Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 498 (2004) 295–301

www.elsevier.com/locate/ejphar

15*d*-prostaglandin J_2 reduces multiple organ failure caused by wall-fragment of Gram-positive and Gram-negative bacteria

Laura Dugo[a,b], Marika Collin[a], Salvatore Cuzzocrea[b], Christoph Thiemermann[a,*]

[a]*Department of Experimental Medicine, Nephrology and Critical Care Medicine, The William Harvey Research Institute, St. Bartholomew's and The Royal London School of Medicine and Dentistry, Charterhouse Square, London EC1M 6BQ, UK*
[b]*Department of Experimental Medicine and Pharmacology, School of Medicine, Torre Biologica Policlinico Universitario, University of Messina, 98100 Messina, Italy*

Received 8 July 2004; accepted 13 July 2004
Available online 20 August 2004

Abstract

Septic shock is still the major cause of death in surgical intensive care units. Both gram-positive (G+) and gram-negative (G−) bacteria have been isolated in the blood of a large portion of septic patients, and these polymicrobial infections often have a higher mortality than infections due to a single organism. Cell wall fragments from G+ and G− bacteria synergise to cause shock and multiple organ dysfunction in vivo (G+/G− shock).

Male Wistar rats were anaesthetised and received a coadministration of wall fragments from G+ and G− bacteria, *Staphilococcus aureus* (*S. aureus*) peptidoglycan [0.3 mg/kg, intravenously (i.v.)] and *Escherichia coli* (*E. coli*) lipopolysaccharide (1 mg/kg, i.v.) or vehicle (saline, 1 ml/kg, i.v.). G+/G− shock for 6 h resulted in an increase in serum levels of creatinine (indicator of renal dysfunction), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (γ-GT), bilirubin (markers for hepatic injury and dysfunction) and creatine kinase (CK, an indicator of neuromuscular, skeletal muscle or cardiac injury). Pretreatment of rats with the peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist 15*d*-prostaglandin J_2 (0.3 mg/kg, i.v., 30 min prior to G+/G−) reduced the multiple organ injury/dysfunction caused by coadministration of peptidoglycan+lipopolysaccharide. The selective PPAR-γ antagonist GW9662 (2-Chloro-5-nitrobenzanilide) (1 mg/kg, i.v., given 45 min prior to G+/G−) abolished the protective effects of 15*d*-prostaglandin J_2. 15*d*- prostaglandin J_2 did not affect the biphasic fall in blood pressure or the increase in heart rate caused by administration of peptidoglycan+lipopolysaccharide. The mechanism(s) of the protective effect of this cyclopentenone prostaglandin are—at least in part—PPAR-γ dependent, as the protection afforded by 15*d*-prostaglandin J_2 was reduced by the PPAR-γ antagonist GW9662. We propose that 15*d*-prostaglandin J_2 or other ligands for PPAR-γ may be useful in the therapy of the organ injury associated with septic shock.

Keywords: Peroxisome-proliferator activated receptor-γ; Lipopolysaccharide; Peptidoglycan; GW9662; (Rat)

1. Introduction

There is evidence that the mortality is substantially higher in septic patients with mixed bacterial infections (polymicrobial infections: 63%) than in those with septic shock caused by only one bacterium (unimicrobial infections: 38%). Most polymicrobial infections comprise of both gram-positive (G+) and gram-negative (G−) bacteria (Weinstein et al., 1983). Interestingly, the morbidity and mortality of intraabdominal infections is significantly greater in the presence of G+ enterococci, although these organisms are conventionally considered to be weak pathogens (Burnett et al., 1995). Experimental evidence suggests that wall-fragments of these organisms synergise with others, including G− bacteria, to cause peritonitis and shock (Matlow et al., 1989).

The cell wall of G+ bacteria contains lipoteichoic acid and peptidoglycan. Lipoteichoic acid is a macroamphiphile

* Corresponding author: Tel.: +44 207 882 6118; fax: +44 207 251 1685.
E-mail address: C.Thiemermann@qmul.ac.uk (C. Thiemermann).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.07.074

(as lipopolysaccharide is for G− bacteria) containing a substituted polyglycero-phosphate backbone attached to a glycolipid (Fischer, 1988). Peptidoglycan is a large polymer that provides stress resistance and shape-determining properties to bacterial cell walls. Several reports demonstrate that lipoteichoic acid and peptidoglycan act in synergy to release tumour necrosis factor-α and interferon-γ, to induce nitric oxide synthase (iNOS) and to cause shock and multiple organ failure in anaesthetized rats and in human whole blood (De Kimpe et al., 1995; Kengatharan et al., 1998; Thiemermann, 2002; Wang et al., 2000). Another mechanism by which some G+ bacteria cause sepsis is by the production of exotoxins that enter the circulation. We have reported recently that peptidoglycan from either pathogenic or nonpathogenic bacteria can also synergise with lipopolysaccharide to cause release of inflammatory mediators, shock and multiple organ injury/dysfunction in vivo (Wray et al., 2001). Thus, models of shock employing wall-fragments of both G+ and G− bacteria may be suitable to mimic the pathophysiology of septic shock caused by mixed bacterial infections.

The cyclopentenone prostaglandin J_2 is formed by dehydration within the cyclopentenone ring of the endogenous prostaglandin D_2. Prostaglandin J_2 is metabolised further to yield Δ^{12}-prostaglandin J_2 and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2. Several members of the cyclopentenone family of prostaglandins possess antiviral, antineoplastic and antiinflammatory properties (Straus and Glass, 2001).

15*d*-prostaglandin J_2 is a ligand for a peroxisome proliferator-activated receptor-γ (PPAR-γ), a nuclear hormone receptor, which regulates gene expression by heterodimerising with the retinoid X receptor. Binding of the activated heterodimer to the promotor region of specific target genes results in either the activation or suppression of the target gene. Various PPAR-γ ligands have been reported to possess antiinflammatory properties in vitro (Jiang et al., 1998) and in vivo (see below). It is possible that PPAR-γ down-regulates the expression of proinflammatory mediators at the transcriptional level by inhibiting the activation of the transcription factors nuclear factor kappa B (nuclear factor-κB) signal transducer and activator of transcription-1 and/or activated protein-1 signalling (Ricote et al., 1998).

Other activities of the cyclopentenone prostaglandins are mediated by the reactive α,β-unsaturated carbonyl group located in the cyclopentenone ring. For instance, 15*d*-prostaglandin J_2 attenuates the activation of nuclear factor-κB by preventing the phosphorylation of its inhibitor protein by inhibitor kinase kinase (IKK; Rossi et al., 1997). It is now widely accepted that 15*d*-prostaglandin J_2 attenuates the nuclear factor-κB-mediated transcriptional activation of many proinflammatory genes by PPAR-γ-dependent and -independent mechanisms (Straus and Glass, 2001). For instance, 15*d*-prostaglandin J_2 attenuates the formation of the cytokines tumour necrosis factor-α and interleukin-12 (Drew and Chavis, 2001), the expression of the adhesion molecules vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1; Pasceri et al., 2000), and the expression of the inducible proinflammatory proteins, cyclooxygenase-2, cytosolic phospholipase A_2 (Tsubouchi et al., 2001) and iNOS (Ricote et al., 1998; Colville-Nash et al., 1998). There is, however, also evidence that 15*d*-prostaglandin J_2 may enhance the formation of the proinflammatory chemokine interleukin-8 in human macrophages/monocytes stimulated with endotoxin in a PPAR-γ-dependent fashion (Zhang et al., 2001). 15*d*-Prostaglandin J_2 also induces the expression of cytoprotective heat shock proteins (Santoro, 2001). Heat shock protein 72 has recently been shown to play a key role in the resolution of the inflammatory process (Ianaro et al., 2001).

The following studies support the view 15*d*-prostaglandin J_2 exerts potent antiinflammatory effects in vivo: 15*d*-prostaglandin J_2 and the PPAR-γ ligand troglitazone reduce the degree of inflammation associated with adjuvant-induced arthritis in female Lewis rats (Kawahito et al., 2000). 15*d*-Prostaglandin J_2 also reduces the tissue injury associated with ischemia–reperfusion of the heart (Wayman et al., 2002) and with acute and chronic inflammation (Cuzzocrea et al., 2002). In addition, 15*d*-prostaglandin J_2 attenuates the development of the colon injury caused by dinitrobenzene sulphonic acid in the rat (Cuzzocrea et al., 2003). 15*d*-Prostaglandin J_2 may, thus, be useful in the therapy of acute and chronic inflammation.

In this study, we report the effects of 15*d*-prostaglandin J_2 on the

(i) renal dysfunction,
(ii) liver injury and dysfunction and
(iii) neuromuscular injury

caused by coadministration of cell wall fragments from G+ and G− bacteria in the rat.

GW9662 (2-Chloro-5-nitrobenzanilide) is a specific PPAR-γ antagonist, with a nanomolar IC_{50} (Leesnitzer et al., 2002), which inhibits the PPAR-γ-mediated suppression by interleukin-4 of osteoclast formation (Bendixen et al., 2001). GW9662 also abolishes the inhibition of osteoprotegerin gene expression in human aortic smooth muscle cells afforded by PPAR-γ activation (Fu et al., 2002). In this study, we have used GW9662 to investigate the role of PPAR-γ in any of the observed effects of 15*d*-prostaglandin J_2 in vivo.

Hence, this study was designed to elucidate the effects of 15*d*-prostaglandin J_2 on the

(i) renal dysfunction,
(ii) liver injury and dysfunction and
(iii) neuromuscular injury caused by G+/G− in the rat,

and to investigate the role of PPAR-γ in these effects by using the specific PPAR-γ receptor antagonist GW9662.

2. Methods

2.1. Surgical procedure

This study was carried out on 38 male Wistar rats (Tuck, Rayleigh, Essex, UK) weighing 240–340 g, receiving a standard diet and water ad libitum. The investigation was performed in accordance with the Home Office *Guidance on the Operation of the Animals (Scientific Procedures) Act 1986*, published by HMSO, London. All animals were anaesthetised with thiopentone sodium [Intraval®, 120 mg/kg, intraperitoneally (i.p.)], and anesthesia was maintained by supplementary injections of thiopentone sodium [approximately 1–2 mg/kg/h, intravenously (i.v.)] as required. The trachea was cannulated to facilitate respiration and rectal temperature was maintained at 37±1 °C with a homeothermic blanket. The right carotid artery was cannulated and connected to a pressure transducer (Senso-Nor 840, Horten, Norway) for the measurement of phasic and mean arterial blood pressure and heart rate, which were displayed on a data acquisition system (Powerlab® Version 4.0.4, AD Instruments, Hastings, UK) installed on a Dell Dimension 4100 personal computer. The jugular vein was cannulated for the administration of drugs and fluid. The bladder was also cannulated to collect urine. Upon completion of the surgical procedure, arterial blood pressure and heart rate were allowed to stabilize for 10 min.

2.2. Experimental design

Animals were assigned to four experimental groups:

(1) Sham Group (Sham). Rats were treated with 10% dimethyl sulfoxide (1 ml/kg, i.v., n=10) without causing G+/G− shock (received saline rather than peptidoglycan+lipopolysaccharide).
(2) G+/G− shock Control Group (Control). Rats were treated with 10% dimethyl sulfoxide (1 ml/kg, i.v., n=11) 30 min prior to administration of *Staphilococcus aureus* peptidoglycan (0.3 mg/kg, i.v.) and *Escherichia coli* lipopolysaccharide (1 mg/kg, i.v., serotype 0127:B8), which were given slowly over 10 min.
(3) G+/G− shock 15d-prostaglandin J_2 Group (15d-prostaglandin J_2). Rats were treated with 15d-prostaglandin J_2 (0.3 mg/kg, i.v., n=10) 30 min before they were subjected to G+/G− shock (as described above).
(4) G+/G− 15d-prostaglandin J_2 GW9662 Group (GW9662). Rats were treated with GW9662 (1 mg/kg, i.v., n=7) 15 min prior to administration of 15d-prostaglandin J_2 (0.3 mg/kg, i.v.), which was given 30 min before the rats were subjected to G+/G− shock (as described previously).

Following administration of peptidoglycan+lipopolysaccharide or saline, rats were given saline (1 ml/kg/h, i.v.) throughout the experiment.

2.3. Quantification of organ function and injury

Six hours after administration of peptidoglycan and lipopolysaccharide, 1.5 ml of blood was collected into a serum gel S/1.3 tube (Sarstedt, Germany) from the catheter placed in the right carotid artery. The blood sample was centrifuged (6000×g for 3 min at room temperature) to separate serum. All serum samples were analysed within 24 h by a contract laboratory for veterinary clinical chemistry (Vetlab Services, Sussex, UK). The following marker enzymes were measured in the serum as biochemical indicators of multiple organ injury/dysfunction:

(1) Renal dysfunction was assessed by measuring the rise in serum levels of creatinine (an indicator of reduced glomerular filtration rate, and hence, renal dysfunction; Thiemermann et al., 1995).
(2) Liver injury was assessed by measuring the rise in serum levels of alanine aminotransferase (ALT, a specific marker for hepatic parenchymal injury), aspartate aminotransferase (AST, a nonspecific marker for hepatic injury), gamma-glutamyl transferase (γ-GT, an early indicator of hepatic injury). Liver dysfunction was assessed by measuring serum levels of bilirubin (an indicator of hepatic excretory function and predictor of the development of liver failure; Baue, 1993; Hewett et al., 1993).
(3) The development of neuromuscular injury was determined by measuring the rise in the serum levels of creatine kinase (CK, an indicator for the development of neuromuscular (skeletal or cardiac) injury (Ruetten et al., 1996).

2.4. Purification of peptidoglycan

Peptidoglycan was isolated from *S. aureus* as previously described (Foster, 1992). Covalently attached proteins were removed by treatment with pronase at 2 mg/ml for 1 h at 60 °C (Atrih et al., 1996). Anionic polymers were removed from the peptidoglycan by the treatment of purified cell walls (10 mg [dry weight]/ml) with hydrofluoric acid (48 vol.%/vol.%) for 24 h at 4 °C. The insoluble peptidoglycan was then washed by centrifugation (14,000×g, 5 min) and was resuspended once in 100 ml of Tris–HCl (pH 8.0) and five times in distilled water until the pH was neutral. The peptidoglycan was then recovered by centrifugation as described above and resuspended in saline (0.9 wt.%/vol.%) prior to sterilization by autoclaving and storage at −20 °C. Peptidoglycan extract was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, with no evidence of any protein whatsoever. Peptidoglycan was also enzymatically digested, and it gave the expected reversed-phase high-pressure liquid chromatography neuropeptide profile with no spurious products.

Table 1
Alteration in mean arterial blood pressure (mm Hg) in the experimental groups studied

Group	Mean arterial blood pressure (mm Hg; means±S.E.M.)							
	Baseline	20 min	1 h	2 h	3 h	4 h	5 h	6 h
Sham	122±6	124±6	120±4	111±5	107±4	105±3	105±4	103±3
Control	132±4	124±3	111±3	117±3	114±3	105±3	98±3	94±3
15*d*-PGJ_2	122±3	110±4[a]	110±2	112±2	109±2	97±3	97±3	91±3
GW9662	127±3	125±11	108±5	124±6	119±6	110±5	101±4	98±4

[a] Significantly different from mean arterial blood pressure in Control ($P<0.05$).

2.5. Materials

15*d*-prostaglandin J_2 was purchased from Merck Biosciences (Beeston, Nottingham, UK). GW9662 was purchased from Alexis (Bingham, Nottingham, UK). *E. coli* lipopolysaccharide (serotype 0127:B8) was obtained from Sigma-Aldrich (Poole, Dorset, UK). Thiopentone sodium (Intraval Sodium®) was obtained from Rhône Mérieux (Harlow, Essex, UK). All chemicals were of the highest commercial grade available. All stock solutions were prepared in nonpyrogenic saline (0.9% NaCl; Baxter Healthcare, Thetford, Norfolk, UK) or 10% dimethyl sulfoxide (Sigma-Aldrich).

2.6. Statistical evaluation

All data are presented as means±standard error of the mean (S.E.M.) of *n* observations, where *n* represents the number of animals or blood samples studied. For repeated measurements (hemodynamics), a two-way analysis of variance (ANOVA) was performed. Data without repeated measurements (multiple organ injury/failure) was analysed by one-way ANOVA, followed by a Dunnett's test for multiple comparisons. A *P*-value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Effects of 15d-prostaglandin J_2 on the circulatory failure caused by peptidoglycan and lipopolysaccharide coadministration

Baseline values of mean arterial blood pressure and heart rate were similar in all of the animal groups studied and ranged from 122±3 to 132±6 mm Hg ($P>0.05$, Table 1) and from 386±10 to 406±12 beats per minute ($P>0.05$, Table 2), respectively. In the sham-operated animals group, injection of vehicle (10% dimethyl sulfoxide; Sham, $n=10$) did not result in any significant alterations in mean arterial blood pressure or heart rate ($P>0.05$, Tables 1 and 2).

Infusion of peptidoglycan (0.3 mg/kg, i.v.) and lipopolysaccharide (1 mg/kg, i.v.) resulted in a modest biphasic fall in mean arterial blood pressure from 132±4 mm Hg (baseline, prior to infusion of peptidoglycan+lipopolysaccharide) to 94±3 mm Hg at 360 min (Table 1). Pretreatment of rats subjected to G+/G− shock with 15*d*-prostaglandin J_2 did not affect the fall in mean arterial blood pressure caused by coadministration of peptidoglycan and lipopolysaccharide ($P>0.05$, Table 1). An increase in heart rate was observed in rats subjected to G+/G− shock and treated with vehicle (Table 2). The observed increase in heart rate was not affected by 15*d*-prostaglandin J_2 (P>0.05, Table 2). Administration of GW9662 did not affect the fall in mean arterial blood pressure or the increase in heart rate caused by coadministration of peptidoglycan and lipopolysaccharide ($P>0.05$, Tables 1 and 2).

3.2. Effects of 15d-prostaglandin J_2 on the multiple organ injury and dysfunction caused by peptidoglycan and lipopolysaccharide coadministration

When compared to rats treated with vehicle rather than peptidoglycan+lipopolysaccharide (Sham), G+/G− shock (Control) resulted in significant rises in the serum levels of creatinine (renal dysfunction, Fig. 1), ALT, AST, γ-GT, bilirubin (liver injury and dysfunction, Figs. 2 and 3) and CK (neuromuscular injury, Fig. 4).

Pretreatment of rats subjected to G+/G− shock with 15*d*-prostaglandin J_2 attenuated the renal dysfunction caused by peptidoglycan+lipopolysaccharide administration ($P<0.05$, Fig. 1). In addition, treatment with 15*d*-prosta-

Table 2
Alteration in heart rate (beats per minute, BPM) in the experimental groups studied

Group	Heart rate (beats per minute; means±S.E.M.)							
	Baseline	20 min	1 h	2 h	3 h	4 h	5 h	6 h
Sham	406±12	405±12	398±13	395±14	390±13	379±14	384±13	379±13
Control	392±9	393±9	413±11	412±8	430±7	453±10[a]	425±8	431±10
15*d*-PGJ_2	486±10	364±9	381±10	388±8	395±8	400±7	401±7	406±10
GW9662	390±13	397±13	388±17	406±14	406±15	417±15	424±16	426±14

[a] Significantly different from heart rate in Sham ($P<0.05$).

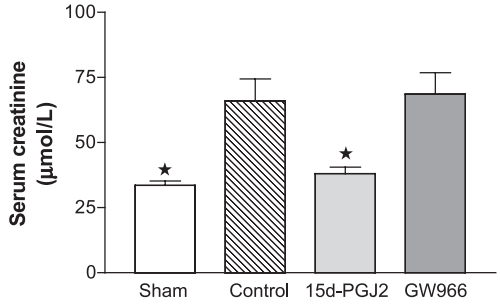

Fig. 1. Alterations in the serum levels of creatinine. Creatinine serum levels in rats subjected to the surgical procedure and pretreated with 10% dimethyl sulfoxide (Sham, n=10). Rats subjected to G+/G− shock (peptidoglycan, 0.3 mg/kg+lipopolysaccharide, 1 mg/kg, i.v., for 6 h) were pretreated with either 10% dimethyl sulfoxide (Control, n=11), 15d-prostaglandin J_2 (15d-prostaglandin J_2, n=10), or 15d-prostaglandin J_2 and GW9662 (GW9662, n=7). (★) P<0.05 when compared with Control by ANOVA followed by Dunnett's post hoc test.

glandin J_2 prior to administration of peptidoglycan+lipopolysaccharide also attenuated the rises in the serum levels of ALT, AST, γ-GT and bilirubin (P<0.05, Figs. 2 and 3), and hence, the liver injury and dysfunction caused by G+/G− shock. The serum level of CK (P<0.05, Fig. 4) was also lower in rats pretreated with 15d-prostaglandin J_2 than in rats subjected only to G+/G− shock. The specific PPAR-γ

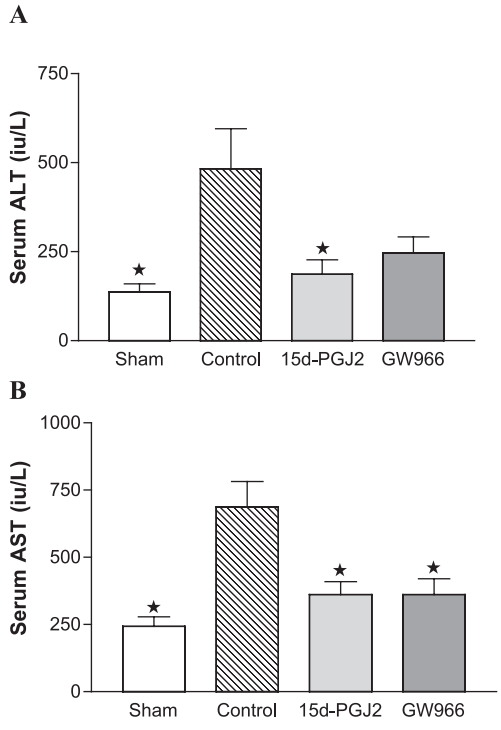

Fig. 2. Alterations in the serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). ALT (A) and AST (B) serum levels in rats subjected to the surgical procedure and pretreated with 10% dimethyl sulfoxide (Sham, n=10). Rats subjected to G+/G− shock (peptidoglycan, 0.3 mg/kg+lipopolysaccharide, 1 mg/kg, i.v., for 6 h) were pretreated with either 10% dimethyl sulfoxide (Control, n=11), 15d-prostaglandin J_2 (15d-prostaglandin J_2, n=10), or 15d-prostaglandin J_2 and GW9662 (GW9662, n=7). (★) P<0.05 when compared with Control by ANOVA followed by Dunnett's post hoc test.

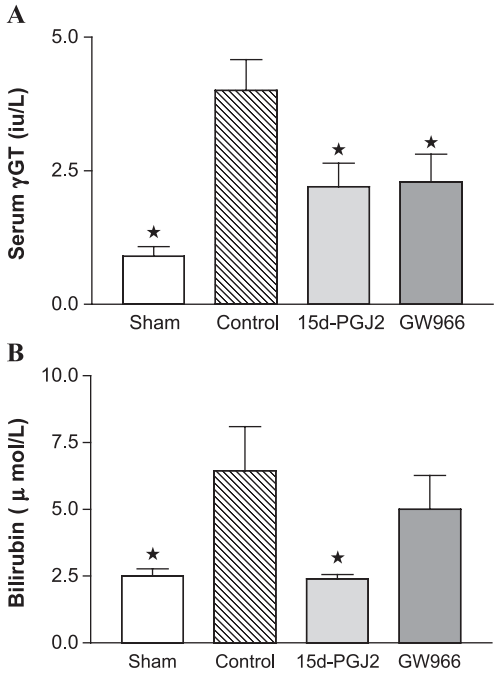

Fig. 3. Alterations in the serum levels of gamma-glutamyltransferase (γ-GT) and bilirubin. γ-GT (A) and bilirubin (B) serum levels in rats subjected to the surgical procedure and pretreated with 10% dimethyl sulfoxide (Sham, n=10). Rats subjected to G+/G− shock (peptidoglycan, 0.3 mg/kg+lipopolysaccharide, 1 mg/kg, i.v., for 6 h) were pretreated with either 10% dimethyl sulfoxide (Control, n=11), 15d-prostaglandin J_2 (15d-prostaglandin J_2, n=10), or 15d-prostaglandin J_2 and GW9662 (GW9662, n=7). (★) P<0.05 when compared with Control by ANOVA followed by Dunnett's post hoc test.

antagonist GW9662 reduced the protective effects afforded by 15d-prostaglandin J_2 on the renal dysfunction (measured as increase in serum creatinine levels, Fig. 1), neuromuscular injury (measured as increase in serum CK level, Fig. 4), indicating that the effect of 15d-prostaglandin J_2 in these organs is at least, in part, mediated by PPAR-γ. GW9662 also partially attenuated the reduction in the degree of liver dysfunction (measured as serum bilirubin

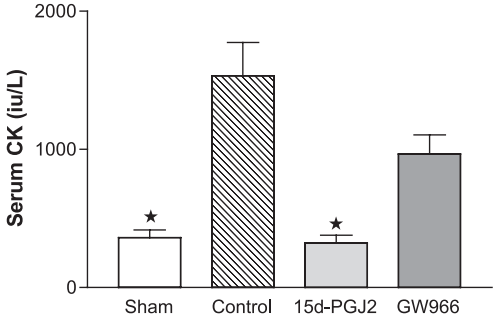

Fig. 4. Alterations in the serum levels of CK. CK serum levels in rats subjected to the surgical procedure and pretreated with 10% dimethyl sulfoxide (Sham, n=10). Rats subjected to G+/G− shock (peptidoglycan, 0.3 mg/kg+lipopolysaccharide, 1 mg/kg, i.v., for 6 h) were pretreated with either 10% dimethyl sulfoxide (Control, n=11), 15d-prostaglandin J_2 (15d-prostaglandin J_2, n=12), or 15d-prostaglandin J_2 and GW9662 (GW9662, n=7). (★) P<0.05 when compared with Control by ANOVA followed by Dunnett's post hoc test.

levels) afforded by 15*d*-prostaglandin J_2 in rats with G+/G− shock (Fig. 3).

4. Discussion

We have previously reported that the cell wall component, peptidoglycan, derived from the pathogenic G+ bacterium *S. aureus*, synergises with a low-dose lipopolysaccharide to cause the development of shock and multiple organ failure in vivo (Wray et al., 2001). We confirm here that coadministration of peptidoglycan and lipopolysaccharide caused a substantial increase in the serum level of creatinine, indicating the development of acute renal dysfunction. G+/G− shock also resulted in a substantial increase in the serum concentrations of ALT, AST, γ-GT and bilirubin, indicating hepatocellular injury and dysfunction. In addition, G+/G− shock was associated with an increase in the serum CK, indicative of neuromuscular (skeletal or cardiac) injury. Neither peptidoglycan nor lipopolysaccharide given alone at the doses used in this study caused a significant increase of any of the parameter discussed above (Thiemermann, 2002). The peptidoglycan fragment NAM-L-Ala-D-isoglutamine has been demonstrated to be essential for the ability of peptidoglycan to synergise with lipoteichoic acid and lipopolysaccharide (Kengatharan et al., 1998; Thiemermann et al., 2002; Wray et al., 2001; Parant et al., 1995). This study provides evidence that pretreatment of rats subjected to G+/G− shock with 15*d*-prostaglandin J_2 attenuates

(i) the renal dysfunction,
(ii) the liver injury and dysfunction, and
(iii) the neuromuscular injury

caused by coadministration of peptidoglycan and lipopolysaccharide. In contrast, 15*d*-prostaglandin J_2 did not affect the circulatory failure caused by peptidoglycan+lipopolysaccharide in the rat. In addition, 15*d*-prostaglandin J_2 reduces the liver injury caused by administration of lipopolysaccharide alone in the rat (Collin and Thiemermann, 2003). Our results are qualitatively similar to those recently reported by Zingarelli et al. (2003), who demonstrated in a rat model of polymicrobial sepsis (cecal ligation and puncture) that

(i) PPAR-γ expression is reduced in lung and thoracic aorta and
(ii) 15*d*-prostaglandin J_2 attenuates the activation of nuclear factor-κB and activated protein-1 associated with sepsis.

To elucidate whether the activation of PPAR-γ mediates the protective effect of 15*d*-prostaglandin J_2 against the multiple organ injury and dysfunction caused by peptidoglycan and lipopolysaccharide administration in the rat, we used the specific PPAR-γ antagonist GW9662. We demonstrate for the first time that GW9662 causes a significant reduction in the protection afforded by 15*d*-prostaglandin J_2 in

(i) renal dysfunction (measured as serum creatinine),
(ii) liver dysfunction (measured as serum bilirubin) and
(iii) neuromuscular injury (measured as serum CK) caused by G+/G− shock.

Thus, we propose that

(1) activation of PPAR-γ by 15*d*-prostaglandin J_2 can protect against the multiple organ injury and dysfunction caused by G+/G− shock and
(2) inhibition of the activation of PPAR-γ contributes to the loss of protection by 15*d*-prostaglandin J_2 after pretreatment with GW9662.

As the protective effects of 15*d*-prostaglandin J_2 on liver injury (ALT and AST) were quite substantial and not reversed by GW9662, it is possible and likely that other, PPAR-γ-independent effects of this cyclopentanone prostaglandin contribute to the observed protective effects. In concert with our findings, there is now evidence that the inhibition by 15*d*-prostaglandin J_2 of the gene expression of cyclooxygenase-2, interleukin-1, interleukin-6, tumour necrosis factor-α and granulocyte–macrophage colony-stimulating factor caused by lipopolysaccharide in human blood monocytes are independent of the activation of PPAR-γ (Hinz et al., 2003). Moreover, the neuroprotective effects of 15*d*-prostaglandin J_2 are also only partially mediated by activation of PPAR-γ (Aoun et al., 2003).

The synergy between peptidoglycan and lipopolysaccharide described here may be of considerable clinical relevance. There are many clinical situations in which these two cell wall fragments might interact and precipitate the development of the systemic inflammatory response syndrome, sepsis or septic shock, conditions known to be associated with high morbidity and mortality. 15*d*-Prostaglandin J_2 or other ligands for PPAR-γ may be useful in the therapy of the organ injury associated with shock and multiple organ injury caused by G+/G− bacteria infections.

References

Aoun, P., Watson, D.G., Simpkins, J.W., 2003. Neuroprotective effects of PPAR-gamma agonists against oxidative insults in HT-22 cells. Eur. J. Pharmacol. 472, 65–71.

Atrih, A., Zollner, P., Allmaier, G., Foster, S.J., 1996. Structural analysis of *Bacillus subtilis* 168 endospore peptidoglycan and its role during differentiation. J. Bacteriol. 178, 6173–6183.

Baue, A.E., 1993. The multiple organ or system failure syndrome. Pathophysiology of Shock, Sepsis, and Organ Failure. Springer Verlag, Berlin, pp. 1004–1018.

Bendixen, A.C., Shevde, N.K., Dienger, K.M., Willson, T.M., Funk, C.D., Pike, J.W., 2001. L-4 inhibits osteoclast formation through a direct action on osteoclast precursors via peroxisome proliferator-activated receptor gamma 1. Proc. Natl. Acad. Sci. U. S. A. 98, 2443–2448.

Burnett, R.J., Haverstock, D.C., Dellinger, E.P., Reinhart, H.H., Bohnen, J.M., Rotstein, O.D., Vogel, S.B., Solomkin, J.S., 1995. Definition of the role of enterococcus in intraabdominal infection: analysis of a prospective randomized trial. Surgery 118, 716–721.

Collin, M., Thiemermann, C., 2003. The PPAR-gamma ligand 15-deoxydelta12,14 prostaglandin J2 reduces the liver injury in endotoxic shock. Eur. J. Pharmacol. 476, 257–258.

Colville-Nash, P.R., Qureshi, S.S., Willis, D., Willoughby, D.A., 1998. Inhibition of inducible nitric oxide synthase by peroxisome proliferator-activated receptor agonists: correlation with induction of heme oxygenase 1. J. Immunol. 161, 978–984.

Cuzzocrea, S., Chatterjee, P.K., Mazzon, E., Dugo, L., Serraino, I., Britti, D., Mazzullo, G., Caputi, A.P., Thiemermann, C., 2002. Pyrrolidine dithiocarbamate attenuates the development of acute and chronic inflammation. Br. J. Pharmacol. 135, 496–510.

Cuzzocrea, S., Ianaro, A., Wayman, N.S., Mazzon, E., Pisano, B., Dugo, L., Serraino, I., Di Paola, R., Chatterjee, P.K., Di Rosa, M., Caputi, A.P., Thiemermann, C., 2003. The cyclopentenone prostaglandin 15-deoxy-delta(12,14)- prostaglandinJ2 attenuates the development of colon injury caused by dinitrobenzene sulphonic acid in the rat. Br. J. Pharmacol. 138, 678–688.

De Kimpe, S.J., Kengatharan, M., Thiemermann, C., Vane, J.R., 1995. The cell wall components peptidoglycan and lipoteichoic acid from *Staphylococcus aureus* act in synergy to cause shock and multiple organ failure. Proc. Natl. Acad. Sci. U. S. A. 92, 10359–10363.

Drew, P.D., Chavis, J.A., 2001. The cyclopentone prostaglandin 15-deoxy-delta(12,14) prostaglandin J2 represses nitric oxide, tumour necrosis factor-alpha, and IL-12 production by microglial cells. J. Neuroimmunol. 115, 28–35.

Fischer, W., 1988. Physiology of lipoteichoic acids in bacteria. Adv. Microb. Physiol. 29, 233–302.

Foster, S.J., 1992. Analysis of the autolysins of *Bacillus subtilis* 168 during vegetative growth and differentiation by using renaturing polyacrylamide gel electrophoresis. J. Bacteriol. 174, 464–470.

Fu, M., Zhang, J., Lin, Y.Y., Zhu, X., Willson, T.M., Chen, Y.E., 2002. Activation of peroxisome proliferator-activated receptor gamma inhibits osteoprotegerin gene expression in human aortic smooth muscle cells. Biochem. Biophys. Res. Commun. 294, 597–601.

Hewett, J.A., Jean, P.A., Kunkel, S.L., Roth, R.A., 1993. Relationship between tumor necrosis factor-alpha and neutrophils in endotoxin-induced liver injury. Am. J. Physiol. 265, G1011–G1015.

Hinz, B., Brune, K., Pahl, A., 2003. 15-Deoxy-delta(12,14)-prostaglandin J2 inhibits the expression of proinflammatory genes in human blood monocytes via a PPAR-gamma-independent mechanism. Biochem. Biophys. Res. Commun. 302, 415–420.

Ianaro, A., Ialenti, A., Maffia, P., Pisano, B., Di Rosa, M., 2001. HSF1/hsp72 pathway as an endogenous anti-inflammatory system. FEBS Lett. 499, 239–244.

Jiang, C., Ting, A.T., Seed, B., 1998. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature 391, 82–86.

Kawahito, Y., Kondo, M., Tsubouchi, Y., Hashiramoto, A., Bishop-Bailey, D., Inoue, K., Kohno, M., Yamada, R., Hla, T., Sano, H., 2000. 15-deoxy-delta(12,14)-PGJ(2) induces synoviocyte apoptosis and suppresses adjuvant-induced arthritis in rats. J. Clin. Invest. 106, 189–197.

Kengatharan, K.M., De Kimpe, S., Robson, C., Foster, S.J., Thiemermann, C., 1998. Mechanism of gram-positive shock: identification of peptidoglycan and lipoteichoic acid moieties essential in the induction of nitric oxide synthase, shock, and multiple organ failure. J. Exp. Med. 188, 305–315.

Leesnitzer, L.M., Parks, D.J., Bledsoe, R.K., Cobb, J.E., Collins, J.L., Consler, T.G., Davis, R.G., Hull-Ryde, E.A., Lenhard, J.M., Patel, L., Plunket, K.D., Shenk, J.L., Stimmel, J.B., Therapontos, C., Willson, T.M., Blanchard, S.G., 2002. Functional consequences of cysteine modification in the ligand binding sites of peroxisome proliferator activated receptors by GW9662. Biochemistry 41, 6640–6650.

Matlow, A.G., Bohnen, J.M., Nohr, C., Christou, N., Meakins, J., 1989. Pathogenicity of enterococci in a rat model of fecal peritonitis. J. Infect. Dis. 160, 142–145.

Parant, M.A., Pouillart, P., Le Contel, C., Parant, F.J., Chedid, L.A., Bahr, G.M., 1995. Selective modulation of lipopolysaccharide-induced death and cytokine production by various muramyl peptides. Infect. Immun. 63, 110–115.

Pasceri, V., Wu, H.D., Willerson, J.T., Yeh, E.T., 2000. Modulation of vascular inflammation in vitro and in vivo by peroxisome proliferator-activated receptor-gamma activators. Circulation 101, 235–238.

Ricote, M., Li, A.C., Willson, T.M., Kelly, C.J., Glass, C.K., 1998. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature 391, 79–82.

Rossi, A., Elia, G., Santoro, M.G., 1997. Inhibition of nuclear factor kappa B by prostaglandin A1: an effect associated with heat shock transcription factor activation. Proc. Natl. Acad. Sci. U. S. A. 94, 746–750.

Ruetten, H., Southan, G.J., Abate, A., Thiemermann, C., 1996. Attenuation of endotoxin-induced multiple organ dysfunction by 1-amino-2-hydroxy-guanidine, a potent inhibitor of inducible nitric oxide synthase. Br. J. Pharmacol. 118, 261–270.

Santoro, M.G., 2001. Heat shock factors and the control of the stress response. Biochem. Pharmacol. 59, 55–63.

Straus, D.S., Glass, C.K., 2001. Cyclopentenone prostaglandins: new insights on biological activities and cellular targets. Med. Res. Rev. 21, 185–210.

Thiemermann, C., 2002. Interactions between lipoteichoic acid and peptidoglycan from *Staphylococcus aureus*: a structural and functional analysis. Microbes Infect. 4, 927–935.

Thiemermann, C., Ruetten, H., Wu, C.C., Vane, J.R., 1995. The multiple organ dysfunction syndrome caused by endotoxin in the rat: attenuation of liver dysfunction by inhibitors of nitric oxide synthase. Br. J. Pharmacol. 116, 2845–2851.

Tsubouchi, Y., Kawahito, Y., Kohno, M., Inoue, K., Hla, T., Sano, H., 2001. Feedback control of the arachidonate cascade in rheumatoid synoviocytes by 15-deoxy-delta(12,14)-prostaglandin J2. Biochem. Biophys. Res. Commun. 283, 750–755.

Wang, J.E., Jorgensen, P.F., Almlof, M., Thiemermann, C., Foster, S.J., Aasen, A.O., Solberg, R., 2000. Peptidoglycan and lipoteichoic acid from *Staphylococcus aureus* induce tumor necrosis factor alpha, interleukin 6 (IL-6), and IL-10 production in both T cells and monocytes in a human whole blood model. Infect. Immun. 68, 3965–3970.

Wayman, N.S., Hattori, Y., McDonald, M.C., Mota-Filipe, H., Cuzzocrea, S., Pisano, B., Chatterjee, P.K., Thiemermann, C., 2002. Ligands of the peroxisome proliferator-activated receptors (PPAR-gamma and PPAR-alpha) reduce myocardial infarct size. FASEB J. 16, 1027–1040.

Weinstein, M.P., Murphy, J.R., Reller, L.B., Lichtenstein, K.A., 1983. The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults: II. Clinical observations, with special reference to factors influencing prognosis. Rev. Infect. Dis. 5, 54–70.

Wray, G.M., Foster, S.J., Hinds, C.J., Thiemermann, C., 2001. A cell wall component from pathogenic and non-pathogenic gram-positive bacteria (peptidoglycan) synergises with endotoxin to cause the release of tumour necrosis factor-alpha, nitric oxide production, shock, and multiple organ injury/dysfunction in the rat. Shock 15, 135–142.

Zhang, X., Wang, J.M., Gong, W.H., Mukaida, N., Young, H.A., 2001. Differential regulation of chemokine gene expression by 15-deoxy-delta 12,14 prostaglandin J2. J. Immunol. 166, 7104–7111.

Zingarelli, B., Sheehan, M., Hake, P.W., O'Connor, M., Denenberg, A., Cook, J.A., 2003. Peroxisome proliferator activator receptor-gamma ligands, 15-deoxy-delta(12,14)-prostaglandin J2 and ciglitazone, reduce systemic inflammation in polymicrobial sepsis by modulation of signal transduction pathways. J. Immunol. 171, 6827–6837.