a	0.34 ± 0.11 ^b	1.38 ± 0.12 ^a

Results are expressed as means ± s.e.m. for 6–7 animals per group.

^a*P* ≤ 0.05 from saline controls; ^b*P* ≤ 0.005 from saline controls; ^c*P* ≤ 0.001 from saline controls; ^d*P* ≤ 0.001 from indomethacin controls; ^e*P* ≤ 0.005 from TNF- α alone.

PgE₂ production by spleen cells when TNF- α was administered chronically. As previously reported (Mahony & Tisdale, 1988), animals become resistant to subsequent injections of TNF- α after the first 24 h with the body weight increasing towards that of controls. At 24 h PgE₂ production by spleen cells was $0.51 \pm 0.04 \text{ ng mg}^{-1}$ wet weight in controls and $1.98 \pm 0.16 \text{ ng mg}^{-1}$ wet weight in TNF- α treated animals, i.e. 3.9 times the control value. This difference was maintained up to 5 days of TNF- α administration (3.7 times the control value) despite the fact that the animals were gaining weight. This suggests that PgE₂ production is not involved in the weight loss produced by TNF- α .

Discussion

There is some evidence to suggest that prostaglandins may be involved in the metabolic effects of TNF- α . An increase in plasma prostaglandin levels has been observed within 1 h of TNF- α administration accompanied by a sharp fall in body temperature and blood glucose levels (Kettlehut *et al.*, 1987). Inhibition of prostaglandin production prevented the hypothermia and changes in blood glucose. Production of PgE₂ is also enhanced after stimulation of mouse osteoblast-like cells (Sato *et al.*, 1987) and endothelial cells (Dayer *et al.*, 1985) with TNF- α . Both TNF- α and interleukin 1 have been shown to stimulate the production of PgE₂ by isolated extensor digitorum longus muscles (Moldawer *et al.*, 1987a). An enhanced release of arachidonic acid by TNF- α from human synovial cells arises by stimulation of phospholipase A₂ and possibly phospholipase C activity (Godfrey *et al.*, 1987). In the present study an enhanced production of PgE₂ was observed in isolated spleen cells taken from TNF- α treated animals, which was significantly greater than saline injected controls within 1 h of treatment. This suggests that PgE₂ may serve as an intermediate for TNF- α effects, although PgE₂ has also been shown to inhibit TNF- α production by macrophages (Kunkel *et al.*, 1988), suggesting a fine control for the regulation of TNF- α production.

A single injection of TNF- α causes a characteristic weight loss consisting of a reduction in food and water intake and a decreased carcass water content. This suggests that at least some of the short-term weight loss associated with TNF- α may be due to dehydration. This appears not to arise from an increased fluid output since TNF- α treated animals excrete significantly less fluid than controls.

The stable prostaglandin E₂ analogue, 16,16-dimethyl PgE₂, produces weight loss in NMRI mice, which is similar to that produced by TNF- α in that it is accompanied by both hypophagia and a decrease in water intake. Body composition analysis shows a similar decrease in total body water in PgE₂ treated mice as in TNF- α treated mice.

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- Indomethacin administration decreases both the weight loss and dehydration after TNF- α , suggesting that the anorectic effect is mediated through a prostaglandin intermediate. However, Marquet *et al.* (1987) showed that, although indomethacin administered before murine TNF- α alleviated the toxic side effects in rats, it had no effect on the excessive wasting produced by high doses of TNF- α . The reason for this disparity is not immediately clear, but could be related to differences between murine and human TNF- α , or to the timing of the indomethacin administration, which in our case required a period of 2 h between indomethacin and TNF- α .
- Despite this stringent time requirement for reversal of weight loss, indomethacin was equally effective in inhibiting PgE₂ production after TNF- α administration at all times from 0.5 to 2 h before TNF- α . These results suggest that prostaglandins are not involved in the weight loss induced by TNF- α .
- This is further confirmed by measurement of PgE₂ production after chronic administration of TNF- α , when the animals become resistant to subsequent injections of TNF- α after the first 24 h (Mahony & Tisdale, 1988). The nature of this tachyphylaxis is not understood at present, but can be overcome by increasing dosage to maintain a constant food intake (Tracey *et al.*, 1988). Despite the inability of repeated treatment of TNF- α to produce continued weight loss it appeared to be equally effective in stimulating spleen PgE₂ production up to 5 days of treatment. This again confirms that prostaglandins are not involved in the anorectic effect of TNF- α . Intracerebroventricular microinfusion of TNF- α has been shown to suppress food intake in rats (Plata-Salaman *et al.*, 1988), possibly by inhibiting glucose-sensitive neurons in the lateral hypothalamic area, and this effect alone may be responsible for the anorexia and weight loss induced by TNF- α .
- Cyclooxygenase inhibitors have been shown to decrease sodium and water excretion in both man (Haylor, 1980) and the rat (Haylor & Lote, 1980), resulting in retention of body fluid. In the present experiments indomethacin had no effect on urine or faeces production either alone or in the presence of TNF- α , nor did it cause appreciable fluid retention above saline infused controls. It did, however, reverse the decrease in body water content caused by TNF- α and also reversed to some extent the weight loss. The mechanism of this effect is currently under investigation.

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