Development of a novel biomarker of free radical damage in reperfusion injury after cardiac arrest

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Abstract In a porcine model of cardiopulmonary resuscitation (CPR), we investigated changes in the plasma levels of 8-iso-PGF$_{2\alpha}$, a marker for oxidative injury, and 15-keto-dihydro-PGF$_{2\alpha}$, an inflammatory response indicator during the post-resuscitation period after cardiac arrest. Twelve piglets were subjected to either 2 or 5 min (VF2 and VF5 group) of ventricular fibrillation (VF) followed by 5 min of closed-chest CPR. Six piglets without cardiac arrest were used as controls. In VF5 group, 8-iso-PGF$_{2\alpha}$ in the jugular bulb plasma (draining the brain) increased four-fold. Jugular bulb 8-iso-PGF$_{2\alpha}$ in the control group remained unchanged. The 15-keto-dihydro-PGF$_{2\alpha}$ also increased four-fold in the VF5 group. Thus, 8-iso-PGF$_{2\alpha}$ and 15-keto-dihydro-PGF$_{2\alpha}$ measurements in jugular bulb plasma may be used as biomarkers for quantification of free radical catalyzed oxidative brain injury and inflammatory response in reperfusion injury.

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1. Introduction

Neurologic outcome after cardiac arrest remains poor. Complicating post-ischemic disturbances could contribute to poor neurologic recovery after circulatory arrest [1]. Oxidative stress caused by lipid or protein oxidation through intracellular free radical generation may aggravate a perturbed membrane function and mitochondrial dysfunction and is believed to contribute to the reperfusion injury [2,3]. Furthermore, local inflammatory mechanisms including the release of cytokines, an upregulation of cell adhesion molecule expression and the subsequent induction of leukocyte accumulation could aggravate the microvascular dysfunction after ischemia [4] and may, therefore, impair the potential for neurologic recovery after circulatory arrest.

A problem related to the assessment of reperfusion injury has been the limitation in available methods for in vivo measurement of free radical generation or end products of free radical catalyzed oxidation of lipids [5]. In 1990, Morrow et al. reported the discovery of isoprostanes, a new family of prostaglandin derivatives biosynthesized in vivo through non-enzymatic free radical catalyzed oxidation of arachidonic acid [6]. It has also been observed that one of the major isoprostanes, 8-iso-prostaglandin F$_{2\alpha}$ (8-iso-PGF$_{2\alpha}$, Fig. 1) is increased in several syndromes that are supposed to be associated with oxidant injury and the measurement of isoprostanes can be used as a reliable method for in vivo measurement of oxidant injury [7–14].

Cyclooxygenase-2 (COX-2), an isoform of cyclooxygenase, has been shown to be expressed in macrophages, epithelial cells and fibroblasts by several pro-inflammatory stimuli (cytokines, growth factors), leading to the release of prostaglandins (Fig. 1) [15–17]. 15-Keto-13,14-dihydro-PGF$_{2\alpha}$, a major metabolite of prostaglandin F$_{2\alpha}$, is increased in inflammatory response and can be used as an indicator for in vivo lipid peroxidation through the cyclooxygenase pathway [18].

We have recently developed highly specific and sensitive radioimmunoassays (RIA) by raising unique antibodies in rabbits against both 8-iso-PGF$_{2\alpha}$ (indicating oxidative injury) and 15-keto-dihydro-PGF$_{2\alpha}$ (indicating inflammatory response) [18,19]. The antibodies discriminate these two very closely related substances. By applying these parameters, we have shown that oxidative modification of arachidonic acid through both non-enzymatic and enzymatic pathways is involved in endotoxin induced inflammation and hepatotoxic induced oxidative injury as demonstrated by significantly increased formation of F$_2$-isoprostane and PGF$_{2\alpha}$ metabolite in plasma and urine [10,12,14]. These results suggest that oxidative injury and inflammation are closely associated in various syndromes, and could be involved in the pathogenesis of other free radical mediated diseases.

In the present study, oxidative injury and inflammatory response in the brain after resuscitation from circulatory arrest were investigated by the measurement of 8-iso-PGF$_{2\alpha}$ and 15-keto-dihydro-PGF$_{2\alpha}$ in plasma collected from the superior bulb of the internal jugular vein (jugular bulb), a direct continuation of the sigmoid sinus which drains the brain. Simultaneous measurement of F$_2$-isoprostane and prostaglandin metabolite in pulmonary arterial blood samples (mixed venous blood from the superior and inferior vena cava and the coronary sinus) provides further information about systemic oxidative reperfusion injury and inflammation, respectively.
2. Materials and methods

2.1. Chemicals

Unlabelled 8-iso-PGF$_{2\alpha}$, 15-K-DH-PGF$_{2\alpha}$ and other related iso-prostanates and prostaglandins were purchased from Cayman Chemicals, Ann Arbor, MI, USA. The tritium labelled 8-iso-PGF$_{2\alpha}$ (specific activity: 608 GBq mmol$^{-1}$) was synthesized and purified as described previously [19]. 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 are well characterized [18,19].

2.2. Animal preparation

The animal experiments were approved by the Animal Ethical Committee, Uppsala University, Sweden and in accordance with a well established protocol from our laboratory [20]. Eighteen Swedish breed piglets of both gender, 11–15 weeks of age and with a mean weight of 24 ± 1 kg were used. Catheters were inserted via a branch of the right external carotid artery into the aortic arch for pressure monitoring, and via the right external jugular vein into a branch of pulmonary artery for blood sampling. A catheter was also inserted into the right atrium for drug administration and another catheter (20 Gauge) was inserted into the left internal jugular vein and passed retrogradely into the jugular bulb for blood sampling.

2.3. Experimental protocol

Nitrous oxide was discontinued after animal preparation and the piglets were ventilated with 30% oxygen in air. After 30 min, baseline values were obtained. The piglets were then randomized into three groups. The first group, denoted VF5, was subjected to 5 min of VF followed by 5 min of closed-chest CPR. The second group, denoted VF2, was subjected to 2 min of VF followed by 5 min of closed-chest CPR. Six animals served as a control group with no further interventions. In the two intervention groups, VF was induced with a brief alternating current shock of 40–60 V administered by two subcutaneous needles. Cardiac arrest was defined as VF on the ECG and the loss of arterial pulsation. Ventilation was stopped at the same time. After the non-intervention period (2 or 5 min) external thoracic compressions (80/min) were applied in the intervention groups and ventilation was resumed with 100% oxygen. A bolus injection of 20 µg/kg epinephrine was administered through the right atrial catheter 4 min after the commencement of CPR. External defibrillatory shocks of 200 J were applied 1 min after epinephrine administration (5 min of closed-chest CPR). Defibrillatory shocks were applied over a maximum period of 5 min. CPR was discontinued if ROSC was not achieved during this time. ROSC was defined as a pulsatile rhythm with a systolic aortic blood pressure $\geq$ 60 mm Hg maintained for at least 10 min. After 5 min of spontaneous circulation the FIO$_2$ was reset to 0.3. Blood samples were collected from the jugular bulb catheter and the pulmonary artery catheter at baseline, 5 min after ROSC, 30 min after ROSC and every 30 min thereafter, up to 6 h of spontaneous circulation. The plasma samples were kept frozen at $-70^\circ$C until analysis.

2.4. Radioimmunoassay of 8-iso-PGF$_{2\alpha}$

The plasma samples were analyzed for 8-iso-PGF$_{2\alpha}$ by a radioimmunoassay.
immunoassay (RIA) at our laboratory as described elsewhere [19]. 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.

2.5. Radioimmunoassay of 15-K-DH-PGF$_{2\alpha}$

The plasma samples were analyzed for 15-K-DH-PGF$_{2\alpha}$ by a RIA at our laboratory as described elsewhere [18]. 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, 0.01%, respectively. The detection limit was about 45 pmol/l.

2.6. Statistical analysis

All data are expressed as mean ± S.E.M. Repeated measures of ANOVAs were performed to test differences between treatment groups over time periods. For the ANOVAs that were statistically significant, the Bonferroni-Dunn multiple comparison procedure was performed to determine how the mean responses among the treatment groups differed.

Fig. 2. Mixed venous plasma levels of 8-iso-PGF$_{2\alpha}$ at baseline and after ROSC (upper panel). Jugular bulb plasma levels of 8-iso-PGF$_{2\alpha}$ at baseline and after ROSC (lower panel). †, group VF5; ■, group VF2; and ▲, control group. * significant difference versus baseline; and §, significant difference between group VF5 versus other groups. Values are expressed as means ± S.E.M.
groups differed. To determine differences within each group over time paired t-tests were used. P values of < 0.05 were considered to indicate a significant difference.

3. Results

3.1. Oxidative injury as determined by plasma 8-iso-PGF$_{2\alpha}$

No differences in baseline levels of mixed venous or jugular bulb 8-iso-PGF$_{2\alpha}$ were observed between the groups. 8-iso-PGF$_{2\alpha}$ in the mixed venous plasma increased significantly in both intervention groups within 5 min after ROSC ($P = 0.005$ and $P = 0.014$ for group VF2 and VF5, respectively, as compared to the baseline, Fig. 2, upper panel). Mixed venous levels of 8-iso-PGF$_{2\alpha}$ returned to baseline within 30 min in the VF2 group and within 60 min in the VF5 group and remained unchanged during the remainder of the study.

The group subjected to 5 min of ventricular fibrillation showed a significant increase in 8-iso-PGF$_{2\alpha}$ levels in the jugular bulb plasma, with a maximum level four-fold higher than baseline during a period of 30 min after ROSC ($P = 0.008$ vs. 0.005).
Thereafter, jugular bulb plasma 8-iso-PGF\textsubscript{2\alpha} decreased gradually and reached the baseline within 150 min. In the VF2 group, a two-fold increase in jugular bulb 8-iso-PGF\textsubscript{2\alpha} was recorded at 5 min after ROSC ($P=0.008$ vs. baseline), which returned to the baseline within 30 min. No increase in jugular bulb or mixed venous plasma level of 8-iso-PGF\textsubscript{2\alpha} was recorded in the control group. During a period of 120 min after ROSC, jugular bulb plasma 8-iso-PGF\textsubscript{2\alpha} was significantly greater in the VF5 group as compared to the VF2 and the control group ($P<0.0001$, VF5 vs. VF2; $P=0.0003$, VF5 vs. control).

### 3.2. Inflammatory response as determined by plasma 15-keto-dihydro-PGF\textsubscript{2\alpha}

Baseline levels of 15-keto-dihydro-PGF\textsubscript{2\alpha} in the jugular bulb or mixed venous plasma did not differ between the groups. After ROSC, mixed venous levels of 15-keto-dihydro-PGF\textsubscript{2\alpha} increased significantly in both intervention groups ($P=0.022$ and $P=0.005$ for group VF2 and VF5, respectively, as compared to the baseline, Fig. 3, upper panel). It decreased thereafter to reach the baseline within 120 min and remained unchanged during the remainder of the study. During the initial 120 min after ROSC, mixed venous plasma 15-keto-dihydro-PGF\textsubscript{2\alpha} was significantly higher in the intervention groups as compared to the control group ($P=0.0003$, VF2 vs. control; $P<0.0001$, VF5 vs. control).

In the group subjected to 5 min of VF, a four-fold increase in jugular bulb 15-keto-dihydro-PGF\textsubscript{2\alpha} was recorded at 5 and 30 min after ROSC, as compared to the baseline ($P=0.0302$, 30 min vs. baseline, Fig. 3, lower panel). Thereafter, jugular bulb 15-keto-dihydro-PGF\textsubscript{2\alpha} decreased gradually and reached baseline after 240 min. A similar pattern was observed in the VF2 group, with a three-fold increase in jugular bulb 15-keto-dihydro-PGF\textsubscript{2\alpha} as compared to baseline at 30 min after ROSC ($P=0.007$). A transient increase in jugular bulb 15-keto-dihydro-PGF\textsubscript{2\alpha} was also recorded in the control group, but not to the same magnitude as in the intervention groups ($P=0.0001$, VF2 vs. control and $P<0.0013$, VF5 vs. control).

### 4. Discussion

To our knowledge, the present study is the first to report an increase both in isoprostane and prostaglandin formation after resuscitation from cardiac arrest. It clearly demonstrates that both 8-iso-PGF\textsubscript{2\alpha} and 15-keto-dihydro-PGF\textsubscript{2\alpha} levels in the jugular bulb plasma are significantly increased during the post-resuscitation period, indicating an association between cerebral ischemia – during cardiac arrest and CPR – and the oxidative injury and inflammatory response after ROSC. Furthermore, prolonged cardiac arrest is shown to increase the magnitude of oxidative reperfusion injury and inflammatory response in the brain, as determined by jugular bulb levels of 8-iso-PGF\textsubscript{2\alpha} and 15-keto-dihydro-PGF\textsubscript{2\alpha}. These also indicate that both free radical and cyclooxygenase catalyzed oxidation of arachidonic acid occurs during reperfusion injury.

The rationale for choosing 2 and 5 min of VF in this study is the evidence available for an association between the duration of circulatory arrest and CPR and the degree of cerebral dysfunction after resuscitation from cardiac arrest [21,22]. Hence, in the group subjected to 5 min of VF greater neurologic damage may be expected, as compared to 2 min of VF or the control group. It is, therefore, conceivable that increased jugular bulb levels of 8-iso-PGF\textsubscript{2\alpha} and 15-keto-dihydro-PGF\textsubscript{2\alpha} recorded in the VF5 group are associated with increased lipid peroxidation in the brain and worse neurologic outcome. This finding indicates that jugular bulb measurements of 8-iso-PGF\textsubscript{2\alpha} and 15-keto-dihydro-PGF\textsubscript{2\alpha} may be used as a possible method for predicting lipid peroxidation mediated neurologic outcome in patients resuscitated from cardiac arrest.

Another important observation was the extended period during which high isoprostane levels were measured in jugular bulb plasma samples (up to 120 min after ROSC). This observation is consistent with results from previous histopathological and physiological studies of post-ischemic progression of neuronal damage [23–25], supporting the theory of a delayed onset of morphological and metabolic neuronal disturbances after anoxia.

A transient increase in mixed venous plasma 8-iso-PGF\textsubscript{2\alpha} after ROSC may be attributed to a systemic reperfusion oxidative damage. Interestingly, mixed venous 8-iso-PGF\textsubscript{2\alpha} did not increase to the same magnitude as in the jugular bulb plasma. Furthermore, because under normal conditions approximately 15% of the mixed venous return originates from the brain, it is conceivable that the increase in mixed venous 8-iso-PGF\textsubscript{2\alpha} is in part caused by the biosynthesis and release of this isoprostane from the brain tissue. However, mixed venous 8-iso-PGF\textsubscript{2\alpha} decreased to baseline soon after the initial increase at 5 min, while jugular bulb levels of 8-iso-PGF\textsubscript{2\alpha} remained high until 150 min. Considering the remarkably short plasma half-life of 8-iso-PGF\textsubscript{2\alpha} [26], the most credible explanation for the difference in jugular bulb and mixed venous 8-iso-PGF\textsubscript{2\alpha} levels is a prolonged and significantly greater post-ischemic free radical catalyzed lipid peroxidation in cerebral tissue, as compared to the whole body.

In conclusion, measurements of 8-iso-PGF\textsubscript{2\alpha} and 15-keto-dihydro-PGF\textsubscript{2\alpha} in the jugular bulb plasma can be served as biomarkers of cerebral oxidative injury and inflammatory response after resuscitation from cardiac arrest. This also opens excellent possibilities to study the role of free radical and cyclooxygenase mediated lipid peroxidation in reperfusion injury.

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References