Intravenous Interferon-β1a for the Treatment of Ischemia-Reperfusion Injury in Acute Myocardial Infarct in Pigs

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ABSTRACT

Background: To investigate the potential of intravenously administered porcine recombinant interferon-β1a (IFN-β1a) for myocardial protection during acute ischemia-reperfusion (IR) injury in an experimental animal model.

Methods: Twenty-two piglets (mean ± standard deviation, 26.7 ± 1.65 kg) were assigned to either the IFN group (n = 12) or the control group (n = 10). IR injury was induced by occluding the distal left descending coronary artery for 30 minutes, with a reperfusion period of 6 h. In the IFN group, the animals received 12.5 µg IFN-β1a intravenously repeatedly; the control group received saline solution. The levels of interleukin-6 (IL-6) and cardiac troponin I (TnI) were measured, and the amount of myocardial damage was quantified by analyzing myocardial apoptosis and the mean fluorescence intensity (MFI) of methylene blue–stained cardiac tissue.

Results: In the IFN group, significantly more premature deaths occurred compared with the control group (25% versus 17%, P = .013). Between the groups, the mean heart rate was higher in the IFN group (102 ± 22 versus 80 ± 20 beats per minute, P = .02). IL-6 and TnI levels were comparable between the groups, with no significant difference, and there was no difference between the study groups in myocardial apoptosis in the infarcted myocardium. The percentage of MFI differed significantly between the IFN and control groups (90.75% ± 4.90% versus 96.02% ± 2.73%, P = .01).

Conclusion: In this acute IR injury animal model, IFN-β1a did not protect the myocardium from IR injury, but rather increased some of the unfavorable outcomes studied.

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INTRODUCTION

Ischemia-reperfusion (IR) injury induced by acute myocardial infarction causes severe damage in the myocardium, and the direct consequences of this damage can be seen in several organs in the human body. At the molecular level, IR injury activates inflammatory responses and metabolic changes through tissue damage, creating an undesirable acidic microenvironment and increasing the risk of electrical imbalance in the myocardium [Hausenloy 2013; Crawford 2017; Heusch 2015]. Myocardial infarction through IR injury causes morbidity and mortality, which is why several therapeutic agents and methods for cardioprotection have been investigated [Barrabes 2016; Foo 2008; Chen 2019].

Interferon-β1a (INF-β1a) is an anti-inflammatory cytokine of the IFN family that is clinically used in treatment of patients with multiple sclerosis [Kasper 2014]. In patients treated for acute respiratory distress syndrome (ARDS), INF-β1a was found to maintain endothelial barrier function in lung tissue through the upregulation of CD73 on endothelial cells [Bellinger 2014]. CD73 is considered the rate-limiting endothelial enzyme that produces local adenosine and the key to preventing capillary leakage and organ damage under ischemic conditions [Kiss 2007; Eltzschig 2004; Eltzschig 2013]. In addition, the immunomodulatory potential of INF-β1a and its anti-inflammatory nature suggest therapeutic potential for other ischemic conditions. In this study, we sought to investigate the role of IFN-β1a in acute myocardial IR injury in an experimental animal model.

METHODS

The animal experiments were approved by the Laboratory Animal Care and Use Committee of the State Provincial Office of Southern Finland (authorization number ESAVI/760/04.10.07/2017). The animals used in this study received humane care according to the Principles of Laboratory Animal Care by the National...
Table 1. Blood Samples at Different Time Points during Operation

| Measurement          | 0-sample, 0000 | 1-sample, 0800 | 2-sample, 0830 | 3-sample, 1430 |
|----------------------|---------------|---------------|---------------|---------------|
| Astrup               | x             | x             |               |               |
| TnT                  |               |               | x             | x             |
| HR                   | x             |               |               |               |
| Oxygen saturation (%)| x             |               |               |               |
| Hemoglobin           |               |               | x             |               |
| Tnl                  |               |               | x             |               |
| IL-6                 |               |               |               | x             |

Society for Medical Research and the Guide for the Care and Use of Laboratory Animals [National Institutes of Health 1996]. This study was conducted using Finnish landrace piglets (n = 22, weight 26.7 ± 1.75 kg, mean ± standard deviation). The animals were randomly assigned to the IFN group (n = 12) or the control group (n = 10). Animals with premature death were excluded from the final analyses. One pig in the IFN group did not complete the entire reperfusion period of 6 hours and missed the final blood sample (3-sample) but was included in the analysis.

Surgical Procedure

The animals were premedicated with an intramuscular injection of 25 mg midazolam (Hameln, Hameln, Germany) and 100 mg xylazine (Rompun Vet; Bayer Animal Health, Leverkusen, Germany). A peripheral vein in the ear was cannulated, and anesthesia was maintained using continuous infusion of 15 to 30 mg · kg⁻¹ · h⁻¹ propofol (Propo Vet; Zoetis Finland Oy, Helsinki, Finland), 1.5 µg · kg⁻¹ · h⁻¹ fentanyl (Janssen-Cilag Oy, Espoo, Finland), and 100 µg · kg⁻¹ · h⁻¹ midazolam boluses. A tracheostomy was performed, and the animal was connected to a respirator. Respiratory settings were adjusted according to the blood gas analyses. Both right external jugular vein and common carotid artery were cannulated openly for central venous line, arterial blood pressure monitoring, and blood sampling. Electrocardiography, oxygen saturation, and arterial blood pressure were monitored throughout the experiment. The bladder was punctured regularly to control adequate urine output. The animals were kept normovolemic with continuous intravenous infusion of Ringer’s solution (200 to 1000 ml/h).

The heart was exposed by midline sternotomy. Myocardial infarct was induced by occluding the distal part of the left anterior descending coronary artery (LAD) after the second diagonal branch with a removable ligature for 30 minutes. Before occlusion and before release of the ligature, 200 mg intravenous lidocaine (Lidocain Orion Pharma, Espoo, Finland) was administered as a slow bolus to prevent arrhythmia. Ischemia was monitored with telemetry as an increase in ST level. In case of arrhythmia, an additional bolus of intravenous lidocaine (100 to 200 mg) was administered. After 6 hours of reperfusion, the experiment was terminated with an intravenous potassium chloride injection (B. Braun Melsungen, Melsungen, Germany).

INF-β1α Treatment

The INF-β1α used in this study was a recombinant porcine cytokine (ab87929; Abcam, Cambridge, UK). In the treatment group (n = 12), the first INF-β1α dose (12.5 µg) was given intravenously in the beginning of the experiment. The second dose of INF-β1α (12.5 µg) was given 8 hours after the first dose, right before ligating the distal LAD.

Blood and Tissue Samples

During the experiment, blood samples were taken at 4 different time points (Table 1). The 0-sample was collected at baseline after induction of anesthesia; the 1-sample 8 hours later, before ischemia; the 2-sample after the release of LAD ligature; and the 3-sample at the end of the experiment. Levels of interleukin-6 (IL-6) (P600B; R&D Systems, Minneapolis, MN) and cardiac troponin I (TnI) (MBS2509024; My Biosource, San Diego, CA) were analyzed using enzyme-linked immunosorbent assay. Arterial blood gas analysis, hemoglobin, and blood cardiac troponin T (TnT) values were controlled using a point-of-care blood analyzer (i-STAT 1; Abbott Laboratories, Princeton, NJ). At the end of the experiment, the ascending aorta was clamped, the ligature on the LAD was tightened, and an injection of methylene blue to the base of the aorta was given to stain myocardium. Myocardial tissue samples from the site of ischemia, border of ischemia, and healthy myocardium were harvested and frozen after termination of the experiment.
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TUNEL Assay

Apoptotic cells within the myocardium were stained with terminal deoxynucleotidyl transferase nick-end labeling (TUNEL) assay (Promega, Madison, WI) using 7-µm-thick frozen sections by the manufacturer’s standard protocol. 4′,6-Diamidino-2-phenylindole (DAPI) was used to visualize all the cell nuclei in the sections. Slides were imaged with an Eclipse Ni-U widefield microscope (Nikon, Tokyo, Japan). ImageJ Fiji software (version 1.51; National Institutes of Health, Bethesda, MD) [Schindelin 2012] was used to quantify the TUNEL-positive cells at the area of infarction (left ventricle, apex) and in the healthy noninfarcted myocardium of the right ventricle.

Table 3. Levels of IL-6 and TNF Measured Postoperatively from Blood Sample and TUNEL-Positive Cells from Infarction Site Compared between Groups*

| Item                      | Control (n = 10) | Treatment (n = 11) | P   |
|---------------------------|-----------------|-------------------|-----|
| Postoperative IL-6        | 129 ± 43        | 253 ± 248         | .132|
| Postoperative TNF         | 19.0 ± 14.7     | 51.3 ± 60.9       | .115|
| TUNEL (%)                 | 15.8 ± 13.7     | 25.0 ± 15.4       | .17 |

*Data are mean ± standard deviation.

Mean Fluorescence Intensity (MFI)

Methylene blue–stained hearts (Figure 1) were fixed in formalin and sliced to expose the left ventricular myocardium, apex, and septal myocardium. Applying ImageJ, the intensity of staining was measured to estimate the intensity of infarction and evaluate the effect of INF-β1a treatment on IR injury. As the absolute values of the intensity between hearts cannot be compared because of the variable intensity of dyeing overall, a percentage share of the intensity was calculated for each heart.

Statistical Analysis

A power analysis assumed that after ligation of distal LAD, the infarcted area was 50% ± 10% of the volume of the left ventricle [Bolli 2018], whereas the area of infarction was estimated as 35% within the IFN group. With these expectations, the estimated number of animals needed was 7 per group. To cover the potential loss of individual subjects due to pigs’ sensitivity to arrhythmia, the number of animals in each group was increased to 10.

Pigs were randomized to receive either INF-β1a or saline solution; there was no selection of pigs before operation. For statistical analysis and considering small group sizes, only comparison between groups was carried out. For continuous variables, analysis of variance or t test was used. McNemar’s or Fisher’s test was used for categorical variables.

RESULTS

In this experiment, the total mortality rate was 21%. There were significantly more premature deaths in the IFN group (25%, n = 4) than in the control group (17%, n = 2; $P = .013$). In all premature deaths, the cause of death was heart failure due to persistent ventricular tachycardia. In the IFN group, persistent tachycardia occurred more frequently, and the mean heart rate (HR) was higher at the time of ischemia (102 ± 22 versus 80 ± 20 beats per minute; $P = .02$) (Table 2). The blood of animals in the IFN group were also more acidic (pH < 7.35) than that in the control group before and after ischemia (before, 7.25 ± 0.19 versus 7.50 ± 0.10, $P = .003$; after, 7.25 ± 0.22 versus 7.50 ± 0.09, $P = .007$), but the difference evened out during the reperfusion period.
Table 4. TUNEL-Positive Cells in Infarcted Myocardium Compared with Healthy Myocardium*

| Item                     | Control (n = 22) | Infarct (n = 22) | P       |
|--------------------------|-----------------|------------------|---------|
| TUNEL                    | 0.32 ± 0.18     | 0.98 ± 0.74      | .000382 |

*Data are mean ± standard deviation.

At the end of the experiment, there were no significant differences between the study groups in IL-6 or TnI levels (Table 3). TUNEL analysis (Figure 2) showed more apoptotic myocardial cells in infarcted tissue compared to non-ischemic, healthy myocardium (0.98% ± 0.74% versus 0.32% ± 0.18% of cells, \( P = .0003 \)) (Table 3) in both groups. However, no significant difference between groups was found in the infarcted myocardium (15.8% ± 13.7% versus 25.0% ± 15.4% for control and treatment groups, respectively, \( P = .17 \)) (Table 4).

**MFI**

The difference in MFI between ischemic apical myocardium and healthy septal myocardium was significantly lower in the IFN group compared with the control group (90.75% ± 4.90% versus 96.02% ± 2.73%, \( P = .01 \)) (Table 5), suggesting more severe ischemia in the apex in the IFN group (Table 5).

### DISCUSSION

In this experimental acute myocardial IR injury study with pigs, INF-β1a did not show cardioprotective qualities. On the contrary: the results of this experiment suggest that INF-β1a administered 8 hours before ischemia increased the occurrence of events with tachycardia and acidosis and possibly increased the magnitude of myocardial damage in acute myocardial IR injury within 6 hours.

In previous studies, INF-β1a has been shown to upregulate the production of adenosine via increased expression of CD73 [Kiss 2007]. CD73 (5’-ecto-nucleotidase) constructs locally active adenosine from adenosine monophosphate (AMP) on the cell surface [Eltzschig 2004]. First, adenosine has been shown to act as a protective agent against IR injury, owing to its anti-inflammatory nature and the ability to precondition endothelium against ischemia and inflammation [Eltzschig 2013; Bolli 2018; Bellingan 2017]. Although several therapies aiming for reperfusion have improved the prognosis of patients with acute myocardial infarction, further studies are required to combat the microvascular damage caused by IR injury. Events such as microvascular obstruction and loss of endothelial integrity result in myocardial edema and hemorrhage at the cellular level [Gao 2017]. Second, adenosine A2 receptor activation has been shown to decrease multiorgan IR injury in extracorporeal cardiopulmonary resuscitation in an experimental porcine model, suggesting even more comprehensive protection [Charles 2019; PRISMS 1998].

INF-β1a has been in clinical use for several years for the treatment of patients with multiple sclerosis [Lee 2015]. It reduces the risk of relapses and decreases the rate of symptoms and the progression of disability. INF-β1a also has endothelial permeability-modulating function in lung tissues, as was portrayed in a study with patients suffering from ARDS [Bellingan 2014]. In that study, INF-β1a decreased mortality by stimulating endothelial permeability-retaining mechanisms, one of them being increased levels of adenosine [Bellingan 2014]. In experimental animal models, INF-β1a reduces IR injury in both cerebral and intestinal tissues [Eltzschig 2013; Lee 2015]. However, INF-β1a has not yet been studied vis-à-vis cardioprotection and reduction of IR injury in large mammals in an acute setting.

In our study, treatment with INF-β1a did not protect the myocardium from apoptosis, and the fluorescence intensity indicated more profound myocardial infarct in treated animals compared with controls. A possible explanation is that the timing and dosage of INF-β1a is crucial for its appropriate function in target tissue. INF-β1a has previously been used in more chronic experimental setups. In an experimental animal study of cerebral IR injury, the infarct size was diminished in the INF-β1a group compared with the control group after 48 hours of follow-up [Malmberg 2011]. Also, a previous study indicated that INF-β1a induces CD73 in a time- and dose-dependent manner, and a longer period of time may be required for INF-β1a to upregulate CD73 and realize its cardioprotective effects, while the initial inflammatory and pyrogenic effects remain [Kuo 2016]. In our study, the first dose of INF-β1a was administered 8 hours before ischemia, and the reperfusion period was limited to 6 hours to mimic a more acute setting. As our previous study indicated, the reperfusion period used in this study is sufficient to introduce differences between the study groups [Karvonen 2020]. Although it is yet unclear by which mechanisms INF-β1a treatment is associated with unwanted effects in this study, it is possible that the benefits of the treatment would emerge in a subacute to chronic IR injury.

In our study, the pigs treated with INF-β1a had significantly higher mortality and higher HR than pigs in the control group. The cause of death in all cases was persistent ventricular tachycardia that did not respond to administration of lidocaine or defibrillation. These findings suggest that in this acute experimental setting, INF-β1a might increase the risk for arrhythmias. Also, metabolic acidosis was more common in the IFN group throughout the experiment, possibly indicating acute-phase inflammatory and pyrogenic reactions induced by INF-β1a. To our knowledge, there are no published studies about use of INF-β1a in an acute myocardial...
infarct model, nor studies in which INF-β1a has been administered 8 hours before ischemia in an acute setting. Our study was begun with the prospect of cardioprotective benefits, as previous studies have shown protective qualities of INF-β1a in IR injury in lung, intestinal, and cerebral tissues [Kiss 2007; Kuo 2016].

Limitations
This study has limitations. First, our results with an experimental animal model cannot be directly applied to the clinical setting. Second, functional imaging was not included in our study. Although such data might have added value, it is not expected to have changed the overall findings. Third, it is possible that earlier dosing of INF-β1a before ischemia might have changed the cardioprotective qualities of INF-β1a, but it would have changed the basic nature of this acute experimental model.

Conclusions
In this experimental porcine model, INF-β1a did not protect myocardium from acute IR injury. Further studies are required to study whether INF-β1a has any clinically meaningful cardioprotective qualities.

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