

Retinol Palmitate Counteracts Oxidative Injury During Experimental Septic Shock

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Abstract

Introduction: Retinols seem to be of clinical importance in ameliorating the clinical consequences of septic shock. These beneficial effects of retinols are suggested to be due to an antioxidant property. The present study was undertaken in order to confirm or rule out such an effect of retinol palmitate (RP) in experimental septic shock by measuring F_2 -isoprostanes and a major prostaglandin $F_{2\alpha}$ metabolite as indicators of oxidative injury and inflammatory response, respectively. **Materials and Methods:** Fourteen anaesthetised pigs were randomly given an injection of RP ($2.300 \text{ IU} \times \text{kg}^{-1}$) or the corresponding volume of vehicle. All pigs received a continuous infusion of *E. coli* endotoxin ($10 \mu\text{g} \times \text{kg}^{-1} \times \text{h}^{-1}$). Blood samples were analysed for lipid peroxidation products (8-iso-PGF_{2 α}), indicating free radical induced oxidative injury and 15-keto-dihydro-PGF_{2 α} indicating cyclooxygenase-mediated inflammatory response). **Results:** Significantly elevated levels of 8-iso-PGF_{2 α} were seen at 3, 5 and 6 hours of endotoxaemia in the vehicle + endotoxin group as compared to RP + endotoxin group. Endotoxin induced cyclooxygenase-mediated inflammatory response was not affected by RP. **Conclusions:** This study is the first one to show that RP counteracts oxidative injury rather than inflammatory response in experimental septic shock. These results may be of importance for the understanding of some beneficial effects of RP during endotoxaemia (i.e. improved systemic haemodynamics and reduced serum levels of endotoxin). Our results may explain the therapeutic effects of nutrients rich in caroten/retinols used in some clinical studies.

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Key words: Endotoxin, Inflammation, Isoprostanes, Lipid peroxidation, Prostaglandins, Septic shock

Introduction

Gram-negative bacteria induced septic shock, a condition with high mortality, is frequently seen in critical care medicine. The wall of gram-negative bacteria consists of endotoxin, which is chemically composed of lipopolysaccharide, and is extremely biologically active. Endotoxin is released during lysis of gram-negative bacteria. Infusion of endotoxin into anaesthetised pigs causes pathophysiological changes and a shock condition resembling that seen during the early phase of human septic shock, which may lead to the systemic inflammatory response syndrome.¹ Endotoxaemia initiates several cascade systems including arachidonic acid metabolism which is induced both by free radicals and cyclooxygenases causing oxidative injury and severe inflammatory response, respectively. Such lipid peroxidation is associated with mortality in experimental septic shock.^{2,3}

We have previously shown that a single injection of retinol palmitate has beneficial effects on circulatory and

respiratory functions during porcine endotoxaemia.^{4,5} Retinol palmitate also exerts potential therapeutic effects in this model⁶ and counteracts the progressive increase of serum endotoxin due to a continuous iv infusion of endotoxin in the pig.⁵ Neutrophil activity is enhanced by vitamin A in conjunction with vitamin E in vivo.⁷ Furthermore, the circulating levels of α -tocopherol decreased during experimental septic shock in pigs. This reduction was greater in the pigs that died as compared to those that survived the standardised endotoxaemic challenge.⁸ Retinol modulates both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism in vitro.⁹ These findings may be clinically relevant. Patients suffering from septic shock and secondary organ dysfunction have low levels of both plasma retinol and plasma tocopherol as well as increased lipid peroxidation as evaluated by plasma thiobarbituric acid-reactive substances.¹⁰

It is well known that arachidonic acid metabolism through non-enzymatic and enzymatic lipid peroxidation pathways

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leads to the formation of isoprostanes and prostaglandins which are reliable parameters of oxidative injury and inflammation, respectively.^{3,11-14} The aim of this study was to determine whether exogenously added retinol palmitate (RP) has any effect on oxidative injury and/or inflammation (by affecting both the non-enzymatic and enzymatic lipid peroxidation) in experimental septic shock in pigs.

Materials and Methods

Materials

Unlabelled prostaglandins, 8-iso-PGF_{2α} and other related isoprostanes were purchased from Cayman Chemicals, Ann Arbor, MI, USA. The tritium labelled 8-iso-PGF_{2α} (specific activity: 608 GBq. mmol⁻¹) was synthesised and purified as described previously.¹³ The tritium labelled 15-keto-dihydro-PGF_{2α} (specific activity: 6.77 TBq. mmol⁻¹) was obtained from Amersham (Buckinghamshire, UK). Antibodies and radioimmunoassays for 8-iso-PGF_{2α} and 15-keto-dihydro-PGF_{2α} were developed and well characterised at our Laboratory.^{13,14}

Radioimmunoassay of 8-iso-PGF_{2α} (Oxidative Injury Indicator)

The plasma samples were analysed for 8-iso-PGF_{2α} by a newly developed radioimmunoassay at our laboratory as described elsewhere.¹³ In brief, unextracted plasma samples were used in the assay. The cross-reactivity of the 8-iso-PGF_{2α} antibody with 15-keto-13,14-dihydro-8-iso-PGF_{2α}, 8-iso-PGF_{2β}, PGF_{2α}, 15-keto-PGF_{2α}, 15-keto-13,14-dihydro-PGF_{2α}, TXB₂, 11β-PGF_{2α}, 9β-PGF_{2α} and 8-iso-PGF_{3α}, 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.

Radioimmunoassay of 15-keto-dihydro-PGF_{2α} (Inflammatory Response Indicator)

The plasma samples were analysed for 15-keto-dihydro-PGF_{2α} by a newly developed radioimmunoassay at our laboratory as described elsewhere.¹⁴ In brief, unextracted plasma samples were used in the assay. The cross-reactivity of the antibody with PGF_{2α}, 15-keto-PGF_{2α}, PGE₂, 15-keto-13,14-dihydro-PGE₂, 8-iso-15-keto-13,14-dihydro-PGF_{2α}, 11β-PGF_{2α}, 9β-PGF_{2α}, TXB₂ and 8-iso-PGF_{3α} 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.

Animals

Fourteen healthy pigs of both sexes, aged 12 to 14 weeks and weighing between 23 and 30 kg (mean 25.5 kg), were used in this experiment, which was approved by the Animal Ethics Committee of the University of Uppsala, Sweden (C 279/96).

Study Protocol

The pigs were randomly given an intramuscular injection of RP (Arovit®, Roche AG, Switzerland; 2.300 IU × kg⁻¹, n = 7), or the corresponding volume of solvent (sterile hydrogenated castor oil, n = 7). Endotoxaemic shock was induced an hour later in all pigs by a continuous intravenous infusion of *Escherichia coli* endotoxin (0111:B4; Sigma Chemical, St Louis, MO) at 10 µg × kg⁻¹ × h⁻¹ during 6 hours.

Animal Experiment

Anaesthesia was induced with Tiletamine-Zolazepam (6 mg × kg⁻¹; Zoletil forte vet®; Boehringer Ingelheim Vetmedica, Ingelheim, Germany) mixed with Xylazin (2.2 mg × kg⁻¹; Rompun Vet®; Bayer, Leverkusen, Germany) and 0.04 mg × kg⁻¹ of atropine and maintained with a continuous intravenous infusion of Pentobarbital natrium® (8 mg × kg⁻¹ × h⁻¹; Apoteksbolaget Sweden). Twenty mg of morphine (Pharmacia Sweden) was injected intravenously, and a tracheotomy was performed. An O₂/N₂O mixture (FiO₂: 0.3) was administered during the surgical preparation followed by O₂ in medical air (FiO₂: 0.3). The bladder was catheterised via a small vesicostomy. Balanced electrolyte solution with 2.5% glucose (Rehydrex™, Pharmacia-Upjohn) was infused at 30 mL × kg⁻¹ × h⁻¹ during the first hour, and thereafter at 10 mL × kg⁻¹ × h⁻¹. The cervical artery was catheterised. This catheter was used for blood sampling and mean arterial blood pressure (MAP) measurement. A central venous catheter and a 7F Swan-Ganz catheter equipped with a thermistor were inserted into the right external jugular vein and into a branch of the pulmonary artery, respectively. Central venous pressure (CVP; mmHg), pulmonary capillary wedge pressure (PCWP; mmHg), mean pulmonary arterial pressure (MPAP), and heart rate (HR; 1 × min⁻¹) were measured. Cardiac output (CO; l × min⁻¹) was calculated by the thermodilution technique. Left ventricular stroke work index (LVSWI) was calculated as MAP × stroke index (stroke index = cardiac output × heart rate⁻¹ × body surface area⁻¹). The amount of blood sampled for analysis was less than 5% of the total blood volume of each pig. 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 stored at -70°C until analysis later. This animal model has been previously described in detail.^{1,4,6}

Statistical Methods

The results were expressed as mean ± SD. A *P* value <0.05 was considered significant. Differences between the two groups were calculated by an analysis of variance model (ANOVA) with factors for treatment, pig and time point. These calculations were based on the individual

values for each pig at baseline (0 h) and each time of measurement. For variables with skewed distributions, logarithmic transformation was performed before statistical analysis.

Results

At inclusion, the pigs did not differ significantly in weight, body surface area, circulatory or laboratory variables. Induction of septic shock was achieved by the infusion of *Escherichia coli* endotoxin with a pronounced increase in MPAP followed by subsequent derangements in respiratory and circulatory variables.

Effect of RP on Oxidative Injury as Measured by Plasma 8-iso-PGF_{2α}

A rapid and significant increase in 8-iso-PGF_{2α} was observed in solvent infused endotoxaemic animals (Fig. 1). The plasma levels of 8-iso-PGF_{2α} increased rapidly from 242 pmol/l to 958 pmol/l within 2 h and thereafter, increased slowly to a peak of 2109 pmol/l at 5 h. At 6 h when the experiment ended, the levels of 8-iso-PGF_{2α} decreased to 1048 pmol/l. Such a rapid increase was not seen in the RP treated endotoxaemic pigs but rather, a slow gradual increase in the basal levels of 8-iso-PGF_{2α} was observed (highest increase was 282 pmol/l to 499 pmol/l from 0 h to 1 h). There was a significant difference ($P = 0.0264$ at 3h, $P = 0.0241$ at 5 h and $P = 0.0276$ at 6 h) in the kinetics of increment of 8-iso-PGF_{2α} from time zero to the following hours between the solvent and RP infused endotoxaemic pigs. We have previously shown that high levels of 8-iso-PGF_{2α} in plasma and urine indicating free radical mediated oxidative injury during porcine endotoxaemia are associated with increased mortality.^{2,3}

Effect of RP on Inflammatory Response as Measured by

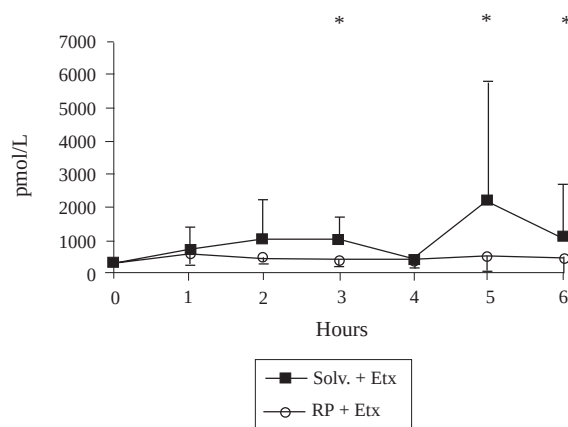


Fig. 1. 8-iso-prostaglandinF_{2α} levels in plasma of endotoxaemic pigs (10 mL × kg⁻¹ × h⁻¹) pretreated with retinol palmitate (2,300 × kg⁻¹) or the corresponding volume of solvent (RP + Etx; n = 7 and Solv. + Etx; n = 7, respectively). "Time 0" = Start of endotoxin infusion.

* $P < 0.05$.

Plasma 15-keto-dihydro-PGF_{2α}

A rapid increase of 15-keto-dihydro-PGF_{2α} was observed both in solvent and RP injected endotoxaemic animals (Fig. 2). The highest increase of plasma 15-keto-dihydro-PGF_{2α} (585 pmol/l at 0 h to 7201 pmol/l at 2 h) was observed at 2 hours after the start of the endotoxin infusion in the solvent group. In the RP group, plasma 15-keto-dihydro-PGF_{2α} levels increased from 979 pmol/l at 0 h to the highest 6331 pmol/l at 1 h and returned to 1251 pmol/l at the end of the experimental period (6 h). The initial increase (between 0 and 2 h) of this metabolite was very similar in the RP and solvent groups. There was no significant difference at any time points up to 6 h; when the changes from time zero were compared after endotoxin infusion between RP and solvent groups.

Discussion

Retinoids have previously been shown to inhibit the generation of superoxide by activated polymorphnuclear leukocytes.¹⁵ To our knowledge, the present study is the first to demonstrate that retinol palmitate exerts an antioxidant effect on in vivo free radicals catalysed lipid peroxidation-mediated oxidative injury which might be beneficial in human septic shock cases. The oxidative injury parameter 8-iso-PGF_{2α} in plasma was significantly decreased in the retinol palmitate treated animals compared to the solvent treated endotoxaemic animals. This effect is thought to be due to an inhibition of F₂-isoprostane formation through a free radical scavenging ability of retinol palmitate and subsequently, a diminished oxidative injury. However, no such inhibition of cyclooxygenase catalysed prostaglandin F_{2α} formation was observed. This further demonstrates that retinol palmitate has a profound effect on radical scavenging and thereby a beneficial effect on

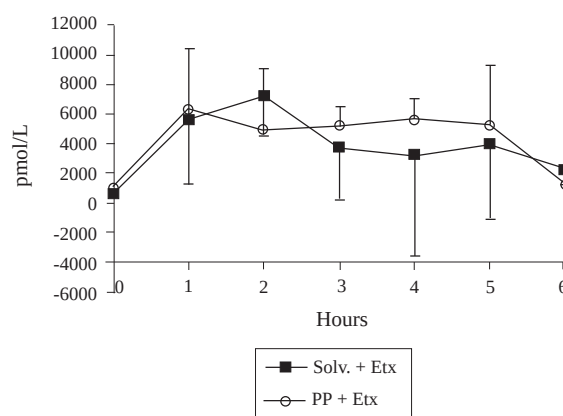


Fig. 2. 15-keto-dihydro-prostaglandinF_{2α} levels in plasma of endotoxaemic pigs (10 mL × kg⁻¹ × h⁻¹) pretreated with retinol palmitate (2,300 × kg⁻¹) or the corresponding volume of solvent (RP + Etx; n = 7 and Solv. + Etx; n = 7, respectively). "Time 0" = Start of endotoxin infusion.

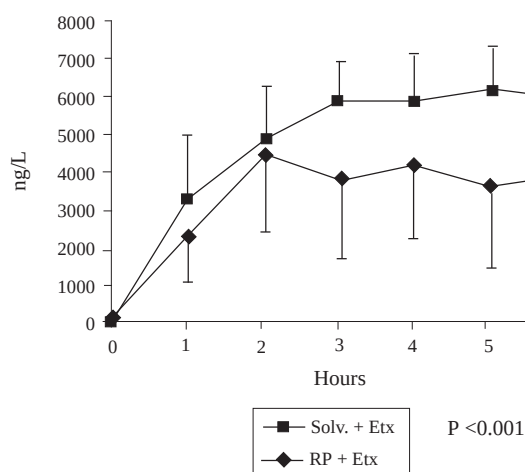


Fig. 3. Endotoxin levels in endotoxaemic pigs ($10 \mu\text{g} \times \text{kg}^{-1} \times \text{h}^{-1}$) pretreated with retinol palmitate $2,300 \times \text{kg}^{-1}$ (RP + Etx; $n = 5$) or the corresponding volume of solvent (Solv. + Etx; $n = 5$). "Time 0" = Start of endotoxin infusion. Reprinted with kind permission from Munksgaard Scientific Journals, Denmark.

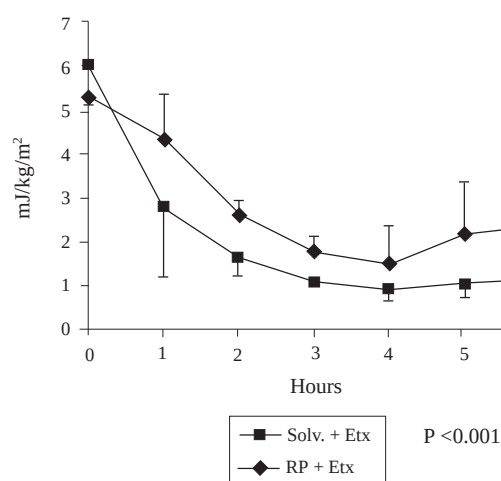


Fig. 4. Left ventricular stroke work index in endotoxaemic pigs ($10 \mu\text{g} \times \text{kg}^{-1} \times \text{h}^{-1}$) pretreated with retinol palmitate $2,300 \times \text{kg}^{-1}$ (RP + Etx; $n = 5$) or the corresponding volume of solvent (Solv. + Etx; $n = 5$). "Time 0" = Start of endotoxin infusion. Reprinted with kind permission from Munksgaard Scientific Journals, Denmark.

oxidative injury rather than on cyclooxygenases mediated inflammatory response. Whether the faster excretion of endotoxin from the circulation after retinol palmitate administration in endotoxaemic pigs shown earlier by our group has any association with the radical scavenging properties of retinol palmitate is not known from this study. However, there is a similarity in the excretion pattern of endotoxin from the blood and the plasma levels of isoprostane. Our results may point towards the possibility that RP may be of importance in preventing some of the clinical consequences of septic shock. In fact, an enteral nutrient rich in vitamin A has been shown to reduce the incidence of septic complications.¹⁶

The present results of the antioxidative effect of RP can explain the beneficial effect of this compound in our previous study in endotoxaemic pigs.⁵ The endotoxin levels in serum (analysed by a chromogenic substrate^{5,17}) reached a plateau after 2 h of endotoxaemia in the RP treated pigs, in contrast to the control group in which the serum endotoxin levels reached a significantly ($P < 0.001$) higher level. This is an interesting observation since the greatest differences in serum endotoxin levels between the two groups of pigs were at 3 h, 5 h and 6 h; i.e. the times when the plasma levels of 8-iso-PGF_{2α} also reached statistical significance (Fig. 3). Biosynthesis and release of prostaglandin-like compounds occur in a pulsatile manner. LVSWI, a measurement of the amount of energy that the left ventricle of the heart transfers to the blood while pumping it into the arteries, was significantly ($P < 0.001$) better preserved during endotoxaemia in the RP treated pigs (Fig. 4). This beneficial effect of RP on LVSWI seems not to be due to cyclooxygenase-mediated myocardial production of metabolites of prostaglandin or thromboxane A₂⁴ but may

be explained by reduced oxidative injury, as evaluated by the decreased formation of 8-iso-PGF_{2α}.

In conclusion, this study shows that retinol palmitate counteracts oxidative injury in experimental septic shock by affecting F₂-isoprostane formation. No such effect was seen in prostaglandin F_{2α} formation, the inflammatory response indicator in experimental septic shock in pigs.

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