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Free Radical Research

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To cite this Article: Samar Basu, Xiaoli Liu, Ala Nozari, Sten Rubertsson, Adriana Miclescu and Lars Wiklund, 'Evidence for Time-dependent Maximum Increase of Free Radical Damage and Eicosanoid Formation in the Brain as Related to Duration of Cardiac Arrest and Cardio-pulmonary Resuscitation,' Free Radical Research, 37:3, 251 - 256

URL: http://dx.doi.org/10.1080/1071576021000043058

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Evidence for Time-dependent Maximum Increase of Free Radical Damage and Eicosanoid Formation in the Brain as Related to Duration of Cardiac Arrest and Cardio-pulmonary Resuscitation

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Accepted by Professor B. Halliwell

(Received 17 June 2002; In revised format 17 September 2002)

Recovery of neurological function in patients following cardiac arrest and cardiopulmonary resuscitation (CPR) is a complex event. Free radical induced oxidative stress is supposed to be involved in this process. We studied levels of 8-iso-PGF2α (indicating oxidative injury) and 15-keto-dihydro-PGF2α (indicating inflammatory response) in venous plasma obtained from the jugular bulb in a porcine model of experimental cardiopulmonary resuscitation (CPR) where 2, 5, 8, 10 or 12 min of ventricular fibrillation (VF) was followed by 5 or 8 min of closed-chest CPR. A significant increase of 8-iso-PGF2α was observed immediately following restoration of spontaneous circulation in all experiments of various duration of VF and CPR. No such increase was seen in a control group. When compared between the groups there was a duration-dependent maximum increase of 8-iso-PGF2α which was greatest in animals subjected to the longest period (VF12 min + CPR8 min) of no or low blood flow. In contrast, the greatest increase of 15-keto-dihydro-PGF2α was observed in the 13 min group (VF8 min + CPR5 min). Thus, a time-dependent cerebral oxidative injury occurs in conjunction which cardiac arrest and CPR.

Keywords: Ischemia reperfusion; Prostaglandins; Isoprostanes; Oxidative injury; Inflammation; Brain

INTRODUCTION

Recovery after cardiac arrest and cardiopulmonary resuscitation (CPR) is often complicated by post-ischaemic derangements and an unfavourable clinical outcome. Although controversy exists about the exact mechanism behind this cerebral injury, the duration of circulatory arrest, CPR and quality of reperfusion seem to be the major causative factors. Free radical mediated oxidative injury has been suspected to be one of the major causes underlying the post-ischaemic reperfusion injury. In addition, local inflammatory mechanisms including the release of cytokines and activation of cyclooxygenases could aggravate the microvascular dysfunction after ischemia and may, therefore, worsen the possibilities for neurologic recovery after restoration of spontaneous circulation (ROSC). We have earlier suggested the there might be an association between the duration of untreated circulatory arrest and CPR, and the degree of cerebral damage. Whether this applies also to the cardiac arrest and CPR of longer duration is still unknown. The hypothesis we wanted to explore was if the total duration of cardiac arrest and CPR was associated with the maximum increase in the jugular bulb plasma concentrations of the eicosanoids, 8-iso-PGF2α (a major F2-isoprostane indicating oxidative injury) and 15-keto-dihydro-PGF2α (a major metabolite of PGF2α indicating inflammatory response) as indicators of the non-enzymatic and enzymatic cerebral...
oxidative processes in conjunction with experimental ischemia–reperfusion.

Isoprostanes biosynthesized from arachidonic acid in vivo, mainly through non-enzymatic free radical catalysed oxidation, are increased in several oxidant injury syndromes. Enzymatic catalysis by cyclooxygenases leading to formation of prostaglandins from arachidonic acid and their involvement in the process of inflammation is well described. Cyclooxygenase-2 has been demonstrated to be expressed in various cells after exposure to several pro-inflammatory stimuli, resulting in the release of prostaglandins. 15-keto-dihydro-PGF2a is increased in inflammation and can be used as an indicator of in vivo lipid oxidation through the cyclooxygenase pathway. We have developed radioimmunoassays through raising specific antibodies against both 8-iso-PGF2a and 15-keto-dihydro-PGF2a. By quantifying these parameters, we have established that oxidative modification of arachidonic acid is associated with hepatotoxicity, septic shock, cardiac arrest and reperfusion, among post-menopausal women.

The aim of the present study was to investigate whether the total duration of cardiac arrest and CPR, i.e. no blood flow or low blood flow, was associated with the magnitude of cerebral oxidative injury and inflammatory response, as measured by the maximum jugular bulb plasma concentration of 8-iso-PGF2a and 15-keto-dihydro-PGF2a, respectively, after resuscitation from circulatory arrest.

MATERIALS AND METHODS

Chemicals

The tritium labelled 8-iso-PGF2a (specific activity: 608 GBq mmol⁻¹) was synthesized and purified as described previously. The tritium labelled 15-keto-dihydro-PGF2a (specific activity: 6.77 TBq mmol⁻¹) was obtained from Amersham (Buckinghamshire, UK). Antibodies against both 8-iso-PGF2a and 15-keto-dihydro-PGF2a were raised at our laboratory and are well characterized.

Animal Preparation

The protocol and care of the animals were approved by the Regional Review Board for Animal Experimentation. Fifty-six Swedish breed piglets of both gender, 11–15 weeks of age were used. Anaesthesia was induced and maintained as previously described, and animal preparation was performed in accordance with a well-established protocol at our research laboratory. A catheter (20 Gauge) was inserted via a branch of the right external carotid artery into the aortic arch for pressure monitoring and blood sampling. A catheter was also inserted into the right atrium for drug administration and another catheter (16 French) was inserted into the left internal jugular vein and passed retrogradely into the jugular bulb for blood sampling. The position of this catheter has been checked by fluoroscopy and regular radiological methods. Hemodynamic data, including standard lead II ECG, systemic arterial blood pressure, and pulmonary artery blood pressure were continuously monitored.

Experimental Protocol

A part of the present results at early time points are partly extracted from previously published data where also the described methods and protocols have been accounted for. Nitrous oxide was discontinued after animal preparation and the piglets were ventilated with 30% oxygen in air. After 30 min, baseline values were obtained. Seven groups of animals were studied. The first group (n = 6) denoted VF2, was subjected to 2 min of untreated ventricular fibrillation followed by 5 min of closed-chest CPR. The second group (n = 6), denoted VF5, was subjected to 5 min of untreated ventricular fibrillation followed by 5 min of closed-chest CPR. The third (n = 14) and fourth (n = 18) groups, denoted VF8, was subjected to 8 min of untreated ventricular fibrillation followed by 5 or 8 min of closed-chest CPR, respectively. The fifth group (n = 8), denoted VF10, was subjected to 10 min of ventricular fibrillation followed by 8 min of closed-chest CPR. The sixth group (n = 3), denoted VF12, was subjected to 12 min of ventricular fibrillation followed by 8 min of closed-chest CPR. Five animals served as controls (group 7) with no further interventions except anaesthesia and placement of catheters. In the six intervention groups, ventricular fibrillation was induced with a brief alternating current shock of 40–60 V administered by two subcutaneous needles. Cardiac arrest was defined by identification of ventricular fibrillation on the ECG and the loss of arterial pulsation, with a systolic aortic blood pressure of <25 mm Hg. Ventilation was stopped at the same time. After the non-intervention period (2, 5, 8, 10 or 12 min) external thoracic compressions were applied in the intervention groups and ventilation was resumed with 100% oxygen. A bolus injection of 20 μg/kg epinephrine or 40 U/kg vasopressin was administered through the right atrial catheter 1–2 min after the commencement of CPR. External defibrillatory shocks of 200 J were applied 1 min after a second administration of epinephrine.
(5 or 8 min of closed-chest CPR). If restoration of spontaneous circulation (ROSC) was not accomplished after three defibrillatory shocks, a second bolus injection of epinephrine was administered through the same route. 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 >60 mm Hg maintained for at least 10 min. After 5 min of spontaneous circulation the FIO2 was reset to 0.3.

Sample Collection

Blood samples were collected from the jugular bulb catheter at baseline, 5 min after ROSC, 30 min after ROSC and every 30 min thereafter, up to 4 h of spontaneous circulation. After centrifugation plasma was collected after which the plasma samples were kept frozen at −70°C pending analysis. The maximum increase of plasma-8-iso-PGF2α and 15-keto-dihydro-PGF2α was used in this report to correlate it with the duration of low or no blood flow which was defined as the total duration of untreated cardiac arrest and CPR up to the point where restoration of spontaneous circulation was achieved.

Radioimmunoassay of 8-iso-PGF2α

Plasma samples were analysed for 8-iso-PGF2α by a radioimmunoassay (RIA) at our laboratory as described elsewhere. In brief, unextracted plasma samples were used in the assay. The cross-reactivity of the 8-iso-PGF2α, antibody with 15-keto-13,14-dihydro-8-iso-PGF2α, 8-iso-PGF2β, PGF2α, 15-keto-PGF2α, 15-keto-13,14-dihydro-PGF2α, TXB2, 11β-PGF2α, 9β-PGF2α and 8-iso-PGF3α, 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-PGF2α

The plasma samples were analysed for 15-keto-dihydro-PGF2α by a RIA at our laboratory as described elsewhere. In brief, unextracted plasma samples were used in the assay. The cross-reactivity of the antibody with PGF2α, 15-keto-PGF2α, PGF2α, 15-keto-13,14-dihydro-PGE2, 8-iso-15-keto-13,14-dihydro-PGF2α, 11β-PGF2α, 9β-PGF2α, TXB2 and 8-iso-PGF3α, 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.

Statistical Analysis

The maximum increase of the two eicosanoids was calculated as a multiple of the control values of each animal. These maximum values were collected for each group of different duration of VF and CPR. The results obtained for all groups were compared using the Kruskal Wallis non-parametrical analysis of variance, and if a statistical difference was detected a post-hoc analysis of each group compared to the control group was performed by the Dunn’s multiple comparisons test. Maximum eicosanoid values were correlated to the total duration of VF and CPR using Pearson correlation. Throughout statistical significance was considered to be present if P < 0.05.

RESULTS

Piglets were subjected to either 2, 5, 8, 10 or 12 min of ventricular fibrillation followed by 5 or 8 min of closed-chest CPR or untreated as controls with no cardiac arrest. Thus, a total time of (VF + CPR) 7, 10, 13, 16, 18 and 20 min was assigned in the evaluation of results.

Cerebral Oxidative Injury as Measured by Jugular Bulb Plasma 8-iso-PGF2α

There was no difference between the groups in baseline values of jugular bulb plasma concentrations of the 8-iso-PGF2α. Jugular bulb plasma levels of 8-iso-PGF2α increased significantly in all intervention groups (VF + CPR duration = 7, 10, 13, 16, 18 and 20 min) compared to baseline values and the control group within 5 min after ROSC. A diagram from various VF duration and 8-iso-PGF2α levels is shown in Fig. 1 (upper panel). The maximum increase of plasma 8-iso-PGF2α was observed 15–30 min after ROSC which subsequently and gradually decreased during the next 150 min. No increase in the level of 8-iso-PGF2α was observed in the control group. Data (maximum increase compared to baseline values) extracted from experiments of various VF and CPR duration are shown in Fig. 2 (left panel). Jugular bulb plasma 8-iso-PGF2α levels increased in a duration-dependent manner with the greatest increase in the intervention group subjected to 12 min of ventricular fibrillation and 8 min of CPR. The levels of 8-iso-PGF2α in this intervention group (VF12) increased up to 33-fold from the baseline during the period 30 min after ROSC, i.e. considerably more than in any of the other groups. The total duration of VF and CPR was correlated to the maximum increase of the 8-iso-PGF2α level (r² = 0.20; P < 0.05). Inter-individual differences in the increase of 8-iso-PGF2α were small in the lowest ventricular fibrillation groups (VF2 and VF5 min) but increased gradually in the groups with longer durations of ventricular...
fibrillation. Out of the animals subjected to a total of 20 min (VF + CPR) of low flow the animal that exhibited signs of severe cerebral blood flow derangements or brain death, as detected by no-flow in the cerebral cortex (laser-doppler flowmetry) at 30 min after ROSC, showed 5-fold increase of 8-iso-PGF$_{2\alpha}$ compared to the basal values at 15 min after ROSC which

![Diagram](image1)

**FIGURE 1** A diagram of mean jugular bulb plasma levels of 8-iso-PGF$_{2\alpha}$ (upper panel) and 15-keto-dihydro-PGF$_{2\alpha}$ (lower panel) at baseline and after ROSC with various VF duration and in control animals as defined in the upper right corner. VF2, ventricular fibrillation of 2 min; VF5, ventricular fibrillation of 5 min; VF8, ventricular fibrillation of 8 min; VF10, ventricular fibrillation of 10 min; VF12, ventricular fibrillation of 12 min. Values in animals not subjected to cardiac arrest are described as controls.

![Diagram](image2)

**FIGURE 2** Levels of maximum increase (in fold) of plasma eicosanoids compared to the baseline (8-iso-PGF$_{2\alpha}$ in left panel; 15-keto-dihydro-PGF$_{2\alpha}$ in right panel) and the various duration of ventricular fibrillation (VF) and cardiopulmonary resuscitation (CPR). Values in animals not subjected to cardiac arrest (control animals = 0) are set to 1-fold. Actual values of fold increase are also mentioned adjacent to the respective increase. X-axis is represented ventricular fibrillation and cardiopulmonary resuscitation of various duration individually and togetherly from separate experiments. Y-axis represent the maximum increase of eicosanoids in fold compared to the baseline in various duration groups of VF and CPR.
was retained until 240 min when the experiment ended.

Cerebral Inflammatory Response as Determined by Jugular Bulb Plasma 15-keto-dihydro-PGF₂a

Baseline levels of 15-keto-dihydro-PGF₂a in the jugular bulb plasma did not differ between the groups. Jugular bulb plasma levels of 15-keto-dihydro-PGF₂a increased significantly in all intervention groups (VF + CPR duration = 7, 10, 13, 16, 18 and 20 min) within 5 min after ROSC as compared to the baseline values. A diagram from various VF duration and 15-keto-dihydro-PGF₂a levels is shown in Fig. 1 (lower panel). A maximum increase of plasma-15-keto-dihydro-PGF₂a was observed 15–60 min after ROSC that decreased gradually within 120 min. A slight increase in the levels of 15-keto-dihydro-PGF₂a was observed at 30 min in the control group but not to the same magnitude as in the intervention groups. Maximum plasma-15-keto-dihydro-PGF₂a levels increased in a non-duration-dependent manner with the greatest increase in the intervention group subjected to 8 min of ventricular fibrillation and 5 min of CPR (Fig. 2; right panel). The total duration of VF plus CPR not being correlated to the maximum 15-keto-dihydro-PGF₂a levels. The levels of 15-keto-dihydro-PGF₂a in the VF8 group increased up to 8-fold from the baseline during a period of 30 min after ROSC. Inter-individual differences of the increase of 15-keto-dihydro-PGF₂a were small in the ventricular fibrillation VF2 and VF5 groups, but increased to a maximum in VF8 groups. Out of the animals subjected to a total of 20 min (VF + CPR) of low flow the animal that exhibited signs of severe cerebral blood flow derangements or brain death at 30 min after ROSC showed a 2-fold increase 15-keto-dihydro-PGF₂a from the basal levels at 15 min after ROSC which rapidly decreased to the basal level at 30 min after ROSC.

DISCUSSION

It is well known that cerebral ischemia and reperfusion/reoxygenation results in an increased rate of formation of reactive radical species like O₂⁻ and H₂O₂. Furthermore, the brain tissue is especially prone to free radical induced oxidative damage both structurally and biochemically due to high content of membrane lipids that are rich in polyunsaturated fatty acids. The present study corroborates with our earlier findings that both jugular bulb F₂-isoprostanes and PGF₂a metabolite may serve as biomarkers of oxidative free radical damage and inflammatory response in the brain following reperfusion injury. The present study, further presents a time-dependent free radical damage of the brain as a consequence of cardiac arrest and CPR duration by demonstrating elevated cerebral isoprostane formation. An elevation of free radical dependent increase of F₂-isoprostanes on the jugular bulb plasma, which further illustrates that the ischemia/reoxygenation injury results in an increased biosynthesis of various reactive species in the brain beyond their normal basal levels. We have earlier shown that there might be a time dependency on the free radical mediated isoprostane formation and the total duration of cardiac arrest and CPR. To our knowledge this study is the first experimental evidence (by assessing various VF and CPR of longer duration) to report this exponential increase is dependent on both the duration of cardiac arrest and CPR, i.e. the total period with impaired cerebral blood flow. Thus, both these factors (duration of cardiac arrest and CPR) seem to be responsible for the increased free radical-mediated lipid peroxidation. Thus, prolonged duration of cardiac arrest and CPR may possibly lead to a worsening of the oxidative injury of brain tissue that could contribute to a possible development of brain death or severe neurological deficits with low survival rate. The slight non-linearity in the relation between the duration of low or no blood flow and the jugular bulb plasma 8-iso-PGF₂a concentration could be explained by the somewhat different experimental protocols as regards administration of the vasopressors in the process of resuscitation. In the present study, we also observed that the inter-individual difference of cerebral oxidative injury is less in animals that subjected to a ventricular fibrillation of 5 min or less. This inter-individual difference in the formation of F₂-isoprostane increased dramatically as the duration of cardiac arrest and CPR was increased which possibly relates to the well-known fact that animals resuscitated after more than 5 min of untreated ventricular fibrillation have an enlarged risk for an increased free radical-mediated oxidative damage in the brain that possibly is reflected in worse neurological outcome. Furthermore, an inter-individual difference of the anti-oxidative defence system to resist free radical mediated damage cannot be ruled out.

When the inflammatory response was evaluated by measuring the major PGF₂a metabolite in the jugular bulb plasma the increase was greatest in the VF8 and CPR5 min groups and less in the groups subjected to longer duration of VF and CPR. This shows that cyclooxygenase-mediated inflammatory response following the free radical mediated oxidative damage in the brain increases up to 8 min of untreated VF plus CPR of 5 min duration. However, the cyclooxygenase derived prostaglandin formation does not increase at the same magnitude as the free-radical mediated isoprostane formation...
which might increase in an uncontrolled manner with wide inter-individual differences when the duration of cardiac arrest and CPR is further increased. One animal that encountered severe cerebral blood flow derangements or brain death had a rapid increase of both the F2-isoprostane and the PGF2α metabolite until the blood flow of the brain decreased to very low values.

In conclusion, cerebral free radical and cyclooxygenase mediated oxidation of arachidonic acid are associated with the ischemia/reperfusion injury during cardiac arrest and CPR. A time dependent free radical damage activity in the brain after prolonged cardiac arrest and CPR would suggest possible benefit of an early application of selective radical scavenger(s) as therapeutic agents following CPR. Shortening the cardiac arrest and CPR duration is a recognised benefit for the brain that at least partly might depend on the free radical induced brain injury.

Acknowledgements

This work was financed by grants from the Geriatrics Research Foundation, Swedish Medical Research Council (project 6579) and the Laerdal Foundation for Acute Medicine. E. Seiby and A. Nordgren are gratefully acknowledged for excellent technical assistance.

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