# Oxidative injury and survival during endotoxemia

S. Basu[a,*], M. Eriksson[b]

[a] Department of Geriatrics, Faculty of Medicine, Uppsala University, S-751 25 Uppsala, Sweden
[b] Department of Anesthesiology and Intensive Care, Faculty of Medicine, Uppsala University, S-751 25 Uppsala, Sweden

Received 21 September 1998

**Abstract This study investigates the plasma levels of 8-iso-$PGF_{2\alpha}$, a non-enzymatic, and 15-K-DH-$PGF_{2\alpha}$, a cyclooxygenase catalyzed oxidation product of arachidonic acid in an experimental porcine endotoxemic shock model. A significant ($P < 0.001$) and rapid appearance and disappearance of $PGF_{2\alpha}$ metabolite after endotoxin infusion was very similar in both non-survival and survival groups indicating an acute progression and recession of inflammation. When oxidative injury was assessed by measuring free 8-iso-$PGF_{2\alpha}$ the levels in plasma increased significantly up to 2 h and remained at this level until death among the non-survivors. This was apparently different from the survivors where the 8-iso-$PGF_{2\alpha}$ levels increased to its height at 1 h, then decreased to the basal levels after 5 h. Thus, free radical and cyclooxygenase catalyzed oxidation of arachidonic acid occurs during endotoxemia. Free radical dependent oxidative injury following endotoxin induced inflammation may be the major cause of organ failure and increased mortality.**

**© 1998 Federation of European Biochemical Societies.**

*Key words:* Inflammation; Oxidative stress; Endotoxin; Isoprostane; Prostaglandin; Septic shock

## 1. Introduction

Oxygen derived free radicals are believed to be important mediators of cellular injury contributing to the development of sepsis or endotoxemic shock, a condition of major concern with a high mortality. The human response to the bacterial endotoxin or lipopolysaccarides, which are found in the cell wall of Gram-negative bacteria and which are released during bacterial death, includes cardiovascular and metabolic disorders and activation of coagulation cascades [1,2]. The increase of free radicals or arachidonic acid metabolites which cause pulmonary and renal vasoconstriction and increase vascular permeability seem to be directly involved in the tissue injury following endotoxin induced septic processes.

Several methods have been developed to measure the products of free radical induced lipid peroxidation to assess oxidant injury in vivo. The limitations of these methods in assessing lipid peroxidation as a mechanism of oxidative injury are common [3]. The non-enzymatic formation of isoprostanes through free radical catalysed oxidation of arachidonic acid or the formation of prostaglandins, mainly prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) through oxidation catalysed by cyclooxygenase, are thought to be unique indices of in vivo oxidative injury or inflammation, respectively [4–6]. 8-Iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), one of the major isoprostanes, increases during free radical mediated tissue damage in human and animal studies [4,5]. Primary prostaglandins, mainly $PGF_{2\alpha}$ increase following endotoxin induced inflammation [6,7], a consequence of cytokine induced cyclooxygenase (COX)-2 gene expression [8].

We have recently developed highly specific and sensitive radioimmunoassays (RIA) through raising unique antibodies in the rabbits against both 8-iso-$PGF_{2\alpha}$ and 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (15-K-DH-$PGF_{2\alpha}$), a major plasma metabolite of $PGF_{2\alpha}$, to be used in the study of a well proven experimental porcine endotoxemic shock model [5,6,9]. The antibodies distinctly discriminate these two very closely related substances which increase during two different important pathophysiological manifestations, namely, oxidative injury and inflammation. The details of the antibody properties and assay development are described elsewhere [5,6]. The ultimate aim of this study was to evaluate whether oxidative injury is involved in the endotoxin induced inflammation in an experimental porcine endotoxemia shock model and also its involvement in survival.

## 2. Materials and methods

### 2.1. Materials

Unlabelled 8-iso-$PGF_{2\alpha}$, 15-K-DH-$PGF_{2\alpha}$ and other related isoprostanes and prostaglandins were purchased from Cayman, Ann Arbor, MI, USA. Tris-HCl, Tris-base, EDTA-disodium salt and bovine γ-globulin were purchased from Sigma (St. Louis, MO, USA). Instagel scintillation cocktail was obtained from Packard Instruments (Meriden, CT, USA). Polyethylene glycol (MW 4000) was purchased from Merck (Germany). Tris-HCl buffer 0.05 M, pH 7.8, was used in the radioimmunoassay. Bovine γ-globulin 0.5% was prepared in the RIA buffer. Unlabelled 8-iso-$PGF_{2\alpha}$, 15-K-DH-$PGF_{2\alpha}$ standards, tritium labelled tracer and working antibody solution were prepared in the RIA buffer. The tritium labelled 8-iso-$PGF_{2\alpha}$ (specific activity 608 GBq $mmol^{-1}$) was synthesized and purified as described previously [5]. The tritium labelled 15-K-DH-$PGF_{2\alpha}$ (specific activity 6.77 TBq $mmol^{-1}$) was obtained from Amersham (Buckinghamshire, UK). Antibodies against both 8-iso-$PGF_{2\alpha}$ and 15-K-DH-$PGF_{2\alpha}$ were raised at our laboratory and well characterized [5,6].

### 2.2. Animal experiment

Endotoxemic septic shock was induced in 10 anesthetized pigs (both sexes; 3–4 months of age, 23–30 kg) by intravenous infusion of *E. coli* (10 μg/kg/h) during 6 h. Anesthesia and surgical procedures were performed according to experimental guidelines from our laboratory [9,10]. Various clinical and blood parameters were checked during the experiments [9,10]. Circulatory and respiratory variables deteriorated during endotoxemia. Blood samples were collected from all animals immediately before the start of the endotoxin infusion and thereafter every hour throughout the experiment. The plasma samples were kept frozen at −70°C until analysis. The animal experiments were approved by the Animal Ethical Committee, Uppsala University, Sweden.

### 2.3. Radioimmunoassay of 8-iso-$PGF_{2\alpha}$

The plasma samples were analyzed for 8-iso-$PGF_{2\alpha}$ by a newly developed radioimmunoassay at our laboratory as described elsewhere

*Corresponding author. Fax: (46) (18) 177976.
E-mail: samar.basu@geriatrik.uu.se

*Abbreviations:* RIA, radioimmunoassay; i.v., intravenous; PG, prostaglandins; TX, thromboxane; COX, cyclooxygenase

0014-5793/98/$19.00 © 1998 Federation of European Biochemical Societies. All rights reserved.
PII: S0014-5793(98)01290-3

[5]. In brief, unextracted plasma samples were used in the assay. The cross-reactivity of the 8-iso-$PGF_{2\alpha}$ antibody with 15-keto-13,14-dihydro-8-iso-$PGF_{2\alpha}$, 8-iso-$PGF_{2\beta}$, $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}$, $TXB_2$, 11β-$PGF_{2\alpha}$, 9β-$PGF_{2\alpha}$ and 8-iso-$PGF_{3\alpha}$, respectively, was 1.7, 9.8, 1.1, 0.01, 0.01, 0.1, 0.03, 1.8 and 0.6%. The detection limit of the assay was about 23 pmol/l.

*2.4. Radioimmunoassay of 15-K-DH-$PGF_{2\alpha}$*

The plasma samples were analyzed for 15-K-DH-$PGF_{2\alpha}$ by a newly developed radioimmunoassay at our laboratory as described elsewhere [6]. In brief, unextracted plasma samples were used in the assay. The cross-reactivity of the antibody with $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, $PGE_2$, 15-keto-13,14-dihydro-$PGE_2$, 8-iso-15-keto-13,14-dihydro-$PGF_{2\alpha}$, 11β-$PGF_{2\alpha}$, 9β-$PGF_{2\alpha}$, $TXB_2$ and 8-iso-$PGF_{3\alpha}$ was 0.02, 0.43, $<0.001$, 0.5, 1.7, $<0.001$, $<0.001$, $<0.001$ and 0.01%, respectively. The detection limit was about 45 pmol/l.

## 3. Results and discussion

*3.1. 15-K-DH-$PGF_{2\alpha}$ levels in plasma (inflammatory index)*

When 15-K-DH-$PGF_{2\alpha}$ was measured in the plasma as an index of inflammation, a significant increase of this metabolite was observed in both groups (non-survivors, $n=5$ and survivors, $n=5$; Fig. 1). A similar increase was previously demonstrated by us for proinflammatory cytokine TNFα levels [9] and platelet microvesicle formation [10] during endotoxemia. Cytokine regulated COX-2 gene expression is known to be involved in induced inflammation [8] as demonstrated in our study by in vivo $PGF_{2\alpha}$ formation. The rapid appearance and disappearance of the $PGF_{2\alpha}$ metabolite after endotoxin infusion was very similar in animals from both groups describing an acute progression and recession of inflammation via activation and deactivation of COX-2, respectively.

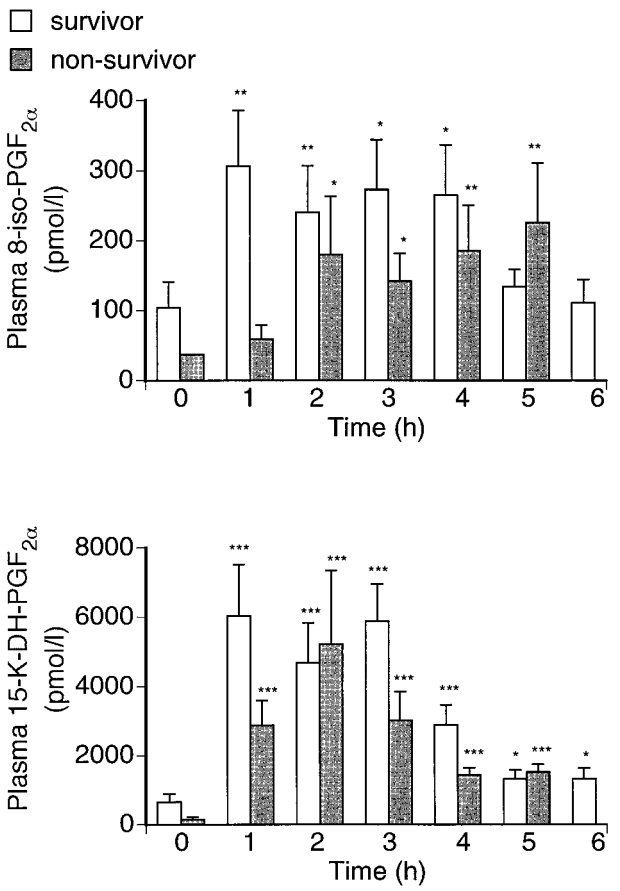



Fig. 1. The mean levels of 8-iso-$PGF_{2\alpha}$ (upper) and 15-K-DH-$PGF_{2\alpha}$ in plasma in surviving and/or non-surviving endotoxemic pigs (*$P<0.05$; **$P<0.01$; ***$P<0.001$).

*3.2. 8-iso-$PGF_{2\alpha}$ levels in plasma (oxidative injury index)*

An increase in 8-iso-$PGF_{2\alpha}$ in plasma as an index of oxidative injury was seen in all animals after endotoxin infusion but with a different time course in the survivors compared with the non-survivors (Fig. 1). The levels of 8-iso-$PGF_{2\alpha}$ in plasma increased up to 2 h and remained at this level until death among the non-survivors. This was apparently different from the survivors where the 8-iso-$PGF_{2\alpha}$ levels increased to its height at 1 h, then decreased to the basal levels after 5 h. This indicates an involvement of severe oxidative injury in the endotoxemic state both in non-survivors and survivors. One animal who died at about 2 h (not included in the figure) had very high levels of 8-iso-$PGF_{2\alpha}$ (34 times higher than the basal level in plasma) and 15-K-DH-$PGF_{2\alpha}$ (22 times in plasma).

These findings reveal that both free radical and cyclooxygenase catalyzed oxidation of arachidonic acid occur during endotoxemia. Free radical dependent oxidative injury following endotoxin induced COX-2 mediated inflammation may be the major cause of organ failure and increased mortality which may also be involved in human septic injury cases or other fatal diseases mediated by oxidative damage. The use of specific antibodies to determine the degree of oxidative modification of arachidonic acid opens excellent possibilities of accurate determination of both oxidative injury and inflammatory state in the pathogenesis of various diseases and clinical conditions.

In conclusion, it was seen that both free 8-iso-$PGF_{2\alpha}$ and 15-K-DH-$PGF_{2\alpha}$ in plasma increased rapidly in an experimental porcine model of endotoxemia indicating free radical and cyclooxygenase catalyzed oxidation of arachidonic acid are well involved in endotoxemia or septic shock. Free radical dependent oxidative injury following endotoxin induced, presumably COX-2 mediated inflammation may be the major cause of organ failure and increased mortality.

*Acknowledgements:* This study is financially supported by the Geriatrics Research Foundation, Swedish Diabetes Foundation, Loo and Hans Ostermans Foundation for Medical Research, Laerdal Foundation of Acute Medicine and Swedish Medical Research Council. The author is especially indebted to Professor Bengt Vessby for valuable discussions.

## References

[1] Burell, R. (1994) Circ. Shock 43, 137–153.
[2] Levi, M., ten Cate, H., van der Poll, T. and van Deventer, S.J. (1993) J. Am. Med. Assoc. 270, 975–979.
[3] Gutteridge, J.M.C. and Halliwell, B. (1990) Trends Biochem. Sci. 15, 129–135.
[4] Morrow, J. and Roberts II, L.J. (1996) Biochem. Pharmacol. 51, 1–9.
[5] Basu, S. (1998) Prostaglandins Leukotrienes Essent. Fatty Acids 58, 319–325.
[6] Basu, S. (1998) Prostaglandins Leukotrienes Essent. Fatty Acids 58, 347–352.
[7] Demling, R.H., Smith, M., Gunther, R., Flynn, J.T. and Gee, M.H. (1981) J. Am. Physiol. Soc. 240, H348–H353.
[8] Xie, W., Chipman, J.G., Robertson, D.L., Erikson, R.L. and Simmons, D.L. (1991) Proc. Natl. Acad. Sci. USA 88, 2692–2696.
[9] Eriksson, M., Lundqvist, K., Larsson, A., Nelson, D., Saldeen, T., Drott, P. and Erikson, Ö. (1997) Acta Anaesth. Scand. 41, 824–829.
[10] Eriksson, M., Nelson, D., Nordgren, A. and Larsson, A. (1998) Acta Anaesth. Scand. 42, 551–557.