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Biomarkers of free radical injury during spinal cord ischemia

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Abstract Plasma and urinary levels of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ were analysed at baseline and during the ischemia-reperfusion period in experimental spinal cord ischemia. A significant and immediate increase of 8-iso-$PGF_{2\alpha}$ in plasma at the start and up to 60 min, and in the urine at 90–150 min following ischemia indicate an association of oxidative injury. The inflammatory response indicator 15-keto-dihydro-$PGF_{2\alpha}$ in plasma increased significantly at the start and up to 60 min after ischemia. No such increase was seen in animals with no spinal cord ischemia. Thus, free radical mediated and cyclooxygenase catalysed products of arachidonic acid are increased during spinal cord ischemia as a consequence of oxidative injury and inflammation.

Key words: Ischemia-reperfusion; Prostaglandin; Isoprostane; Spinal cord; Oxidative injury; Inflammation

1. Introduction

Spinal cord ischemia resulting in paraplegia remains the most devastating complication following thoracoabdominal aortic replacement [1]. Oxidative injury caused by lipid or protein oxidation through intracellular free radical formation is believed to contribute to ischemic-reperfusion injury [2,3]. The present hypothesis is whether experimental spinal cord ischemia can cause any increase in the non-enzymatic and enzymatic oxidative processes in the body.

Isoprostanes biosynthesised from arachidonic acid in vivo mainly through non-enzymatic free radical catalysed oxidation [4,5] and are elevated in several oxidant injury syndromes [4–11]. Cyclooxygenase-2 (COX-2) has been shown to be expressed in various cells by several pro-inflammatory stimuli leading to the release of prostaglandins (PGs) [12,13]. 15-Keto-dihydro-$PGF_{2\alpha}$, a major metabolite of $PGF_{2\alpha}$, is increased in inflammatory response and can be used as an indicator of in vivo lipid peroxidation through the COX pathway [14]. We have developed specific radioimmunoassays through raising antibodies against both 8-iso-$PGF_{2\alpha}$ (a major isoprostane indicating oxidative injury) and 15-keto-dihydro-$PGF_{2\alpha}$ (indicating inflammatory response) [14,15]. By quantifying these parameters, we have shown that oxidative modification of arachidonic acid is involved in hepatotoxicity [9], septic shock [8,10], after cardiac arrest [16] and rheumatic diseases [11]. This implicates that oxidative injury and inflammation are closely associated in these conditions but with different kinetics of formation of arachidonic acid metabolites due to diverse biochemical regulatory systems that are involved.

The goal of this study was to investigate oxidative injury and inflammatory response following spinal cord ischemia-reperfusion as measured by plasma and urinary 8-iso-$PGF_{2\alpha}$, and plasma 15-keto-dihydro-$PGF_{2\alpha}$ in a well-described model of experimental ischemia-reperfusion in pigs. The grade of ischemia was continuously monitored in cerebrospinal fluid (CSF) by intrathecal PO_2 and PCO_2 correlated with the ultrastructural changes in the spinal cord after clamping and declamping of the descending aorta [17].

2. Materials and methods

2.1. Animal preparation and experimental model

The experiments were performed in 11 Swedish domestic pigs weighing 23.3–37.4 kg. The experimental model was previously described [17]. In brief, following general anaesthesia and surgical preparation, a limited distal thoracic laminectomy was performed, and over an arterial-needle introducer a multiparameter PO_2, PCO_2, pH and temperature sensor (Paratrend 7) was introduced into the intrathecal space for continuous CSF monitoring of PO_2, PCO_2 and pH. A plastic tube was inserted as an aortic shunt via the proximal left subclavian artery close to the aortic arch and to the distal abdominal aorta via proximal left common iliac artery. The thoracic aorta just distal to the left subclavian artery was clamped and the distal aortic perfusion was restored after 3 min through the aortic shunt. The right subclavian artery was also clamped followed by interruption of all the lumbar arteries with ligatures except for L1, and 3 min were allowed for CSF recordings before placement of the distal aortic clamp below the level of L1. Animals were divided into two groups depending on the level of the distal aortic cross-clamping (DAXC): Group A ($n = 8$, spinal cord ischemia): DAXC caudal to the level of L1 and perfusion of only the visceral arteries through the aortic shunt; group B ($n = 3$, no spinal cord ischemia): DAXC cranial to the level of T12 and perfusion of the spinal cord (T12-L1) together with the visceral arteries via the aortic shunt. DAXC was 60 min and was followed by a 1-h reperfusion period. Paratrend recordings were continuously obtained during AXC and reperfusion. Central hemodynamic measurements were also obtained before AXC, before declamping, 30 and 60 min after declamping. At the end of the declamping period, 1000 ml isotonic saline (37°C) were perfused followed by paraformaldehyde (1%) and glutaraldehyde (2%) in Millonig (300 mOsm buffer) solution with a pH of 7.4. During the perfusion procedure distal aorta below L3 and all visceral arteries were clamped, giving the solution solely to the organs supplied by intercostal, subclavian and carotid arteries. The spinal cord specimens were then harvested from the cervical and lower thoracic spinal cord through laminectomies. Study approval was obtained from the local Ethics Committee of the Uppsala University.

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Abbreviations: PG, prostaglandin; TXB_2, thromboxane B_2; CSF, cerebrospinal fluid; DAXC, distal aortic cross-clamping; COX, cyclooxygenase

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2.2. Sample collection

Blood samples were collected from superior *vena cava* at the baseline, after start of ischemia (aortic cross-clamping), 15, 30 and 60 min after double cross-clamping and 30, 60 and 90 min after declamping and reperfusion (=90, 120 and 150 min after start of ischemia). Urinary samples were collected from the urinary bladder by a catheter at the same time points as for the blood samples collection. Samples were stored frozen at −70°C until analysis.

2.3. Radioimmunoassays of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$

Plasma and/or urinary samples were analysed for 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ by radioimmunoassay as described elsewhere [14,15]. 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-$PGF_{2\alpha}$, thromboxane B_2 (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. The cross-reactivity of the 15-keto-dihydro-$PGF_{2\alpha}$ 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, 0.01%, respectively. The detection limit of the assay was about 45 pmol/l.

2.4. Statistical analysis

The results were expressed as mean ± S.D. A P value <0.05 was considered to be 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 point of measurement. For variables with skewed distributions, logarithmic transformation was performed before statistical analysis.

3. Results

3.1. Oxidative injury as measured by plasma and urinary 8-iso-$PGF_{2\alpha}$

Baseline levels of 8-iso-$PGF_{2\alpha}$ in the plasma and urine did not differ between the groups. A significant difference in the concentration of 8-iso-$PGF_{2\alpha}$ in both plasma and urine was observed between groups A and B at various time points after ischemia. 8-Iso-$PGF_{2\alpha}$ increased significantly in plasma in animals with spinal cord ischemia (group A) after the start of clamping of the proximal and distal part of decending thoracic aorta ($P=0.03$) and continued to be significantly higher than the baseline values ($P=0.02$ at 15 min; $P=0.03$ at 30 min; $P=0.03$ at 60 min) until 60 min of ischemia but not at the following reperfusion period after declamping of the aorta

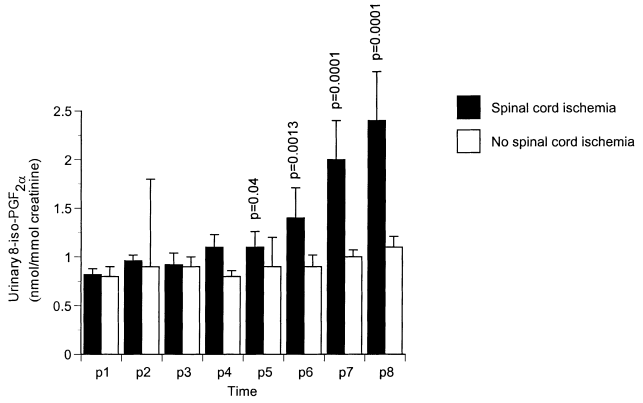

Fig. 2. The urinary levels of free 8-iso-$PGF_{2\alpha}$ in animals at different times (p1 = baseline; p2 = aortic cross-clamping; p3, p4, p5 = 15, 30, 60 min after double cross-clamping; p6, p7, p8 = 30, 60, 90 min after aortic declamping and reperfusion) with or without spinal cord ischemia (P values are denoted on the bars).

(Fig. 1). Similarly, the urinary concentration of 8-iso-$PGF_{2\alpha}$ increased significantly ($P=0.04$ at 60 min; $P=0.0013$ at 90 min; $P=0.0001$ at 120 min; $P=0.0001$ at 150 min after start of ischemia) from 60 min to the end of the experiment at 150 min after the start of ischemia (=90 min after the start of declamping of the aorta and reperfusion period) (Fig. 2). No such difference was found in the animals with no spinal cord ischemia (group B). Neither there was a significant difference in plasma 8-iso-$PGF_{2\alpha}$ levels during the reperfusion period compared to the baseline values.

3.2. Inflammatory response as measured by plasma 15-keto-dihydro-$PGF_{2\alpha}$

Baseline levels of 15-keto-dihydro-$PGF_{2\alpha}$ as an indicator of primary $PGF_{2\alpha}$ release in the plasma did not differ between the groups. After clamping the thoracic aorta prostaglandin metabolite levels increased in both groups (A and B) but with different fashion and magnitude between the groups. The increase was significant, much higher and longer in duration in the animals (group A) with spinal cord ischemia ($P=0.006$ at the start; $P<0.0001$ at 15 min; $P<0.0001$ at 30 min; $P=0.04$ at 60 min after the ischemia starts) compared to

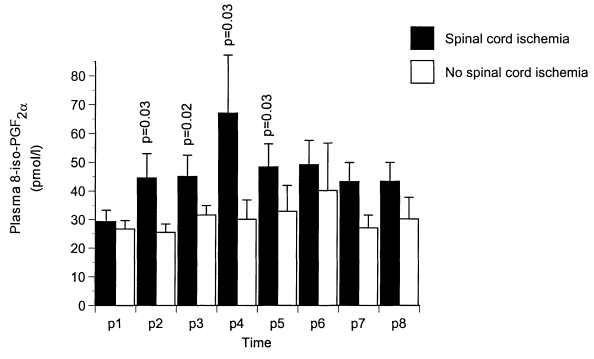

Fig. 1. The levels of free 8-iso-$PGF_{2\alpha}$ in peripheral plasma at different times (p1 = baseline; p2 = aortic cross-clamping; p3, p4, p5 = 15, 30, 60 min after double cross-clamping; p6, p7, p8 = 30, 60, 90 min after aortic declamping and reperfusion) in animals with or without spinal cord ischemia (P values are denoted on the bars).

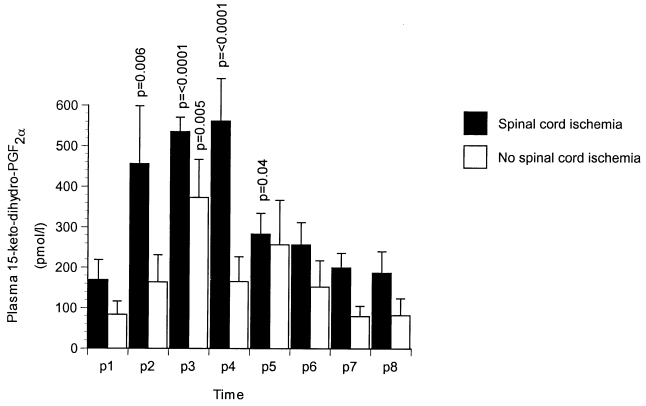

Fig. 3. The levels of 15-keto-dihydro-$PGF_{2\alpha}$ in peripheral plasma at different times (p1 = baseline; p2 = aortic cross-clamping; p3, p4, p5 = 15, 30, 60 min after double cross-clamping; p6, p7, p8 = 30, 60, 90 min after aortic declamping and reperfusion) in animals with or without spinal cord ischemia (P values are denoted on the bars).

the animals (group B) with no spinal cord ischemia ($P = 0.005$ at 15 min after start of ischemia) (Fig. 3). No significant increase of plasma 15-keto-dihydro-$PGF_{2\alpha}$ was observed during the reperfusion period after declamping of the thoracic aorta in either of the groups.

4. Discussion

This study describes a rapid appearance of oxidative stress and inflammatory response caused by cross-clamping of the thoracic aorta to achieve an experimental spinal cord ischemia. Since free radicals are involved in the oxidative injury which might cause enhanced lipid peroxidation [16] this study investigates the role of lipid peroxidation in spinal cord ischemia-reperfusion. To our knowledge, this report is the earliest to describe an increase of both isoprostane and prostaglandin formation during spinal cord ischemia. It demonstrated that both 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ in the plasma are increased instantly and continued during 60 min of the ischemic period indicating an association of oxidative stress and inflammation during the clamping of the proximal and distal descending thoracic aorta. When urinary 8-iso-$PGF_{2\alpha}$ was taken into consideration as an indication of excretion of 8-iso-$PGF_{2\alpha}$, an excellent correlation was found between the plasma and urinary measurement of this compound due to a small time difference in the appearance and disappearance in blood and urine which corroborates with previous studies [9,18]. These indicate that both free radical and COX mediated oxidation take place immediately after an ischemia which further proves that involvement of free radical and COX catalysation of arachidonic acid is a rapid process when an obstruction of the major spontaneous circulation is achieved. Those animals who did not achieve spinal cord ischemia might suffered of surgical trauma as evidenced by a mild rise of 15-keto-dihydro-$PGF_{2\alpha}$ metabolite in plasma at 15 min after aortic double cross-clamping.

We have previously reported that the oxidative stress parameter 8-iso-$PGF_{2\alpha}$ arose in the circulation within 2 h in rats after oral administration of CCl_4 and which was followed by inflammatory $PGF_{2\alpha}$ release [9]. In experimental septic shock, $PGF_{2\alpha}$ levels increased within half-an hour which was followed by an increase of 8-iso-$PGF_{2\alpha}$ [8,10]. However, this study shows that both the oxidative stress and inflammatory response parameters appear instantly in plasma and continue to release during the whole ischemic period and slowly returned to their basal levels at the end of the reperfusion period. A rapid increase of both F_2-isoprostanes and $PGF_{2\alpha}$ metabolite levels in plasma has also been shown following resuscitation after cardiac arrest [16]. Together, it indicates that by shunting major aorta the induction of free radicals related reactions in body appear to be an instant process. Thus, although the oxidative stress and inflammatory response are closely linked the kinetics of appearance and disappearance of free radical induced or COX catalysed oxidative state is a process which mainly depends on the type of insult that body had experienced.

The cross-reactivity of 15-keto-dihydro-$PGF_{2\alpha}$ to 8-iso-$PGF_{2\alpha}$ antibody is only 0.01%. However, in this study we have seen that the levels of 15-keto-dihydro-$PGF_{2\alpha}$ increased about 10 times more than the levels of 8-iso-$PGF_{2\alpha}$ in plasma at time points 15, 30, 60 min after aortic double cross-clamping in the spinal cord ischemia group. These increases of 8-iso-$PGF_{2\alpha}$ at these time points are not due to the cross-reactivity of the 8-iso-$PGF_{2\alpha}$ antibody by the 15-keto-dihydro-$PGF_{2\alpha}$ levels in the plasma as evidenced by our recent study where induction of $PGF_{2\alpha}$ formation by conjugated linoleic acid is inhibited by COX-2 inhibitor but not the induction of 8-iso-$PGF_{2\alpha}$ formation [19]. This indicates that even if the levels of 15-keto-dihydro-$PGF_{2\alpha}$ arise an order of magnitude above those of F_2-isoprostane in this study the cross-reactivity of 0.01% (8-iso-$PGF_{2\alpha}$ antibody to 15-keto-dihydro-$PGF_{2\alpha}$) does not affect the levels of isoprostanes.

In conclusion, free radical and COX catalysed oxidation of arachidonic acid are involved in spinal cord ischemia. Enhanced oxidative injury and inflammatory response as demonstrated by a rapid increase of both 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ in the body fluids play an eminent role in spinal cord ischemia and thus can be used as biomarkers of ischemic injury in the spinal cord.

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