Induction of CD73 Prevents Death after Emergency Open Aortic Surgery for a Ruptured Abdominal Aortic Aneurysm – A Randomized, Double-blind, Placebo-controlled Study

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Abstract

Background: Mortality remains high after emergency open surgery for a ruptured abdominal aortic aneurysm (RAAA). The aim of the present study was to assess, if intravenous (IV) Interferon (IFN) beta-1a improve survival after surgery by up-regulating Cluster of differentiation (CD73).

Methods: This is a multi-center phase II double-blind, 2:1 randomized, parallel group comparison of the efficacy and safety of IV IFN beta-1a vs. placebo for the prevention of death after open surgery for an infra-renal RAAA. All study patients presented a confirmed infra-renal RAAA, survived the primary emergency surgery and were treated with IFN beta-1a (10µg) or matching placebo for 6 days after surgery. Major exclusion criteria included irreversible hemorrhagic shock, chronic renal replacement therapy, diagnosed liver cirrhosis, severe congestive heart failure, advanced malignant disease, primary attempt of endovascular aortic repair (EVAR), and per-operative suprarenal clamping over 30 minutes. Main outcome measure was all-cause mortality at day 30 (D30) from initial emergency aortic reconstruction.

Results: The study was pre-maturely stopped due to a reported drug-drug interaction and was left under-powered. Out of 40 randomized patients 38 were included in the outcome analyses (27 IFN beta-1a and 11 placebo). There was no statistically significant difference between treatment groups at baseline. However, from surgery more open-abdomen and intestinal ischemia was present in the IFN beta-1a arm. D30 all-cause mortality was 22.2% (6/27) in the IFN beta-1a arm and 18.2% (2/11) in the placebo arm (OR 1.30; 95% CI, 0.21 – 8.19). The most common adverse event relating to the IFN beta-1a was pyrexia (20.7% in the IFN beta-1a arm vs. 9.1% in the placebo arm). High level of serum CD73 associated with survival ($P = 0.001$) whereas the use of glucocorticoids and the presence of IFN beta neutralizing antibodies associated with a poor CD73 response and survival.

Conclusions: Due to the size of the study and several confounding factors a benefit from IFN beta-1a could not be determined. Survival after open RAAA surgery associated strongly with up-regulation of serum CD73. The use of glucocorticoids and IFN beta neutralizing antibodies blocked the up regulation of CD73.

Trial registration: ClinicalTrials.gov NCT03119701. Registered 19/04/2017 (retrospectively registered).

Key Points

Question: Can the induction of CD73 using intravenous interferon beta-1a improve survival after emergency open surgery for a ruptured abdominal aortic aneurysm?

Findings: In this randomized controlled trial the up-regulation of CD73 associated with day-28 survival after patients had survived initial emergency aortic reconstruction. Glucocorticoid use and IFN beta neutralizing antibodies blocked the up regulation of CD73 and was associated with higher post-operative mortality.

Meaning: Intravenous interferon beta-1a is potential organ protective treatment in states of ischemia and inflammation but needs further testing. The key learning is it should not be used together with glucocorticoids.

Introduction

A ruptured abdominal aortic aneurysm (RAAA) is a medical crisis. The only lifesaving treatment is immediate aortic surgery. Roughly half of RAAA patients die before reaching the hospital, and despite successful surgery another 30 – 40% of these patients die post-surgery in intensive care due to multi-organ failure (MOF)¹. The cause of MOF is systemic inflammatory response brought forth by inflammatory mediators that are released from stressed and dying cells. Although operative techniques and intensive care have developed during the past decades, little change has occurred in post-operative mortality for open surgery of RAAA. Endovascular aortic repair (EVAR), which does not require the cross-clamping of the aorta and does not produce surgical trauma to the extent as open-repair, has emerged as new surgical option. The post-operative systemic inflammatory response is considered to be milder after EVAR compared to open repair, which could lead to less MOF and better survival². CD73 is considered a key organ protective endothelial molecule in states of hypoxia and inflammation^{3,4}. In vascular beds CD73 is the rate limiting enzyme in producing anti-inflammatory adenosine from pro-inflammatory adenosine triphosphate (ATP), which is released from stressed and dying cells⁵. CD73 is up regulated on the endothelium by hypoxia itself, or by type I interferons (IFNs)⁶. IFN beta-1a is known to induce CD73 and attenuate inflammation in critical care settings if given intravenously (IV)⁷. IV IFN beta-1a is still an investigational medicinal product but considered a viable treatment option for adult respiratory distress syndrome (ARDS), Middle East respiratory syndrome (MERS) and coronavirus (COVID-19)⁸. In the current trial we set out to test the ability of IV IFN beta-1a to prevent MOF and death after emergency aortic reconstruction for RAAA in a randomized and placebo-controlled setting. The study was pre-maturely stopped after an interim analysis, that was performed because another study concerning the use of IV IFN beta-1a in ARDS reported that the concomitant use of glucocorticoids inhibits IFN beta-1a from up-regulating CD73^{9,10}. Glucocorticoids are widely used in intensive care due to their profound anti-inflammatory and hemodynamic properties, especially in septic shock, although they have shown contradictory results in patient outcomes in a number of trials¹¹. More recently in the era of COVID-19 literature suggest using type I IFNs and glucocorticoids in a sequenced manner, respectively¹². Hence, the current study did not have power to evaluate the efficacy of IV IFN beta-1a to prevent death in RAAA patients, but the study does provide important information concerning CD73 as an organ protective molecule.

Methods

Study Design

This was a multicentre, phase II, 2:1 randomised, double-blind, parallel group comparison of the efficacy and safety of IV IFN beta-1a compared to placebo in the prevention of death in adult patients surviving emergency open surgery for an infra-renal RAAA.

The study was designed to enrol patients who presented with a RAAA diagnosed by ultrasound or computed tomography (CT) scan in the emergency room. All ruptures were confirmed (=retroperitoneal haematoma) in operation, and only confirmed ruptures were included. Patients underwent screening when a diagnosis of RAAA had been made. Before entering the study, patients were to be assessed to ensure that all eligibility criteria were met. Study specific procedures were performed only after obtaining written informed consent from the patient itself, if applicable in the emergency department, or from the next of kin.

Patients meeting all the inclusion and none of the exclusion criteria at screening were randomly assigned to treatment with IFN beta-1a or placebo in the randomisation ratio of 2:1 (IFN beta-1a: placebo). The treatment assigned to each patient was determined according to a computer-generated randomisation list within an electronic case report form (eCRF). Following randomisation, after successful surgery, the first dose of the study drug was administered at arrival to the intensive care unit (ICU). No longer than 4 hours could pass after arriving to the ICU before administering the study treatment. This allowed some time for assessing the stability of the patient and seeking consent from next of kin, without unnecessarily prolonging the beginning of the treatment. Consecutively the patients were treated daily with 10µg of IFN beta-1a or matching placebo as an IV bolus followed by a 5 ml flush of sterile saline for up to 6 days every 24 hours (+/-2 hours) starting from the first dose. Treatment could be stopped earlier if the patient improved and was discharged from the ICU. The main analysis and reporting used D30 follow-up. The trial was conducted according to Good Clinical Practice and ICH Guidelines according to the Declaration of Helsinki. The trial was first approved by the institutional review board Ethics Committee of the Hospital District of South-West Finland and of then at each participating site (identifier ETMK:62/1800/2014), and informed consent was obtained before any study procedure in accordance with local processes. The present study was registered on ClinicalTrials.gov NCT03119701 on 19/04/2017. The study was monitored by an independent Data Monitoring Committee that reviewed on-going safety data in an unblinded manner.

Eligibility Criteria

Patients older than 18 years with emergency open infra-renal RAAA surgery were included for the study. The infra-renal RAAA was confirmed by imaging or during emergency surgery. Temporary clamping above the renal arteries to control the surgical situation did not lead to exclusion unless total suprarenal clamping time exceeded 30 minutes. This was because kidney function was assessed as an endpoint and prolonged clamping could imbalance study groups.

Major exclusion criteria were; irreversible hemorrhagic shock, unconsciousness at arrival or during screening, chronic renal replacement therapy, diagnosed liver cirrhosis, severe congestive heart failure

defined as NYHA class IV, advanced malignant disease, EVAR first attempt, known hypersensitivity to natural or recombinant IFN or on-going treatment with IFN alpha or beta.

Outcome measures

The primary outcome was all-cause mortality at day 30 (D30). Key secondary endpoints included organ failure-free days at D30, ventilator free days at D30, days on hemodialysis, length of ICU stay, length of hospital stay, and all-cause mortality at D90. Adverse events (serious and non-serious) were recorded between the informed consent and D30.

To assess the biological activity and effect of the study drug, Myxovirus Resistance Protein A (MxA) and serum CD73 were measured during study days 1-13 with developed enzyme-linked immunosorbent assays as described previously⁹. The former is a specific biomarker of IFN beta-1a activity, the latter is the molecular target of IV IFN beta-1a.

Statistical Analysis

A sample size of 129 eligible patients for the primary efficacy analysis, with a 2:1 ratio to active (86) and placebo (43), was planned to detect the difference of 22% between the two treatment groups with 80 % power using 2-sided test with 0,05 significance level. Because of the planned interim analyses, the calculations assume that 2 sequential tests are made, and the O'Brien-Fleming spending function is used to determine the test boundaries. The sample size calculations were to be re-evaluated after interim analyses at 40 and 84 evaluable patients. Logistic regression model was used for the analyses of observed mortality rates in the two treatment groups. Data for the primary efficacy endpoint was presented for key, pre-defined subgroups of patients: patients with concomitant use of glucocorticoids with study drug, CD73 responders (two-fold increase from baseline value), and actual treated patients receiving at least two doses of study drug, i.e. Per Protocol Set (PPS). To assess the association of CD73 response with glucocorticoid use, average and maximum CD73 values from days 3-13, i.e. after at least two doses of study drug, between overlapping glucocorticoid use and non-use were tested with t-test (using Satterthwaite t-test in case the variances were unequal). The same test was used to assess the association of CD73 values between patients alive and not alive at D30. Further exploratory analyses concerning CD73 are described along with Supplemental Results.

Descriptive statistics were provided for all treatment-emergent adverse events (TEAEs). TEAEs were summarised using frequency and patient-based tables. All safety analyses were conducted for the entire enrolled patient population, i.e. Full Analyses Set (FAS). All statistical tests were performed as two-sided and at a significance level of 0.05.

Results

Study Participants

Patients were enrolled between February 2017 and September 2019 at 9 sites in Finland and the Baltic countries. A total of 50 patients were screened, out of which 40 patients fulfilled all inclusion criteria and were randomly assigned and received treatment in the study at the time of the interim analysis: 29 patients were allocated into the IFN beta-1a group and 11 patients into the placebo group. A total of 30 (75.0%) patients completed 6-day dosing (22 [75.9%] and 8 [72.7%] patients in the IFN beta-1a and placebo groups, respectively). All 40 (100.0%) randomised patients were included in the FAS population and 38 (95.0%) in the PPS population evaluating drug efficacy. The two patients that were excluded from the PPS population died within 24 hours after surgery, which does not allow sufficient time for the study drug to work and be evaluated for efficacy. These patients were evaluated for safety and detailed description of these patients can be found in the online Supplement. No patient discontinued the study or was lost for follow-up (Figure 1).

The overall mean age of the study subjects was 73.6 (SD, 8.95) years and similar in both active treatment and placebo groups. The percentage of male patients enrolled in the study was higher compared with female patients. All the patients were Caucasian. Overall, the demographic characteristics were similar in both treatment groups (Table 1). The median duration of the index surgery for RAAA was 181 mins, and similar between the treatment groups. The median total volume substitution, total blood substitution and total crystalloid substitution for patients were rather similar between the treatment groups, although there was a tendency of more fluids and platelets used in the active arm. In approximately half of the study patients (16 [42.1%]) the abdomen was left open at the index surgery: more frequently in the active group (13 [48.1%]) compared to the placebo group (3 [27.3%]).

Table 1
Baseline demographics and procedural characteristics

	IFN beta-1a N = 27	Placebo N = 11	p-value
Demographics			
Age	73.4 (SD, 9.6)	73.9 (SD, 7.6)	P=0.87
Sex (male)	85 %	100 %	P=0.30
Hypertension	16 (59%)	5 (46%)	P=0.49
Atrial fibrillation	4 (15%)	4 (36%)	P=0.20
Coronary artery disease	4 (15%)	1 (9%)	P=1.00
Hypercholesterolemia	9 (33%)	3 (27%)	P=1.00
Diabetes	5 (19%)	2 (18%)	P=1.00
COPD	5 (19%)	2 (18%)	P=1.00
Asthma	3 (11%)	1 (9%)	P=1.00
Major surgical features			
Duration of surgery (min)	186 (SD, 76)	169 (SD, 55)	P=0.49
RBC substitution (mL)	1573 (SD, 946)	1378 (SD, 972)	P=0.57
Platelet substitution (mL)	357 (SD, 603)	173 (SD, 212)	P=0.18
Total volume substitution (mL)	7159 (SD, 4133)	6294 (SD, 1938)	P=0.39
Left renal vein ligated	3 (11%)	0 (0%)	P=0.54
Clamp above the renal arteries	2 (7%)	1 (9%)	P=1.00
Open abdomen	13 (48%)	3 (27%)	P=0.30
COPD = chronic obstructive pulmonary disease, RBC = red blood cell			

Outcomes and biomarkers

At this data cut-off 38 patients (27 in the active arm and 11 in the placebo arm) were included in the efficacy analyses. Two patients were excluded from efficacy analyses, because they died within 24 hours after arriving in the ICU post-surgery, and this is too early of IFN beta to induce CD73. Both excluded patients had been allocated to the IFN beta-1a treatment group. The Data Monitoring Committee carefully examined these two cases. Both patients were deeply hypotensive and anuric already from surgery due to underlying illnesses and massive bleeding. Overall treatment was ceased within 24 hours of surgery. Both deaths were attributed to be a cause of the underlying condition and not the study drug (please see online

Supplement for further details). There was no statistical difference in the primary endpoint between study groups in the entire safety population (FAS, N = 40) or efficacy population (PPS, N = 38) (Table 2). All-cause mortality at D30 was 22.2% (6 of 27 patients) in the interferon beta (FP-1201-lyo) group and 18.2% (2 of 11 patients) in the placebo group (OR 1.30; 95% CI, 0.21 – 8.19). No secondary efficacy endpoint showed a significant difference between study groups either. However, subgroup analyses revealed that there was a trend of increased mortality in patients that received glucocorticoids during the study treatment. This was seen in both active and placebo arms (Table 2). D30 mortality was 19% in patients not receiving overlapping glucocorticoids with the study drug compared to 33.3% in patients receiving glucocorticoids in the active arm. In similar fashion D30 mortality was 14.3% vs 25% depending on glucocorticoid use in the placebo arm. Further biomarker analyses measuring serum CD73 could not demonstrate significant difference between study groups (Figure 2A), but showed that an elevation in CD73 significantly associated with survival at D30 ($P = 0.001$) (Figure 2B). This was seen in the entire patient population (Table 2). Overlapping use of glucocorticoids with the study drug was associated with no CD73 response. This was statistically significant especially in the active arm ($P = 0.002$) (Figure 2C). Details on the use glucocorticoids together with IFN beta-1a are presented in Supplemental Table S1. Supplemental Figure S1 and S2 illustrates the head-to-head comparison of the entire population divided into CD73 responders and non-responders, which show the survival benefit and improvement in SOFA score following a CD73 response. Figure S2 also illustrates that both groups have similar baseline severity according SOFA score. MxA values are presented in Supplemental Figure S3. Their values are not that affected by glucocorticoids, which is in line with existing knowledge.

Table 2
Primary outcome for the treatment groups and key sub-groups

		Active	Placebo	Total
	At D30	N (%)	N (%)	N (%)
All	Alive	21 (78.8%)	9 (81.8%)	30 (78.9%)
	Dead	6 (22.2%)	2 (18.2%)	8 (21.1%)
Sub-group				
Glucocorticoids	Alive	4 (66.7%)	3 (75%)	7 (70%)
	Dead	2 (33.3%)	1 (25%)	3 (30%)
No glucocorticoids	Alive	17 (81%)	6 (85.7%)	23 (82.1%)
	Dead	4 (19%)	1 (14.3%)	5 (17.9%)
Sub-group				
CD73 responder	Alive	8 (100%)	1 (100%)	9 (100%)
CD73 non-responder	Dead	6 (31.6%)	2 (20%)	8 (27.6%)
	Alive	13 (68.4%)	8 (80%)	21 (72.4%)

Safety

For the safety analyses, 40 patients (29 patients in the active arm and 11 patients in the placebo arm) were exposed to study drug, and 75.9% and 72.7% of patients in the FP-1201-lyo and placebo group, respectively, completed 6 days of study treatment. There were no differences between groups in treatment exposure duration.

Altogether, 36 (90.0%) patients experienced treatment-emergent adverse events (TEAEs) during the study. Overall, the number and percentage of patients experiencing at least 1 TEAE were similar in the 2 treatment groups (27 [93.1%] patients in the IFN beta-1a group compared with 9 [81.8%] patients in the placebo group). Pyrexia and atrial fibrillation were the most common TEAEs, with a different incidence in both treatment groups: pyrexia occurred at a higher frequency in the IFN beta-1a group (6 [20.7%] patients vs. 1 [9.1%] patient in the placebo group), whereas new onset atrial fibrillation occurred at a higher frequency in the placebo group (3 [27.3%] patients in the placebo group and 5 [17.2%] patients in the IFN beta-1a group). Serious TEAEs were reported in 16 (40%) of patients. The number and percentage of patients experiencing at least 1 serious TEAE were higher in the IFN beta-1a group compared with the placebo group (13 [44.8%] patients in the active group compared with 3 [27.3%] patients in the placebo group). None of these differences were statistically significant. The most common TEAEs concerned gastrointestinal ischemia and perforations, which formed the clear majority (7 out of 13) of the reported serious TEAEs in the active arm. Out of these 4/7 had open abdomen from surgery and 5/7 of these

patients died. A clear feature was, that 6/7 of these patients with intestinal ischemia were patients that did not have a CD73 response. In the placebo arm 1 patient had intestinal ischemia and died. TEAEs leading to discontinuation of the study drug occurred in 6 (15%) patients overall, of which 5 [17.2%] were from the IFN beta-1a compared to 1 [9.1%] in the placebo (Table 3). Liver enzyme increases were the most common TEAE leading to discontinuation, which occurred in the IFN beta-1a group only (2 [5.0%] patients). In summary, 9 (22.5%) patients died during the study, plus an additional 2 patients shortly after surgery. Overall, the incidence of fatal outcomes was similar in the treatment groups and no clear safety concern was observed.

Table 3

Treatment-emergent adverse events leading to discontinuation of study drug in the safety population

System Organ Class	Reported Term	IFN beta-1a	Placebo
		(N=29) N (%)	(N=11) N (%)
Any TEAE	Total	5 (17.2)	1 (9.1)
Cardiac disorders	Total	1 (3.4)	0 (0.0)
	Myocardial ischaemia	1 (3.4)	0 (0.0)
Gastrointestinal disorders	Total	0 (0.0)	1 (9.1)
	Intestinal ischaemia	0 (0.0)	1 (9.1)
General disorders	Total	2 (6.9)	0 (0.0)
	Multiple organ dysfunction syndrome	1 (3.4)	0 (0.0)
	Pyrexia	1 (3.4)	0 (0.0)
Laboratory investigations	Total	3 (10.3)	0 (0.0)
	Hepatic enzyme increased	2 (6.9)	0 (0.0)
	Liver function test abnormal	1 (3.4)	0 (0.0)

IFN beta neutralizing antibodies

The interaction of glucocorticoids with IFN beta did not fully seem to explain why certain individual did not respond to the IFN beta-1a treatment. As a part of the studies safety analyses, the development of IFN beta neutralizing antibodies (NABS) was monitored. We did not see any increase in the development IFN beta NABS during treatment, but 45% of patient had elevated titres of NABS at baseline, which was unexpected. These titres normalized by day 30, i.e. IFN beta-1a treatment did not produce NABS. Elevated baseline titres of NABS associated significantly with lower levels of both CD73 ($P = 0.04$) and MxA ($P = 0.02$) during the study in multivariate model of repeated measurement (RM ANCOVA). In the multivariable model the presence of NABS was strongest and only statistically significant variable that affected CD73

levels during the observation period (baseline to day 13). Please see online Supplement for further details and Supplemental Figures S3 & S4. Overall, in the study population D30 mortality was 14% in patient without NABS and 29% in patients that had elevated levels of NABS.

Discussion

In the INFORAAA trial we randomly assigned infra-renal RAAA patients to receive either 10µg of IV IFN beta-1a or matching placebo for up to 6 days during their ICU stay after surviving emergency aortic reconstruction. The aim was to prevent death by inducing CD73. The IFN beta-1a treatment was well tolerated and did not show any clear safety concern in this extremely ill and hazardous patient population. IFN beta-1a treatment was especially associated with pyrexia, which is a known adverse effect of type I IFNs⁹, and to a small extent an increase in liver enzymes. Liver failure could be a concern in this extremely ill patient population, but mainly a small number of cases with increased liver enzymes were observed. Intestinal ischemia and perforations are rather common in RAAA patients¹³, and there was a slightly higher incidence of these in the active arm. Patients with gastrointestinal ischemia were mainly (6/7) patients that had no CD73 response. CD73 has been shown to be a key molecule to protect also from gastrointestinal ischemia¹⁴. Overall, the active arm seems to have had more surgical challenges looking at procedure duration, volume and platelet substitution, and incidence of left renal vein ligation, together with not being able to close the abdomen in quite a high number of patients.

Altogether, IV IFN beta-1a did not show efficacy in the prevention of death in this study and its ability induce CD73 was hindered by confounding factors. Overlapping use of glucocorticoids during the intervention period and the existence IFN beta neutralizing antibodies appear to have prevented the desired IFN beta-1a induction of CD73, and thus, preventing organ protection. The same drug-drug interaction has recently been reported in an ARDS study⁹ and shown to happen in primary human pulmonary endothelial cells because glucocorticoids block the formation of transcription factor binding to the interferon response element in the nucleus¹⁰. Also recently, a high level IFN neutralizing antibodies have been reported to associate with severe COVID-19¹⁵, a condition leading to ARDS and MOF. It has been postulated that the presence of IFN neutralizing antibodies, which increase with age, are a cause that leads to severe COVID-19^{15,16}. However, here in a blood sample taken after a ruptured aorta we find a surprisingly high rate of IFN neutralizing antibodies, in 45% of patients, which are in the midst of an inflammatory storm. The question is, are this IFN neutralizing antibodies the cause or result of the witnessed inflammatory and vascular crisis.

A remarkable zero-mortality was observed in patients that had a clear CD73 response. Even though the study population was relatively small, such a clear-cut observation is striking and highlights CD73's role as an organ protective and possibly lifesaving molecule in ischemic conditions¹⁷⁻¹⁸. A notable observation, which conquers to current knowledge, is that hypoxia itself is a strong inducer of CD73^{14,19}. This was observed also in the placebo arm where elevation in CD73 values were seen in similar fashion as in the IFN beta-1arm 3-4 days after surgery. However, it did not happen in similar intensity as in the IFN

beta-1a group. Inducing CD73 with IFN beta-1a or hypoxia requires time, because it happens through response elements in the gene encoding for CD73, which then leads to protein transcription and expression of the protein¹⁹. One can consider this as a form high altitude acclimatization, which also takes time. CD73 has been shown to increase at altitude or hypoxemic conditions²⁰, and to be crucial to prevent hypoxia induced vascular leakage³. Remote ischemic pre-condition, i.e. introducing intermittent hypoxic periods to a tourniquet limb, also induces systemic CD73 and has been sought to be organ protective when done before coronary artery bypass grafting (CABG)²¹. So far large clinical trials investigating ischemic pre-conditioning in CABG have been disappointing²², but then again ischemic periods to the limb have mainly been applied during anesthesia induction, which does not allow sufficient time for induction of CD73 prior to surgery. Pre-operative statin treatment has been shown to be beneficial prior to CABG and other major cardiovascular surgery²³. Amongst their many effects, statins are also known to stabilize endothelial CD73²⁴. From dose finding studies with intravenous IFN beta 1, it is known that induction of prominent pharmacodynamic effect happens within 2 to 3 daily doses if measured using MxA from circulating lymphocytes, i.e. the anti-viral effect of IFN beta⁷. Concerning induction of CD73, it is likely that induction happens in a similar time frame than with MxA, but quantifying CD73 elevations is more complex. In vitro a similar time frame for CD73 induction has been shown on human endothelial cells⁷. Direct measurement of CD73 expression on the endothelium is not possible, and measuring endothelium shed serum CD73 is a surrogate marker reflecting endothelial happenings in a delayed fashion. Ideally, patients would benefit the most from early administration of IV IFN beta-1a before the ischemic insult and inflammatory responses. However, this would have to be explored in a different setting than RAAA. For RAAA patients any mean to improve the turnover of extracellular ATP into adenosine is likely to benefit patients after they have survived the immediate crisis and surgery²⁰.

Limitations

Investigating a pharmaceutical intervention in a population of RAAA patients is extremely challenging. Firstly, the incidence of RAAA is low and variable, which makes enrollment a challenge. This study was slow in recruiting, partly due to the increasing number of EVARs performed for RAAA instead of open surgery. Open surgery instead of EVAR was chosen because the systemic inflammatory response is significantly bigger after open surgery²¹. Secondly, patients are very volatile and suffer a higher number of complications, especially surgical complications, which makes the study outcome measures sensitive to challenges faced in the index surgery. Thirdly, investigators and healthcare professionals looking after these patients operate under highly stressful and demanding conditions, which makes overall running and monitoring the trial challenging. The study was designed to manage all these variables to the best possible extent to capture a coherent and comparable population that could still receive the study drug and possibly benefit from it in comparison to placebo. Thus, we left out impending ruptures, thoracoabdominal aneurysms, or patients that were not likely to survive, because they would add significant variability to the outcome and imbalance between groups. We also chose not to administer the study drug before surgery, for example in the emergency department, because subsequent blood loss and

surgical features could have altered the drug effect even further and led to a higher degree of early dropouts and un-certainty on the drug effect. Nonetheless, the biggest limitation of this study to draw any definitive conclusions concerning its original aim is that it is significantly under-powered due to premature interruption.

Conclusions

The current results further highlight the role of CD73 as an organ protective molecule under states of acute ischemia and inflammation. Hypoxia itself is an efficient inducer of CD73. In acute settings such RAAA our natural responses can be sufficient to induce an adequate CD73 response and the additional benefit IFN beta-1a may have in this setting remains elusive. Efficacy and safety of IV IFN beta-1a for the prevention of death after open surgery for an infra-renal RAAA could not be established in this prematurely stopped study. Future trial should take into account potential interaction with glucocorticoids.

Abbreviations

ARDS Adult Respiratory Distress Syndrome

ATP Adenosine triphosphate

CD73 Cluster of Differentiation

COPD chronic obstructive pulmonary disease

COVID-19 Coronavirus Disease 2019

CT Computed Tomography

D30 Day 30

D90 Day 90

eCRF electronic Case Report Form

EVAR Endovascular aortic repair

FAS Full Analyses Set

FP-1201-lyo Interferon beta

ICU Intensive Care Unit

IFN Interferon

IV Intravenous

MERS Middle East Respiratory Syndrome

MOF Multi-organ failure

MxA Myxovirus Resistance Protein A

NYHA New York Heart Association

PPS Per Protocol Set

RAAA Ruptured Abdominal Aortic Aneurysm

RBC red blood cell

TEAE Treatment-Emergent Adverse Events

Declarations

***Ethics approval and consent to participate:**

The trial was approved by the institutional review board of each participating site, and informed consent was obtained before any study procedure in accordance with local processes.

***Consent for publication:**

The present article is original and bases on data that has not been published or communicated to a society or meeting at any form. All authors have given their consent for publication.

***Availability of data and materials:**

The study data is available on request 4Pharma Ltd. (Turku, Finland) Timo Huttunen.
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***Competing interests:**

Dr Jalkanen and Karvonen own stock and are employed by Faron Pharmaceuticals Ltd. Other authors have no competing interest.

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***Authors' contributions:**

Author Contributions: Drs Hakovirta, Jalkanen, Karvonen and Venermo had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Jalkanen, Hakovirta, Karvonen, Venermo

Acquisition, analysis, or interpretation of the data: All authors.

Drafting of the manuscript: Hakovirta, Jalkanen, Karvonen, Venermo

Critical revision of the manuscript for important intellectual content: All authors

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References

1. Laine, M. T., Laukontaus, S. J., Kantonen, I. & Venermo, M. Population-based study of ruptured abdominal aortic aneurysm. *Br J Surg*, **103** (12), 1634–1639 (2016).
2. IMPROVE Trial Investigators. Comparative clinical effectiveness and cost effectiveness of endovascular strategy v open repair for ruptured abdominal aortic aneurysm: three year results of the IMPROVE randomised trial. *BMJ*.2017;359.
3. Thompson, L. *et al.* Crucial role for ecto-5'-nucleotidase (CD73) in vascular leakage during hypoxia. *J Exp Med*, **200**, 1395–1405 (2004).
4. Eltzschig, H., Sitkovsky, M. & Robson, S. Purinergic signaling during inflammation. *NEJM*, **367** (24), 2322–2333 (2012).
5. Minor, M., Alcedo, K. P., Battaglia, R. A. & Snider, N. T. Cell type- and tissue-specific functions of ecto-5'-nucleotidase (CD73). *Am J Physiol Cell Physiol*, **317** (6), 1079–1092 (2019).
6. Kiss, J. *et al.* Interferon-beta protects from acute lung injury via upregulation of CD73. *Eur J Immunol*, **37** (12), 334–338 (2007).
7. Bellingan, G. *et al.* The effect of intravenous interferon-beta-1a (FP-1201) on lung CD73 expression and on acute lung injury mortality: an open-label study. *The Lancet Respiratory Medicine*, **2**, 98–107 (2014).
8. Jalkanen, J., Hollmén, M. & Jalkanen, S. Interferon beta-1a for COVID-19: critical importance of the administration route. *Crit Care*, **24** (1), 335 (2020).

9. Ranieri, V. M. *et al.* Effect of Intravenous Interferon β -1a on Death and Days Free From Mechanical Ventilation Among Patients With Moderate to Severe Acute Respiratory Distress Syndrome: A Randomized Clinical Trial. *JAMA*, **323** (8), 725–733 (2020).
10. Jalkanen, J. *et al.* Glucocorticoids inhibit type I IFN beta signaling and the upregulation of CD73 in human lung. *Intensive Care Med*, **46** (10), 1937–1940 (2020).
11. Carlet, J., Payen, D. & Opal, S. M. Steroids for sepsis and ARDS: this eternal controversy remains with COVID-19. *Lancet*, **396**, e61–62 (2020).
12. Feuillet, V., Canard, B. & Trautmann, A. Combining anti-virals and immunomodulators to fight COVID-19. *Trends Immunol*, **42** (1), 31–44 (2021 Jan).
13. Bown, M. J. *et al.* The post-operative mortality of ruptured abdominal aortic aneurysm repair. *Eur J Vasc Endovasc Surg*, **27** (1), 65–74 (2004).
14. Hart, M. *et al.* Hypoxia-inducible factor-1 alpha dependant protection from intestinal ischemia/reperfusion injury involves ecto-5'-nucleotidase (CD73) and the A2B adenosine receptor. *J Immunol*, **186**, 4367–4374 (2011).
15. Zhang, Q. *et al.* Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science*. 2020 Oct 23;370(6515):eabd4570
16. van der Wijst, M. G. P. *et al.* Type I interferon autoantibodies are associated with systemic immune alterations in patients with COVID-19. *Sci Transl Med*, **24**, eabh2624 (2021 Aug).
17. Grenz, A. *et al.* Protective role of ecto-5'-nucleotidase (CD73) in renal ischemia. *J Am Soc Nephrol*, **18** (3), 833–845 (2007).
18. Eckle, T., Krahn, T. & Grenz, A. Cardioprotection by ecto-5'-nucleotidase (CD73) and A2B adenosine receptors. *Circulation*, **115** (12), 1581–1590 (2007).
19. Jalkanen, S. & Salmi, M. VAP-1 and CD73, endothelial surface enzymes in leukocyte extravasation. *Arterioscler Thromb Vasc Biol*, **28** (1), 18–26 (2008).
20. Liu, H. *et al.* Beneficial Role of Erythrocyte Adenosine A2B Receptor-Mediated AMP-Activated Protein Kinase Activation in High-Altitude Hypoxia. *Circulation*. 2016 Aug 2;134(5):405-21.
21. Cai, Z., Luo, W., Zhan, H. & Semenza, G. L. Hypoxia-inducible factor 1 is required for remote ischemic preconditioning of the heart. *Proc Natl Acad Sci*, **110**, 17462–17467 (2013).
22. Hausenloy, D. J. & Yellon, D. M. Targeting Myocardial Reperfusion Injury–The Search Continues. *N Engl J Med*, **373**, 1073–1075 (2015).
23. Liakopoulos, O. J. *et al.* Preoperative statin therapy for patients undergoing cardiac surgery. *Cochrane Database Syst Rev*, **4**, CD008493 (2012).
24. Ledoux, S. Lovastatin Enhances Ecto-5'-Nucleotidase Activity and Cell Surface Expression in Endothelial Cells: Implication of Rho-Family GTPases. *Circ Res*, **90**, 420–427 (2002).
25. Cauwels, A. *et al.* Extracellular ATP drives systemic inflammation, tissue damage and mortality. *Cell Death Dis*, **5**, e1102 (2014).

26. Makar, R. *et al.* The inflammatory response to ruptured abdominal aortic aneurysm is altered by endovascular repair. *Int J Vasc Med.*2013;482728.

Figures

Figure 1

CONSORT diagram

Figure 2

Individual CD73 responses A) in the active vs. placebo arms, B) in patients that survived vs. patients that died by D30 (i.e. all study patients) (Satterthwaite t-test, $p = 0.001$), and C) in patients that received vs. did not receive overlapping glucocorticoids with IV IFN beta-1a (i.e. active arm only) (Satterthwaite t-test, $p = 0.002$)

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