Endotoxin Augments Hemodynamic and Metabolic Effects of Formyl-Methionyl-Leucyl-Phenylalanine (FMLP) in Rabbits

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Abstract. *The chemotactic factor formyl-methionyl-leucyl-phenylalanine (FMLP) when injected in rabbits causes dose-dependent transient hypotension, as well as neutropenia, thrombocytopenia and a decrease in systemic vascular resistance, as previously shown. Since both FMLP and endotoxin are elaborated at sites of infection by certain bacteria, whether they act in concert to produce shock was examined. Animals were pretreated with 380 μg/kg of E. coli endotoxin, 24 hours before the infusion of 10^{-9} moles FMLP and were compared with animals pretreated only with saline and administered FMLP. Within 3 min after FMLP, endotoxin-pretreated animals developed a significant fall in MAP ($p<0.001$) and neutrophils ($p<0.001$) compared to controls. Statistically significant lactic acidemia, with reduced HCO^-_3 levels also developed in the endotoxin-pretreated group after FMLP injection. These results indicate that endotoxin apparently induces a prepared state, thus facilitating the hemodynamic and cellular effects of FMLP in this model.*

Sepsis is an infection-induced syndrome characterized by a generalized inflammatory state (1). Although activation of the immune system during microbial invasion is generally protective, septic shock develops in a number of patients as a consequence of excessive or poorly regulated immune response to the offending organism (Gram-negative or Gram-positive bacteria, fungi, viruses, or microbial toxins) (2). Many mechanisms are involved in the pathogenesis of septic shock, including the release of cytokines (3), the

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activation of neutrophils (4), monocytes and microvascular endothelial cells (5), as well as the activation of neuroendocrine reflexes (6) and plasma protein cascade systems, such as the complement system (7), the intrinsic and extrinsic pathways of coagulation and the fibrinolytic system (8). Although the lipopolysaccharide of the gram-negative bacterial wall (endotoxin) and the peptidoglycan of gram-positive bacterial wall are the most extensively studied (1), several other agents, like the formyl-peptide, formyl-methionyl-leucyl-phenylalanine (FMLP), also promote the inflammatory response (9).

The bacterial N-formyl peptides, such as N-formyl-Met-Leu-Phe (fMLF), are some of the first identified and most potent chemoattractants for phagocytic leukocytes (10). Two fMLF receptors, the high affinity formyl peptide receptor (FPR) and its low affinity variant FPR-like 1 (FPRL1), belong to the seven-transmembrane, Gi protein-coupled receptor superfamily which also includes chemokine receptors (11). Apart from their ability to bind bacterial chemotactic peptides, the physiological role of these receptors in humans remains unclear (12). FMLP induces activation of leukocytes, initiates chemotaxis, stimulates cell aggregation, enzyme secretion, arachidonic acid and oxidative metabolism (9, 13, 14), and causes systemic hypotension associated with a decrease in systemic vascular resistance (15, 16). Some of these actions resemble those seen after exposure of animals or cells to endotoxins (1, 3, 17). Leukopenia, thrombocytopenia and hypotension follow infusion of both substances *in vivo*, but FMLP-mediated hypotension is rapid in onset and transient (16), while that of endotoxin generally occurs late and is persistent (18).

Fehr *et al.* have documented that FMLP could substitute for the place of the second endotoxin injection in provoking the generalized Shwartzman reaction in rabbits (19). In addition, Goldman *et al.* demonstrated that rabbit neutrophils pretreated with endotoxin exhibited an increase in the receptor density for FMLP (20). Endotoxin-pretreated neutrophils can then be stimulated by FMLP to produce enhanced injury of microvascular endothelial cells

(21) and animals exposed to FMLP after endotoxin pretreatment developed acute lung injury (22).

The purpose of this study was to examine the possible interaction between FMLP and endotoxin in producing the hemodynamic and metabolic alterations typical of septic shock in the rabbit.

Materials and Methods

New Zealand white rabbits weighing 2.5 to 3.5 kg were used in this study. The experimental protocol is shown in Table I. Surgical procedures were performed on lightly anesthetized animals with a mixture of ketamine (Vetalar, Parke Davis, 60 mg/kg) and xylazine (Rompun, Mobay Co., 7 mg/kg). Polyethylene catheters were placed within the right atrium and the aortic arch *via* the right jugular vein and the left carotid artery, respectively. To prevent dislodgment of the catheters during recovery, the animals wore Harvard soft-mesh jackets. The animals were divided into four experimental groups (Table I). After the operation (time-24 h), 16 animals received 1.0 ml of normal saline intravenously (group SAL+FMLP) and all the other the animals (n=37) were injected with endotoxin (*E. coli* 026:B6, Sigma Chemical Co., 380 μg/kg). This dose of endotoxin was chosen because it caused no immediate deleterious effects and 100% of the animals recovered uneventfully if no further interventions were undertaken. The animals were allowed to recover in their cages for 24 h. The next day, each animal was kept awake in a standard rabbit cage for the duration of the experiment. The arterial line was connected to a Statham transducer and blood pressure and pulse rates were continuously recorded on a Beckman Dynograph. This cannula was also used to withdraw blood samples for the determination of blood cell counts, blood gases, pH, plasma lactate, fibrinogen and prothrombin time. The venous line was used to administer the chemotactic factor FMLP (10^{-9} moles) in groups SAL+FMLP (n=16) and ENDO+FMLP (n=6) and appropriate dilutions of DMSO in saline (group ENDO+DMSO, n=17) or endotoxin (380 μg/kg, group ENDO+ENDO, n=14, Table I). Throughout the experiment, replacement volumes of isotonic saline (usually 30 ml) were infused. Both lines were kept patent with heparinized saline.

FMLP (Sigma Chemical Co.) was prepared as a 10^{-2} M stock solution using DMSO as the solvent. Dilution of 10^{-9} moles FMLP was prepared daily. Endotoxin (*E. coli* 026:B6) solution in normal saline was also prepared daily at the appropriate dose of 380 μg/kg for each animal. Blood cell counts were determined by a Coulter Counter (Model S-Plus, Coulter Electronics, Inc.), blood gases and pH by a pH-blood gases Analyzer (Model 813, Instrumentation Laboratory) and lactate by an ACA III-discrete Clinical Analyzer (Clinical Systems, DuPont Co). Clotting factors were measured by standard methods.

Statistical analysis. The data analysis was performed with an SAS statistical program. One way ANOVA followed by Dunnett's test or one way ANOVA on the ranks followed by Dunn's test (when data's distribution was not normal) were used to compare baseline levels with levels at the different time-points within the same group during the experiment. Comparisons of the means at the same time-point between two experimental groups were made by the *t*-test or Wilcoxon's rank sum test (when data distribution was not normal), and between multiple groups with one way ANOVA followed by Student-Newman-Keuls test or one way ANOVA on the ranks

Table I. *Experimental protocol.*

Time	SAL+FMLP (n=16)	ENDO+DMSO (n=6)	ENDO+FMLP (n=17)	ENDO+ENDO (n=14)
-24 h	Anesthesia and catheter placement			
-24 h (1st injection)	Saline	Endotoxin	Endotoxin	Endotoxin
0 h (2nd injection)	FMLP	DMSO	FMLP	Endotoxin

followed by Dunn's test (for comparisons between means when data distribution was not normal). Data are expressed as mean±SD. A two-tailed $p < 0.05$ was considered statistically significant.

Results

Effect of endotoxin pretreatment on FMLP administration. To investigate the effect of endotoxin preatreatment on FMPL administration the SAL+FMLP group was compared to the to group ENDO+FMLP group. As shown in Figure 1A, the two groups presented similar mean arterial pressure (MAP) levels at time 0, before the second injection of the protocol. Three minutes after the administration of 10^{-9} moles FMLP, a significant but transient reduction in MAP (from 87±3.8 to 54±4.7 mmHg, $p < 0.05$) developed in the SAL+FMLP animals. FMLP given ENDO+FMLP animals, led to a drop in MAP to 52% of the levels at time 0 (from 79±2.9 to 41±3.2 mmHg, $p < 0.0001$). This effect was acute, with maximal hypotension at 3 min after FMLP. There was a full recovery within 15 min, followed by a second phase of progressive decline with statistically lower levels of MAP compared to SAL+FMLP for the rest of the experiment

As shown in Figure 1A, 3 min after the administration of FMLP a significant drop in neutrophil levels was observed (from 3715±362 at time 0 to 907±218/mm³, $p < 0.0001$) in SAL+FMLP. In ENDO+FMLP, neutrophil levels were also profoundly reduced (from 3376±635 to 132±50/mm³, $p < 0.001$). Neutrophil counts in this group were significantly lower compared to SAL+FMLP ($p < 0.01$). The lowest neutrophil counts occurred 3 min after the FMLP administration and a slight recovery was shown within 15 min, which was sustained for the rest of the experiment.

Thrombocytopenia (from 421±18 at –24 to 80±16x10³/mm³ at 0, $p < 0.001$) was found in the ENDO+FMLP animals and FMLP caused no any further alteration in the platelet counts throughout the experiment. A decrease in platelet counts (from 341±24 at time 0 to 187±32.6x10³/mm³, $p < 0.001$) was observed 3 min after FMLP administration in animals of the SAL+FMLP group, but platelets were always significantly higher than in the ENDO+FMLP group throughout the experiment (Figure 1A).
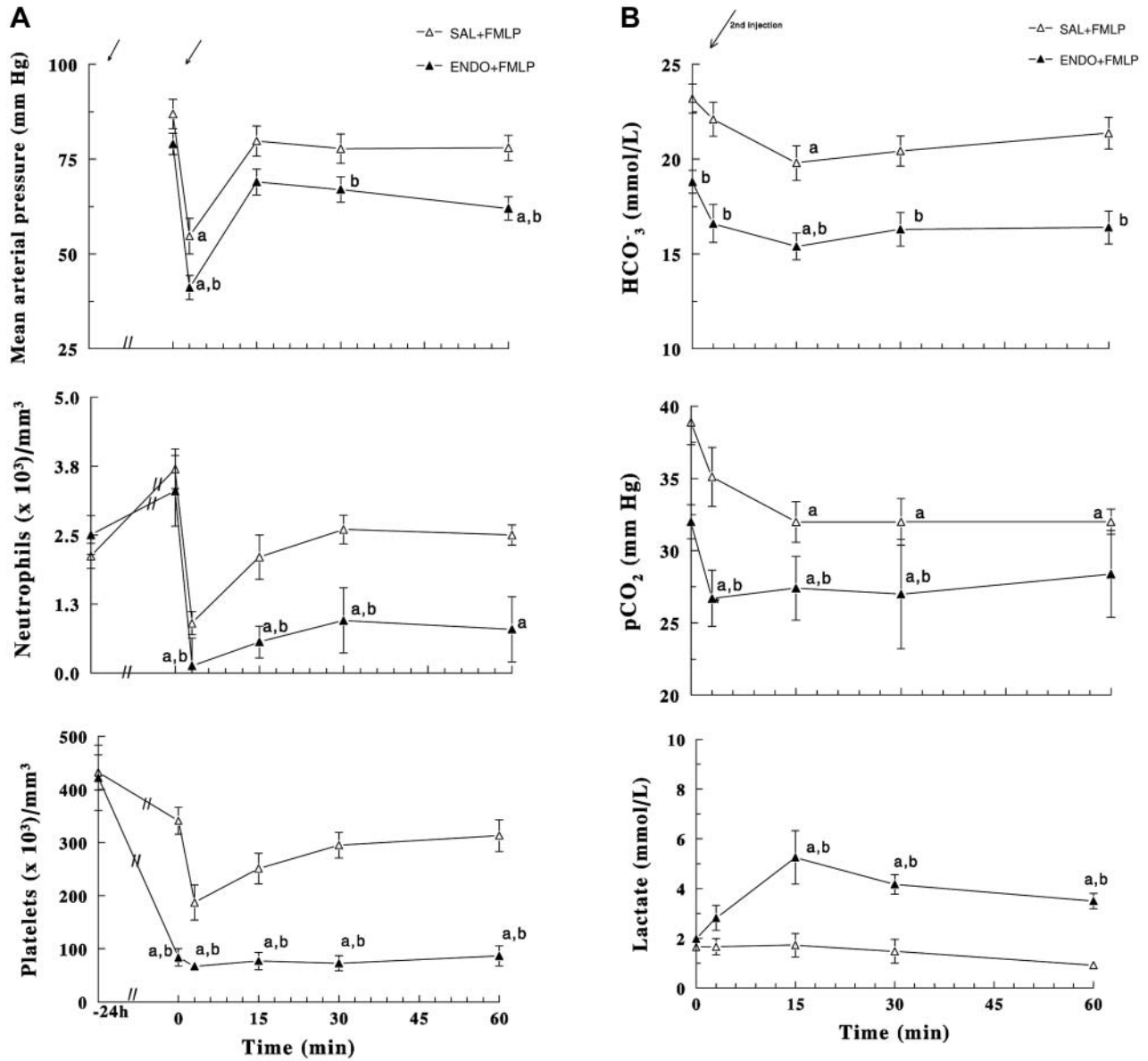
Figure 1. *Mean arterial pressure, neutrophil and platelet levels (A) and arterial HCO$^-_3$, pCO$_2$ and lactate levels (B) in the 2 experimental groups: SAL+FMLP (n=16): 10^{-9} moles FMLP (2nd injection of the protocol at 0 h) in saline-pretreated animals (1st injection at baseline, -24 h) and ENDO+FMLP (n=17): 10^{-9} moles FMLP (2nd injection of the protocol at 0 h) in 380 μg/kg endotoxin-pretreated animals (1st injection at baseline, -24 h). Data are mean±SE, $^ap<0.05$ from time 0 of the same group, $^bp<0.05$ from SAL+FMLP, at the same time-point.*

Pretreatment of rabbits with endotoxin, caused significantly lower levels of bicarbonate (HCO$^-_3$) and blood pCO$_2$ at time 0, compared to saline-pretreated animals ($p<0.001$, Figure 1B). FMLP in ENDO+FMLP animals, induced a further reduction of HCO$^-_3$ (from 18.9±0.7 to 15.4±0.7 mmol/L, $p<0.001$) and pCO$_2$ (32.3±1.2 to 26.7±1.9 mmHg, $p<0.05$) and a marked elevation of plasma lactate (from 2±0.2 to 5.3±1.1 mmol/L, $p<0.05$, Figure 1B), sustained up to the end of the experiment.

Animals in SAL+FMLP also demonstrated a slight and progressive reduction in HCO$^-_3$ levels; this was less profound however. This reduction could be explained by saline infusion to replace volume following blood withdrawal. A progressive decrease in the arterial pCO$_2$ levels was also noted (Figure 1B).

The endotoxin-pretreated animals had significantly higher heart rate values at time 0 (Table II) than saline pretreated animals. After FMLP infusion, a further increase in heart

Table II. *Arterial pO$_2$ and pH levels (mm Hg).*

Arterial pO$_2$

Time	SAL+ FMLP (n=16)	ENDO+ DMSO (n=6)	ENDO+ FMLP (n=17)	ENDO+ ENDO (n=14)
0	108.5±4.5	106.8±3.5	115±3.3	117±3.11
3	105.8±3.2	117.6±3.5	109.8±6.2	122.7±2.7
15	110.1±3.8	114±4.6	116±5.2	123.2±2.3
30	113.3±5.1	106±5.1	113±3.9	102±7.6
60	102.7±3.7	109.4±7.3	111±3.7	109.7±7.6

Arterial pH

0	7.42±0.02	7.38±0.01	7.39±0.01	7.42±0.01
3	7.44±0.02	7.39±0.01	7.43±0.01	7.44±0.1
15	7.44±0.02	7.44±0.01	7.36±0.04	7.47±0.0.01
30	7.45±0.01	7.41±0.02	7.42±0.03	7.43±0.02
60	7.44±0.01	7.43±0.01	7.402±0.03	7.409±0.04

Values are means±SE.

Table III. *Effect of endotoxin preatreatment on the heart rate (pulses/min) of the FMPL-treated animals.*

Time	SAL+FMLP	ENDO+FMLP
0	237±6	280±11[b]
3	240±6	304±9[a,b]
15	249±6	299±13[b]
30	266±9	299±14[b]
60	267±9	294±18

Values are means±SE; [a]$p<0.05$ from time 0 of the same group, [b]$p<0.05$ from SAL+FMLP, at the same time-point.

rate was observed in the ENDO+FMLP animals, statistically different from that in the SAL+FMLP animals ($p<0.01$). None of the animals demonstrated any changes in arterial pH or pO$_2$ levels (Table II).

Effect of FMLP on endotoxin pretreated animals. There was no significant change in MAP levels in the ENDO+DMSO or ENDO+ENDO animals (Figure 2A), while in the ENDO+FMLP group an acute and transient hypotensive effect at 3 min post FMLP was noted with MAP levels significantly lower than in all other groups ($p<0.01$). A progressive delayed decline in MAP was also noted in the ENDO+ENDO group, parallel to the second phase of progressive MAP decline in the ENDO+FMLP group.

Administration of endotoxin or FMLP induced a similar degree of neutropenia in the endotoxin-pretreated animals, as no difference was noted between neutrophil levels between the ENDO+ENDO and ENDO+FMLP animals (Figure 2A). Neutrophil counts in these groups were statistically lower compared to the neutrophil counts of ENDO+DMSO animals after the second injection and throughout the experiment.

Blood platelet counts of the three experimental groups are shown in the lower panel of Figure 2A. Immediately before the second injection of the protocol (time 0), all endotoxin-pretreated groups presented a statistically significant decrease in blood platelet counts from the baseline levels (time-24, $p<0.001$, in all groups). FMLP administered to rabbits after challenge with endotoxin (ENDO+FMLP), did not cause any further alteration in the platelet counts throughout the experiment.

FMLP administered to ENDO+FMLP animals, induced reductions in HCO$^-_3$ and pCO$_2$ which were similar to the second endotoxin injection in ENDO+ENDO animals. These changes were sustained up to the end of the experiment. Lactate levels were elevated both in ENDO+FMLP and ENDO+ENDO groups, but in the latter group a continuous increase was noted which reached statistical difference from ENDO+FMLP at the end of the experiment (Figure 2B).

While no changes were demonstrated in arterial pH or pO$_2$ levels (Table II), after FMLP infusion, an increase in heart rate was observed in the ENDO+FMLP animals compared to the ENDO+DMSO animals ($p<0.05$), but was similar to that in ENDO+ENDO animals (Table III).

Discussion

The synthetic chemotactic peptide FMLP, structurally related to bacterial chemotactins, has been found to activate human and rabbit neutrophils (9), macrophages (23) monocytes and endothelial cells (12, 24, 25). FMLP, the major chemoattractant synthesized by *E. coli* (10) and several related peptides were isolated from cultures of streptococci (26, 27) and staphylococci (18). Therefore, it is possible that, in cases of bacterial infection, the inflammatory response may be promoted, at least in part, by these agents. In the present work, animals were subjected to 380 μg/kg endotoxin injection, followed by 10^{-9} moles FMLP. The dose of endotoxin used in this experiment did not affect the MAP, as reported by others (21, 22). The subsequent administration of 10^{-9} moles FMLP induced a biphasic profound decrease in MAP. From our data, it is clear that endotoxin pretreatment potentiates the hypotensive, neutropenic, thombopenic effect of FMLP and leads to lactic acidosis accompanied by compensatory alveolar hyperventilation. Endotoxin produces hypotension at relatively high doses and several substances released during endotoxemia such as arachidonic acid metabolites, cytokines and platelet-activating factor (1, 3) and nitric oxide (28, 29) have been implicated in its pathogenesis.
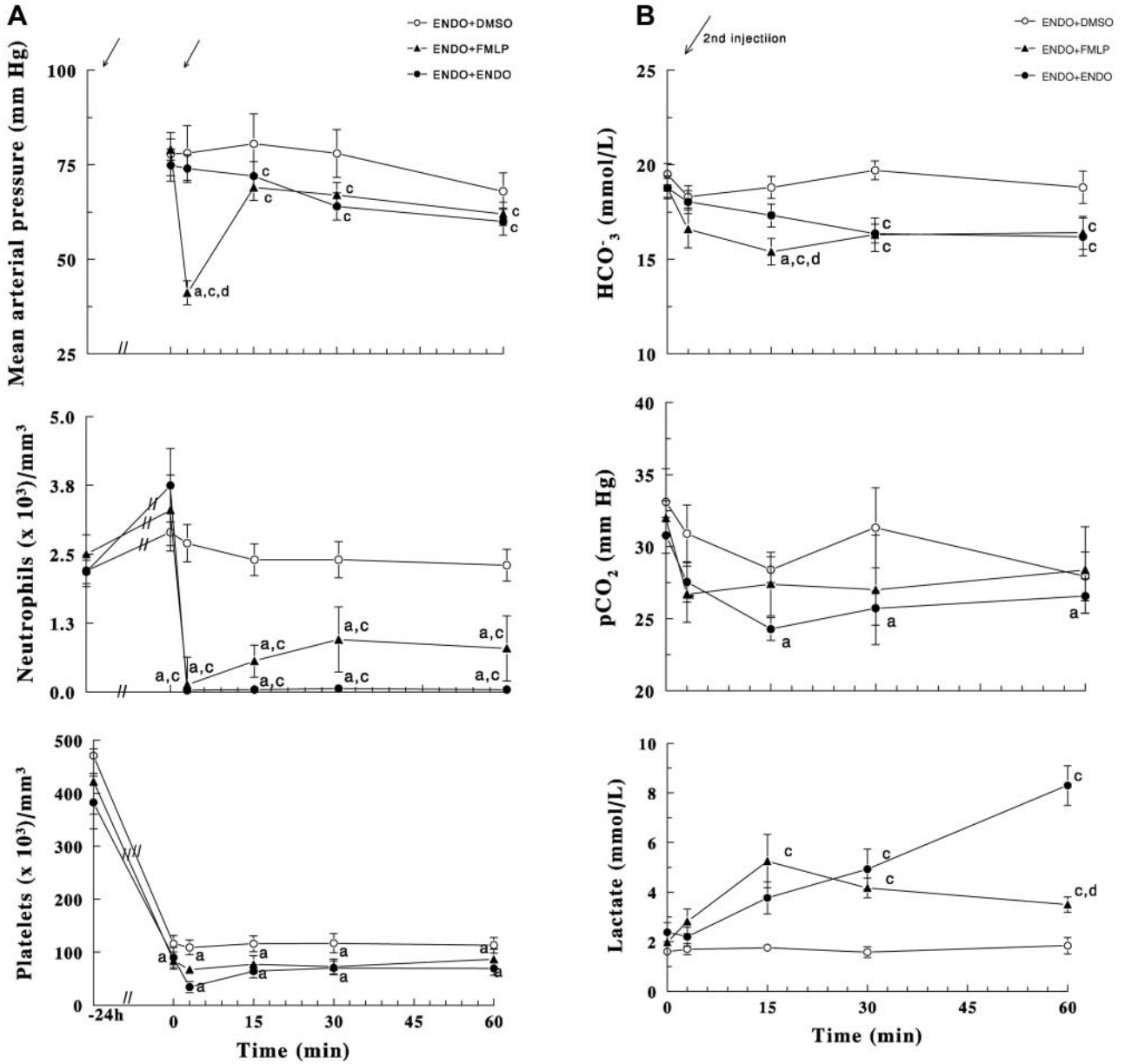
Figure 2. *Mean arterial pressure, neutrophil and platelets levels (A) and arterial HCO^-_3, pCO_2 and lactate levels (B) in the 3 experimental groups: ENDO+FMLP (n=17): 10-9 moles FMLP (2nd injection of the protocol at 0 h) in 380 μg/kg endotoxin-pretreated animals (1st injection at baseline, -24 h), ENDO+ DMSO (n=6): DMSO (2nd injection of the protocol at 0 h) in 380 μg/kg endotoxin-pretreated animals (1st injection at baseline, -24 h) and ENDO+ENDO (n=14): 380 μg/kg endotoxin (2nd injection of the protocol at 0 h), in 380 μg/kg endotoxin-pretreated animals (1st injection at baseline, -24 h). Arrows indicate time of the injections. Values are means±SE; [a]p<0.05 from time 0 of the same group, [c]p<0.05 from ENDO+DMSO and [d]p<0.05 from ENDO+ENDO, at the same time-point.*

Endotoxin-induces also neutropenia within minutes after injection with a complete recovery by 6 hours (30) and systemic administration of FMLP also causes a reversible and dose-related decrease in circulating neutrophils (16). Pretreatment with a low dose of endotoxin increased the neutropenic effect of FMLP. A

second endotoxin administration in endotoxin-pretreated animals also caused a similar degree of neutropenia but did not induce acute hypotension, an observation indicating that the first phase of FMLP-induced hypotension is neutrophil-independent as we have previously shown (16).

A reversible and dose-related decrease in circulating platelets follows FMLP administration in rabbits (16), although we were not able to demonstrate FMLP-receptor sites on human or rabbit thrombocytes, (Frederick DS and McCormick JR, unpublished data). The release of platelet activating factor (PAF) from rabbit basophils or mast cells may mediate this response (31). Thrombo-cytopenia has also been observed in experimental animals after treatment with endotoxin, and this effect is probably due to platelet aggregation following endotoxin which was demonstrated, *in vivo*, in rabbits and rats (32). In the present study, endotoxin-pretreated rabbits continued to have very low platelet levels 24 hours after endotoxin and FMLP failed to further reduce the platelet counts.

Another interesting finding of this study was the prominent lactic acidemia which developed in the endotoxin-pretreated animals, associated with a significant fall in arterial bicarbonate levels. We previously demonstrated that a similar degree of lactic acidemia occurs after injection of a 5-fold higher dose of FMLP alone to rabbits (16). It is also known that endotoxin causes an immediate increase in lactate concentration as a part of a generalized alteration in carbohydrate metabolism (33). The shift from aerobic to anaerobic metabolism, indicated by the high lactate levels in these animals, may be the result either of a deficit in oxygen utilization related to systemic hypoperfusion, or of a direct defect in intermediary metabolism caused by the endotoxin-FMLP treatment. Endotoxin is a potent stimulus of TNF and IL-1 release from macrophages, which have been implicated as mediators of the metabolic changes observed in endotoxic shock (3). It is possible that this cascade of cytokines is responsible for the potentiation of the metabolic effects of FMLP administration in the endotoxin-pretreated animals.

Several investigators have been working on the potential interaction of endotoxin and FMLP as they both are elaborated at sites of infection by certain bacteria. Fehr *et al.* (19) reported that FMLP can mimic the second, provoking endotoxin injection in the generalized Shwartzman reaction. FMLP causes dose-dependent edema and vasoconstriction in isolated lungs from endotoxin-pretreated rats, mediated by eicosanoids released from cells trapped in the lung circulation (22). An identical effect of FMLP has been observed in lungs after injection of tumor necrosis factor, an effect thought to be mediated by PAF and thromboxane (34). More recently it has been demonstrated that FMLP administered 24 h after intratracheal endotoxin resulted in acute lung injury (35).

It is possible that a cellular mechanism is involved in the endotoxin-induced potentiation of the FMLP effect. Rabbit neutrophils pretreated with endotoxin exhibit increased FMLP receptor density (20) and endotoxin at very low concentrations greatly enhances the endothelial injury caused by FMLP stimulated neutrophils (21). TNF-α, a cytokine produced by macrophages in response to endotoxin, modulates the affinity of FMLP receptors on human neutrophils and stimulates FMLP-mediated activity, expressed with superoxide anion production (36). FMLP also induces gene expression and release of IL-8 (37). Interestingly, recent data provides evidence that in humans endotoxin desensitizes monocytes, by down-regulating CD14 (38). Thus, the second phase of sustained hypotension after FMLP in endotoxin-preatreated animals, similar to the hypotension after the second injection of endotoxin in the same animals, could be the result of a compromised state induced in these animals by endotoxin pretreatment *via* one of the above mechanisms.

In summary, we showed that endotoxin pretreatment potentiated the hypotensive, neutropenic and thombopenic effect of FMLP and induced a lactic acidosis compensated by alveolar hyperventialation. FMLP caused an effect similar to the second endotoxin injection in endotoxin-pretreated animals, with the exception of the induction of acute but transient hypotension and lactic acidosis. These results indicate that, in our model, endotoxin pretreatment induces a prepared state, thus potentiating the hemodynamic and metabolic effects of FMLP, as well as the effect of FMLP on blood cells.

Acknowledgements

References

1   Ahmed N and Christou N: Systemic inflammatory response syndrome: interactions between cells and the endothelium. Shock *6*: S39-42, 1996.

2   Klosterhalfen B, Horstmann-Juhgemann K, Vogel P *et al*: Time course of various inflammatory mediators during recurrent endotoxemia. Biochem Pharmacol *43*: 2103-2109, 1992.

3   Ulloa L and Tracey KJ: The "cytokine profile": a code for sepsis. Trends Mol Med *11*: 56-63, 2005.

4   Yuo A, Kitagawa S, Kasahara T *et al*: Stimulation and priming of human neutrophils by interleukin-8: cooperation with tumor necrosis factor and colony-stimulating factors. Blood *78*: 2708-2714, 1991.

5   Gougerot Podicalo MA, Elbim C and Chollet Martin S: Modulation of the oxidative burst of human neutrophils by pro- and anti-inflammatory cytokines. Pathol Biol Paris *44*: 36-41, 1996.

6   Van den Berghe G, de Zegher F and Bouillon R: Acute and prolonged critical illness as different neuroendocrine paradigms. J Clin Endocrinol Metab *83*: 1827-1834, 1998.

7   Sprung CL, Schultz DR, Marcial E *et al*: Complement activation in septic shock patients. Crit Care Med *14*: 525-528, 1986.

8   Corrigan JJ, Ray WL and May N: Changes in the blood coagulation system associated with septicemia. N Engl J Med *279*: 851-856, 1968.

9   Becker EL: The formylpeptide receptor of the neutrophil. A search and conserve operation. Am J Pathol *129*: 16-24, 1987.

10 Marasco WA, Phan SH, Krutzch H *et al*: Purification and indetification of formyl-methionyl-leucyl-phenylalanine as the major peptide neutrophil chemotactic factor produced by *escherichia coli*. J Biol Chem *259*: 5430-5439, 1984.

11 Sahagun-Ruiz A, Colla JS, Juhn J, Gao JL, Murphy PM and McDermott DH: Contrasting evolution of the human leukocyte N-formylpeptide receptor subtypes FPR and FPRL1R. Genes Immun *2*: 335-342, 2001.

12 Le Y, Iribarren P, Zhou Y, Gong W, Hu J, Zhang X and Wang JM: Silencing the formylpeptide receptor FPR by short-interfering RNA. Mol Pharmacol *66*: 1022-1028, 2004.

13 Dalpiaz A, Spisani S, Biondi C, Fabbri E, Nalli M and Ferretti ME: Studies on human neutrophil biological functions by means of formyl-peptide receptor agonists and antagonists. Curr Drug Targets Immune Endocr Metabol Disord *3*: 33-42, 2003.

14 Tiffany HL, Lavigne MC, Cui YH, Wang JM, Leto TL, Gao JL and Murphy PM: Amyloid-beta induces chemotaxis and oxidant stress by acting at formylpeptide receptor 2, a G protein-coupled receptor expressed in phagocytes and brain. J Biol Chem *276*: 23645-23652, 2001.

15 Saito H, Matsuo M, Naminohira S *et al*: Effect of N-formyl-L-methionyl-L-leucyl-L-phenylalanine and its analogues on blood pressure. J Pharm Dyn *6*: 141-144, 1983.

16 Jonsson M, Tzanela M and Kolbeck RC: Hemodynamic and metabolic effects of intravenous formyl-methionyl-leucyl-phenylalanine (FMLP) in rabbits. In Vivo *11*: 133-140, 1997.

17 Bone RC: Toward a theory regarding the pathogenesis of the systemic inflammatory response syndrome: what we do and do not know about cytokine regulation. Crit Care Med *24*: 163-172, 1996.

18 Wichterman KA, Baue AE and Chaudry IH: Sepsis and septic shock – a review of laboratory models and a proposal. J Surg Res *29*: 189-201, 1980.

19 Fehr J, Dahinden C and Russi R: Formylated chemotactic peptides can mimic the secondary, provoking endotoxin injection in the generalized Shwartzman reaction. J Infec Dis *150*: 160-161, 1984.

20 Goldman DW, Enkel H, Gifford LA *et al*: Lipopolysaccharide modulates receptors for leukotriene B4, C5a, and formyl-methionyl-leucyl-phenylalanine on rabbit polymorphonuclear leukocytes. J Immunol *137*: 1971-1976, 1986.

21 Smedly LA, Tonnesen MG, Sandhaus RA *et al*: Neutrophil-mediated injury to endothelial cells. Enhancement by endotoxin and essential role of neutrophil elastase. J Clin Invest *77*: 1233-1243, 1986.

22 Voelkel NF, Czartolomna J, Simpson J *et al*: FMLP causes eicosanoid-dependent vasoconstriction and edema in lungs from endotoxin-primed rats. Am Rev Respir Dis *145*: 701-711, 1992.

23 Snyderman R and Fudman EJ: Demonstration of a chemotactic factor receptor on macrophages. J Immunol *124*: 2754-2757, 1980.

24 Doherty DE, Haslett C, Tonnesen MG *et al*: Human monocyte adherence: A primary effect of chemotactic factors on the monocyte to stimulate adherence to human endothelium. J Immunol *138*: 1762-1771, 1987.

25 Selvatici R, Falzarano S, Traniello S, Pagani Zecchini G and Spisani S: Formylpeptides trigger selective molecular pathways that are required in the physiological functions of human neutrophils. Cell Signal *15*: 377-383, 2003.

26 Miyake Y, Yasuhara T, Fuikui K *et al*: Purification and characterization of neutrophil chemotactic factors of streptococcus sanguis. Biochem Biophys Acta *758*: 181-186, 1983.

27 Rot A, Henderson LE, Coperland TD *et al*: A series of six ligands for the human formyl peptide receptor: Tetrapeptides with high chemotactic potency and efficacy. Proc Natl Acad Sci USA *84*: 7967-7971, 1987.

28 Meyer J, Traber LD, Nelson S *et al*: Reversal of hyperdynamic response to continuous endotoxin administration by inhibition of NO synthesis. J Appl Physiol *73*: 324-328, 1992.

29 Thiemermann C and Van J: Inhibition of Nitric Oxide Synthesis reduces the hypotension induced by bacterial lipopolysaccharides in the rat *in vivo*. Eur J Pharm *182*: 591-595, 1990.

30 Stetson CA: Studies on the mechanism of the Shwartzman phenomenon. Certain factors involved in the production of the local hemorrhagic necrosis. J Exp Med *93*: 489-504, 1951.

31 Stewart AG, Dubbin PN, Harris T *et al*: Platelet activating factor may act as a second messenger in the release of eicosanoids and superoxide anions from leukocytes and endothelial cells. Proc Natl Acad Sci USA *87*: 3215-3219, 1990.

32 Qi M and Jones SB: Contribution of platelet activating factor to hemodynamic and sympathetic responses to bacterial endotoxin in conscious rats. Circ Shock *32*: 153-163, 1990.

33 Bellomo R, Kellum JA and Pinsky MR: Transvisceral lactate fluxes during endotoxemia. Chest *110*: 198-204, 1996.

34 Johnson A, Hocking DC and Ferro TJ: Tumor necrosis factor-alpha primes pulmonary hemodynamic response to N-formyl-L-methionyl-L-leucyl-L-phenylalanine. Am J Physiol *261*: H996-1004, 1991.

35 Mitsuhashi H, Asano S, Nonaka T *et al*: Potency of truncated secretory leukoprotease inhibitor assesed in acute lung injury models in hamsters. J Pharmacol Exp Ther *282*: 1005-1010, 1997.

36 Atkinson YH, Marasco WA, Lopez AF *et al*: Recombinant human tumor necrosis factor-α. Regulation of N-formyl-methionyl-leucyl-phenylalanine receptor affinity and function on human neutrophils. J Clin Invest *81*: 759-765, 1988.

37 Cassatella MA, Bazzoni F, Ceska M *et al*: IL-8 production by human polymorphonuclear leukocytes. The chemoattractant formyl-methionyl-leucyl-phenylalanine induces the gene expression and release of IL-8 through a pertussis toxin-sensitive pathway. J Immunol *148*: 3216-3220, 1992.

38 Hojman H, Lounsbury D, Harris H *et al*: Immunodepressive effects of LPS on monocyte CD14 *in vivo*. J Surg Res *69*: 7-10, 1997.