ARENA Trial Protocol

Background

Acute lung injury (ALI) is a common devastating clinical syndrome characterised by life-threatening respiratory failure requiring mechanical ventilation and multiple organ failure and is a major cause of morbidity and mortality. ALI occurs in response to a variety of insults, such as trauma, severe sepsis and transfusion with blood products. It affects all age groups; has a high mortality of up to 30-50% (1, 2) and causes a long-term reduction in quality of life for survivors (3). ALI has significant resource implications, prolonging intensive care unit (ICU) and hospital stay, and requiring rehabilitation in the community (4). The cost per ICU bed-day in the UK exceeds £1800 and delivery of critical care to patients with ALI accounts for a significant proportion of ICU capacity. Only 54% of survivors are able to return to work 12 months after hospital discharge (5). The high incidence, mortality, long-term consequences and high economic costs mean that ALI is an extremely important problem.

Mechanisms of ALI

ALI is an inflammatory condition, characterised by neutrophil (6) and macrophage mediated (7) injury associated with the release of inflammatory cytokines and proteases, particularly matrix metalloproteinases (MMPs) and oxidative stress. This uncontrolled local inflammatory response causes alveolar epithelial and capillary endothelial barrier damage, outpouring of protein rich fluid into the alveolar space leading to the development of non-cardiogenic pulmonary oedema, with accompanying widespread activation of the coagulation cascade, leading to microvascular thrombosis and fibroproliferation (8).

Platelets have an increasingly recognised role in the inflammatory response of ALI. They contain α-granules (containing chemokines such as heparin binding protein, growth factors including IGF1 and TGFb, and clotting factors) and dense granules (containing ADP/ATP, calcium and histamine) (9, 10). Platelet activation and degranulation within the lung can therefore lead to neutrophil and monocyte chemoattraction and pulmonary inflammation.

Inhaled lipopolysaccharide as an *in vivo* model of acute lung injury

We and others have established that inhalation of low dose inhaled lipopolysaccharide (LPS) results in a local inflammatory response in the alveolar compartment of healthy humans, as reflected by influx of neutrophils and an increase in the concentrations of cytokines in bronchoalveolar lavage (BAL) fluid, without causing adverse events (11-15) and is a useful in vivo model of acute lung injury. Inhalation of 50 μg (11) and 60 μg (16) have been reported to cause transient pyrexia ($0.7^{o}C$) and symptoms (malaise, myalgia, shivers, fatigue, headache and cough)lasting less than 4 hours with no reduction in lung function as measured by FEV1 and FVC. No serious adverse events were reported. In a previous study, inhalation of 100μg did not induce clinically significant adverse signs or symptoms and was not associated with significant changes in FEV1 and FVC (15). Indeed, LPS dosesgreater than 100μg have been tolerated well by healthy subjects (17).

We have established this *in vivo* model of lung injury, whereby healthy subjects inhale 50 μg of E. coli LPS and subsequently undergo bronchoalveolar lavage (BAL) 6 hours later (11). Reflecting the mechanisms implicated in the development of ALI, we have shown qualitatively similar (but quantitatively significantly less) inflammation to that seen in the alveolus of patients with ALI, with increased pulmonary cytokines

such as IL-8, IL-1β, and TNFα, evidence of pulmonary cell specific injury/activation as well as a systemic inflammatory response as measured by plasma CRP. We have used this model to provide pilot data for 2 clinical trials in lung injury.
Firstly using this model in which healthy subjects were pre-treated with simvastatin for 3 days prior to LPS inhalation, simvastatin resulted in anti-inflammatory and antiprotease activity (11). This led to a phase 2 study (HARP) of simvastatin in ALI in which it was demonstrated that simvastatin reduced pulmonary inflammation and improved surrogate clinical outcomes in patients with ALI (18). This has now progressed to National Institutes of Health Research, Efficacy and Mechanism Evaluation programme funded multi-centre study of simvastatin in ALI called HARP-2.
In the second study, which was funded by the Medical Research Council, we again utilised this *in vivo* model to demonstrate that recombinant human keratinocyte growth factor promoted production of epithelial repair factors in the alveolar space and this data was utilised to design a phase 2 clinical trial for KGF treatment in patients with ALI which is ongoing. Therefore this is a safe, well tolerated model which yields clinically relevant data to determine efficacy of potential therapies to inform design of phase 2 clinical trials inpatients with ALI.

The role of aspirin in modulating platelet activation and inflammation
Aspirin (acetylsalicylic acid) has been used in clinical practice for over 100 years. It inhibits cyclo-oxygenase enzymes, therefore preventing the formation of lipid mediators including thromboxane A2 (TxA2) and pro-inflammatory prostaglandins from arachidonic acid. TxA2 is required for platelet degranulation and aggregation. Production of pro-inflammatory prostaglandins from arachidonic acid is mediated by cyclo-oxygenase-2 (COX-2), an enzyme induced in inflammatory and endothelial cells by cytokines, growth factors and bacterial products including lipopolysaccharide (LPS) (19-21). PGE_2 is a key downstream pro-inflammatory product of COX-2 activation. It can act in an auto- and paracrine fashion on local inflammatory and parenchymal cells to increase intracellular cAMP and hence drive nuclear translocation of NFkB and thus the production of many pro-inflammatory cytokines, including TNFa and IL-8. Lipoxins are a group of anti-inflammatory eicosanoids derived from arachidonic acid which act via the lipoxin A_4 receptor (LXA4R) on leukocytes to inhibit free-radical formation, and reduce activation of the proinflammatorytranscription factors AP-1 and NFkB (22), all of which are implicated in the development and perpetuation of ALI. Aspirin can induce the production of a lipoxin (“aspirin-triggered 15-epi-lipoxin A4”, also known as “ATL”) (23).

Experimental data in ALI
Platelet activation has been shown to mediate neutrophil-recruitment to the lung in an acid-induced murine model of lung injury which is inhibited by pre-treatment with aspirin (1mg/g intra-peritoneally 15 minutes prior to induction of ALI) (24). Platelet depletion in a mouse model of lung injury (LPS + transfusion-related injury) reduced lung oedema and histologic markers of injury, and increased survival (25). The effect was reproduced by pre-treatment with aspirin (100 µg/g intra-peritoneally prior to injury) (25). In a murine model of ALI, aspirin-triggered epi-lipoxin A4 (ATL) treatment reduced pulmonary oedema, neutrophil infiltration, and lung IL-6 levels and promoted neutrophil apoptosis and clearance by macrophages (26). ATL also inhibits LPS induced IL-8 release by neutrophils (22), In humans aspirin prevents delayed neutrophil apoptosis in the period post coronary artery bypass grafting (27):

delayed neutrophil apoptosis and persisting inflammation is a feature of ALI. Finally, aspirin inhibits cytokine-induced degradation of the NFkB inhibitor IkBa in lung epithelium(28).

Observational data predicting beneficial effect of aspirin in the critically ill

In a recent observational study, subjects admitted to medical ICU without ALI at the point of admission, but who were on anti-platelet (aspirin or clopidogrel) treatment, were shown to have over 50% reduction in the development of ALI compared with those not on anti-platelet treatment (29). A mortality benefit was seen in a further retrospective cohort study in which subjects on anti-platelet therapy (the majority of whom received aspirin at a dose up to 160mg daily) were compared with those who were not at the time of admission, even among patients at perceived higher bleeding risk (30). Finally in a cohort of patients admitted with community acquired pneumonia, those on anti-platelets (the majority of whom received aspirin at a dose less than 100mg daily) had a significantly lower rate of need for ICU admission (31). All studies were adjusted for confounding variables including statin treatment, which may affect development of organ dysfunction in the critically ill (18). These data suggest that clinically relevant doses of aspirin may attenuate the development of ALI.

Therefore on the basis of in vitro and animal data as well as clinical studies, aspirin may be a potential therapy for patients with ALI. We postulate that aspirin may inhibit platelet driven neutrophil and monocyte chemoattraction, as well as decrease the production of pro-inflammatory lipid mediators and increase anti-inflammatory lipoxins.

Rationale for the aspirin dose and duration

Aspirin has been widely used in a range of patients with a variety of clinical conditions. The normal therapeutic dose of aspirin ranges from low dose with a predominantly anti-platelet effect (75mg once daily) to higher doses associated with an anti-inflammatory action (up to 2400mg daily) (32).

Data from animal models of acute lung injury indicate aspirin may be beneficial in animal models of ALI. However, in these studies, high doses were used. No animal study has compared low and high dose aspirin. While lower doses of aspirin were reported in the observational cohort studies in the critically ill where aspirin was associated with benefit (29-31), again there is no data investigating the effects of both low and higher dose aspirin.

If aspirin decreases pulmonary inflammation, it is important to determine if both low dose aspirin with a predominantly anti-platelet effect and higher doses are effective. While on the basis of available data, both low and high dose aspirin are safe, particularly given the duration of treatment is only 7 days, it is acknowledged that the risk of side effects are dose related. Furthermore, as gastric stress ulceration occurs in the critically ill and the risk might be increased with higher doses of aspirin, it would be an important to know if low dose aspirin was effective.

Reflecting the pharmacokinetics of aspirin, a 7-day dose regimen (rather than a single dose) has been selected to ensure steady state is achieved to more closely reflect the clinical scenario.

Therefore this current study will investigate if aspirin (administered as 75mg once daily or 600mg 12 hourly for 7 days) modulates pathogenic mechanisms important in the development of ALI.
If effective these data would be important to inform the dose to be used in subsequent phase 2 clinical trials in patients with ALI.

Aspirin has acceptable side effects
Side effects of aspirin are usually mild and infrequent as listed in the British National Fomularly (BNF). In the multicentre PAIN study (33) in 2900 patients who received aspirin (up to 3000mg daily for 7 days) the most common adverse events were abdominal discomfort (6.8%), dyspepsia (3.1%), nausea (2.5%), headache (1.3%) with all adverse events rare (<1.0%). The main risk factor identified for adverse events is the use of concomitant medications (34).
As healthy volunteers with no history of peptic ulcer disease or concomitant medication will be recruited the risk of developing these side effects will therefore be mitigated. We do not expect any of the volunteers to have any significant risk by participating in this study. The higher dose has been used safely in previous healthy volunteer studies with aspirin and platelet aggregation studies (35).

There are no effective pharmacological therapies for ALI
The Cochrane systematic review of pharmacological treatments that included 22 studies of 14 different drugs concluded that “effective pharmacotherapy for ALI is extremely limited, with insufficient evidence to support any specific intervention” (36).
The National Heart, Lung and Blood Institute Working Group considered the future research directions in ALI in 2002 and concluded that clinical trials underpinned by mechanistic investigations were essential to develop new therapies for ALI (37).

Hypothesis
The hypothesis to be tested is whether treatment with aspirin will reduce pulmonary and systemic inflammation in healthy subjects induced by inhaled LPS i.e. an *in vivo* model of acute lung injury.

Trial design
Prospective, randomised, double-blind, allocation concealed, placebo-controlled single centre, clinical study of aspirin in healthy subjects exposed to LPS inhalation.

Population
Healthy subjects will be recruited by advertising. They will be invited to partake in screening which will consist of a history, physical examination, blood investigations, pregnancy test (if appropriate), ECG and measurement of lung function with spirometry (FEV_1 and FVC).
Subjects will be eligible to participate in the study if they fulfil the following criteria:

Inclusion criteria:
1. Healthy subjects

Exclusion criteria:
1. Age < 18 years
2. Pregnancy or breast feeding or woman of childbearing potential not using adequate contraception.

3. Participation in a clinical trial of an investigational medicinal product within 30 days
4. Consent declined
5. Aspirin or non steroidal anti-inflammatory (NSAID) use in the past 4 weeks
6. History of asthma
7. Known aspirin or NSAID hypersensitivity
8. History of peptic ulcer disease
9. Platelet count < 150 x 10^6/ml
10. Aspirin resistance

Aspirin resistance is uncommon and therefore it is not anticipated that it will have a significant impact on the study.

Intervention
Subjects will be randomised to aspirin 75mg once daily or 600mg 12 hourly or placebo (1:1:1) orally for 7 days prior to inhalation of LPS.

Outcomes
As this is a phase 2 clinical study, several outcomes will be evaluated to determine whether treatment with aspirin shows efficacy for important biologic outcomes.

Primary outcome
The primary outcome for this study is BAL IL-8 concentration at 6hours following LPS administration.
This endpoint was chosen as dysregulated alveolar inflammation is important in the development of lung injury and aspirin has a range of anti-inflammatory effects. Specifically IL-8 is a potent neutrophil chemoattractant present in the alveolar space early in course of ALI (38), treatment with anti-IL-8 monoclonal antibody in experimental animal models of lung injury has been shown to attenuate injury (39) and increased plasma IL-8 is associated with mortality in ALI (40) suggesting that it is important in the pathophysiology of lung injury.

Secondary outcomes
The secondary outcomes are to investigate if pre-treatment with aspirin will modulate:
1. Alveolar inflammatory response
2. Plasma inflammatory response
3. Intracellular signalling activity in the alveolar space
4. Indices of alveolar epithelial and endothelial function and injury
5. Lipid inflammatory mediators

Trial procedures
Figure 1 demonstrates the assessments to be performed at given time periods.

Figure 1. Timing of assessments

TIME	Baseline	Day 7 T = -15 mins	Day 7 T = 0 hr	Day 7 T = 6-7 hrs	Day 8 T = 24 hrs
Eligibility criteria	*				
Consent	*				
Demographics	*				
Baseline haematology and biochemistry	*	*			*
ECG	*				
Lung function (FEV1 and FVC)	*	*		*	*
Symptom assessment and vital signs		*		*	*
Aspirin responsiveness	*	*			
Specialised blood tests		*		*	*
LPS inhalation			*		
Bronchoalveolar lavage (BAL)				*	
Adverse event assessment		*	*	*	*

Informed consent

The Chief Investigator is responsible for ensuring that informed consent for trial participation is given by each subject. An appropriately trained doctor may take consent. If no consent is given a subject cannot be randomised into the trial. The subject will be asked to sign the consent form which will then be countersigned by the person taking consent and will be retained in the trial site file. Subjects may withdraw from the trial at any time without prejudice.

Baseline assessment

The following will be performed:
Participant demographics (date of birth, gender, height and weight)

Vital signs (pulse, blood pressure, respiratory rate and tympanic temperature)
Laboratory assessments: haematological parameters, biochemical parameters.
Pulmonary function (FEV1 and FVC)
Check inclusion and exclusion criteria

Subject registration and randomisation procedure
After informed consent, the researcher will contact the clinical trials pharmacist who will allocate a unique subject number to the subject and randomise the subject in a 1:1:1 ratio to the designated treatment group. The clinical trials pharmacist will dispense the trial drugs.
Patients will be randomised to receive aspirin 75mg once daily or 600mg 12 hourly or placebo. The study medication will have an identical appearance. Blinding of the aspirin and placebo will be achieved by encapsulation.
To maintain blinding each subject will be given 4 containers of study medication, 2 morning containers A and B and 2 evening containers C and D. Subjects will be instructed to take one capsule from each container in the morning and one capsule from each container in the evening.

Patients randomised to aspirin 75mg will receive 1 container containing aspirin 75mg and 1 container containing placebo for the morning and 2 containers containing placebo for the evening.

Patients randomised to aspirin 600mg 12 hourly will receive 2 containers of aspirin 300mg each for the morning and 2 containers of aspirin 300mg each for the evening.

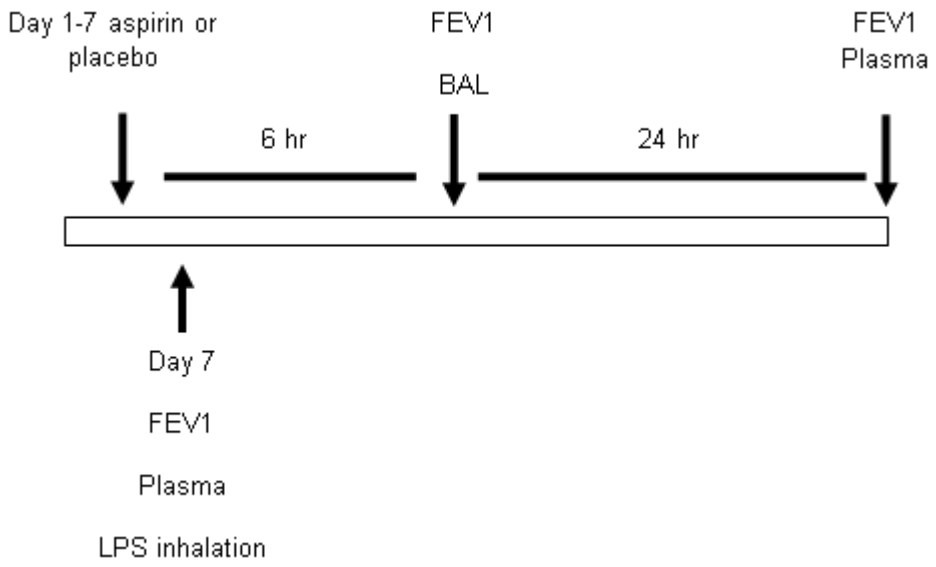
Patients randomised to placebo will receive 4 containers of placebo, 2 containers containing placebo for the morning and 2 containers containing placebo for the evening.

Study procedures for unblinding
The investigator or treating physician may unblind a participant's treatment assignment in the case of an emergency, when knowledge of the study treatment is essential for the appropriate clinical management or welfare of the subject. Should a treating clinician require emergency unblinding, the investigator will be contacted via an emergency contact telephone number. The investigator will contact the clinical trials pharmacist at the Belfast City Hospital during working hours or if out of working hours the on call pharmacist for the Belfast Health and Social Care Trust for emergency unblinding.
The date and reason for the unblinding must be recorded in the CRF.

Standardised management
All subjects during the treatment period will not take any prescribed or "over the counter" medications.

The study procedure is summarised below:

Figure 2



Day 7
The subject will attend hospital. Vital signs, clinical laboratory assessments and pulmonary function will be measured. Blood for research assays will be taken.

Subjects will then inhale 50 µg LPS (from *Escherichia coli* O26:B6 Sigma) via an automatic inhalation-synchronised dosimeter nebuliser. The lyophilised LPS will be reconstituted under sterile conditions at the pharmacy at Celerion, Belfast (Clinical Research Organisation) using endotoxin-free, sterile saline prior to nebulisation.

Bronchoscopy and BAL will be undertaken at 6 hours. Prior to bronchoscopy and BAL, lung function will be measured. Participants will be closely monitored during and after bronchoscopy. In brief, subjects will receive light sedation and topical lignocaine to anaesthetise the vocal cords and airways. The bronchoscope will be wedged in a segment of the middle lobe and 3 x 60 ml aliquots of normal saline instilled and then aspirated by suction in keeping with standard procedures. Samples will be labelled with the subject's unique subject number, transferred to the laboratory on ice. Total cell count will also be performed as well as cytospin preparation on an aliquot of pooled uncentrifuged BALF. BAL fluid will be processed as previously described (11, 41). The BALF will be stored at -80 °C until analysis. At 24 subjects will return and lung function will be measured, blood sampling will be performed. Samples will be stored beyond study completion. As new scientific data become available we will be able to use this resource of stored samples to investigate if this new data is relevant to ALI pending additional ethical approval.

Laboratory measurements
Measurements will include

1. Alveolar inflammatory response biomarkers which may include but are not limited to the measurement of BAL cytokines (including but not limited to TNFα, IL1β, IL6, IL8), proteases and antiproteases, HO1, coagulation factors (including but not limited to thrombin-antithrombin complex, tissue factor, protein C, thrombomodulin and plasminogen activator inhibitor1), and RAGE ligands. Identification of specific cellular populations within the BAL (using but not limited to cytospins, flow cytometry, ELISpot assays, *in vitro* cell expansion).

2. Plasma inflammatory response biomarkers which may include but are not limited to measurement of plasma CRP, cytokines (including but not limited to TNFα, IL1β, IL6, IL8), proteases and antiproteases, HO1, adhesion and activation molecule expression (including but not limited to sICAM1), coagulation factors (including but not limited to thrombin-antithrombin complex, tissue factor, protein C, thrombomodulin and plasminogen activator inhibitor1), and RAGE ligands.

3. Intracellular signalling activity in the alveolar space which may include but not limited to the measurement of BAL total and phosphorylated p38, ERK and JNK MAPKs and STAT -1/-3 from leucocyte extracts. Activated and total IκBα and β will be measured in cytoplasmic extracts and NFκβ and AP-1 in nuclear extracts
.
4. Indices of alveolar epithelial and endothelial function and injury in plasma and BAL which may include but not be limited to the measurement RAGE, Ang I/II, SP-D, vWF, PCP3 as well as total protein, plasma albumin, α2-macroglobulin, protein permeability (albumin:α2-macroglobulin ratio).

5. Lipid inflammatory mediators in plasma and BAL which may include but not be limited to the measurement of thromboxane B2, prostaglandin E metabolite and 15-epi-lipoxin A4.

BAL from subjects (with and without aspirin) will also be tested on primary cultures of fresh human neutrophils and monocytes to determine surrogate markers of inflammation which may include but not be limited to the measurement of activation (shape change, CD11b surface expression, superoxide release), adhesion and transmigration, cytokine release and MMP production, rate of apoptosis and for monocytes their ability to phagocytose.

Alveolar macrophages will be isolated from BAL to study the effects of aspirin administration and LPS inhalation on alveolar macrophage function, which may include but not be limited to the measurement of inflammatory mediator release and apoptosis as well as response to anti-protease peptides *in vitro*.

Identification of specific cellular populations within the blood (using but not limited to cytospins, flow cytometry, ELISpot assays, *in vitro* cell expansion) and measuring transcriptome changes within these cell population.

Statistical considerations
Sample size
BAL IL-8 concentration in response to LPS is 389+/-94pg/ml (11). Aspirin can reduce systemic IL-8 by up to 30% (42). Assuming a similar treatment effect of 30% in pulmonary IL-8 with aspirin, a sample size of 11 completed subjects in each group will have 80% power to show a 30% reduction in IL-8 at a two tailed significance level of 0.05 between placebo and each aspirin group. Combining the aspirin groups will have a 90% power to show a 30% reduction in IL-8 at a two tailed significance level of 0.05 between placebo and the aspirin groups. The recruitment will continue until 33 completed volunteers have been recruited to ensure that the study is adequately powered. In addition 22 healthy volunteers will be recruited who will act

as controls; 11 will undergo the BAL only and 11 will undergo BAL after LPS inhalation only. These 22 healthy volunteers will have the same data collected as the other 33 volunteers.

Statistical analysis

Data will be analysed by GraphPad Prism using t tests for parametric data and Mann-Whitney test for non-parametric data, comparing placebo and aspirin-treated groups for both models. The a priori analysis plan will be to compare the combined aspirin and placebo groups. A secondary analysis is to determine if there is a dose dependent response. The study will be analysed with an intention to treat so all randomised patients will be included in the analysis. However a secondary analysis will be undertaken taking into consideration volunteers who show aspirin resistance. A statistical analysis protocol will be written by the trial statistician before the end of the trial. All the power calculations and methodology for data analysis have been confirmed by Cliona McDowell, Biostatiscian, Clinical Research Support Centre.

Risks to subject

Overall aspirin is a well tolerated drug. Side effects of aspirin are usually mild and infrequent as listed in the British National Formulary (BNF). In the multicentre PAIN study (33) in 2900 patients who received aspirin (up to 3000mg daily for 7 days) the most common adverse events were abdominal discomfort (6.8%), dyspepsia (3.1%), nausea (2.5%), headache (1.3%) with all adverse events rare (<1.0%). The main risk factors identified for adverse events the use of concomitant medications (34).
As healthy volunteers with no history of peptic ulcer disease or concomitant medication will be recruited the risk of developing these side effects will therefore be mitigated. We do not expect any of the volunteers to have any significant risk by participating in this study. The higher dose has been used safely in previous healthy volunteer studies with aspirin and platelet aggregation studies (35).

Participants will be closely monitored during and after the bronchoscopy and BAL. Participants will receive light sedation and local anaesthesia (to prevent discomfort). Bronchoscopy and BAL can rarely be associated with low oxygen levels. Prior to bronchoscopy subjects will be given supplemental oxygen. The test will be stopped if the oxygen levels fall significantly. Predefined stopping criteria are established and if oxygen levels, as measured by pulse oximetry falls to <93% from baseline bronchoscopy and BAL will be stopped. As the subject receives light sedation, they need to be accompanied home and will not be able to drive for the remainder of the day. Bronchoscopy and BAL will be undertaken in the bronchoscopy suite at the Belfast City Hospital. All the necessary facilities to safely undertake this procedure are available.
Subjects can experience cough and fever within 24 hours following LPS inhalation or bronchoscopy and BAL. Subjects will be advised how to manage symptoms (paracetamol as anti-pyretic and analgesia) and given a telephone number to contact a member of the study team if any symptoms develop after leaving hospital. Subjects will receive a follow up telephone consultation 7 days after completing the study to ensure their well being. We have experience in using low dose nebulised LPS at a dose of 50μg as a model of lung injury without any serious adverse events.

Pharmacovigilance

Definition of Adverse Events

The EU Clinical Trials Directive 2001/20 provides the definitions given in Table 1.

Table 1. Terms and Definitions for Adverse Events

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	Any untoward and unintended response in a participant to an investigational medicinal product, which is related to any dose administered to that participant.
Unexpected Adverse Reaction (UAR)	An adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out in the Summary of Product Characteristics (SPC) for that product (for products with a marketing authorisation)
Serious Adverse Event (SAE) **Serious Adverse Reaction (SAR)** **Suspected Unexpected Serious Adverse Reaction (SUSAR)**	Respectively, any adverse event, adverse reaction or unexpected adverse reaction that: • results in death • is life-threatening • requires hospitalisation or prolongation of existing hospitalisation* • results in persistent or significant disability or incapacity • consists of a congenital anomaly or birth defect is any other important medical event(s) that carries a real, not hypothetical, risk of one of the outcomes above and suspected transmission via a medicinal product of an infectious agent.

AE Reporting

The chief Investigator or their delegated investigator is responsible for recording adverse events observed during the study period.

The investigator should attempt, if possible, to establish a diagnosis based on the subject's signs and symptoms. When a diagnosis for the reported signs or symptoms is known, the investigator should report the diagnosis as the adverse event, rather than reporting the individual symptoms.

The investigator should follow all adverse events observed during the study until they are resolved or stabilized, or the events are otherwise explained. All adverse events should be treated appropriately. Treatment may include one or more of the following: no action taken (i.e., further observation only); non-drug therapy given; discontinuation of study drug; subject's hospitalized. The action taken to treat the adverse event will be recorded in the CRF.

For both adverse events and serious adverse events, the adverse event report page in the CRF will be completed.

The Chief Investigator must assess seriousness, causality and expectedness for any adverse events in keeping with regulatory requirements.

The investigator must record the adverse events, seriousness, regardless of

relationship to study drug as well as duration (start and end dates).
Adverse events are recorded at each study time point and tabulated for inclusion in an annual safety report to the sponsor, MHRA and Research Ethics Committee.

Suspected Unexpected Serious Adverse Reactions (SUSARs)
Suspected Unexpected Serious Adverse Reactions (SUSARs) are SAEs that are unexpected i.e. are not consistent with the Summary of Product Characteristics and are considered to be caused by the study drug. SUSARs will be recorded and reported in line with UK statutory requirements for clinical trials involving Investigational Medicinal Products (IMP).
The Chief Investigator is responsible for reporting adverse events to the sponsor within required timelines. The Chief Investigator must report serious adverse events to the sponsor within 24 hours of becoming aware of the event.
The sponsor is responsible for reporting adverse events to the MHRA and Research Ethics Committee, within the specified timelines as per the regulatory requirements.

End of Trial
The trial will end when the completed number of patients have been recruited and completed follow-up.
The trial will be stopped prematurely if:
• Mandated by the Ethics Committee
• Mandated by the MHRA
• Mandated by the sponsor eg following recommendations from the DMEC
• Funding for the trial ceases

The Research Ethics Committee that originally gave a favourable opinion of the trial and the MHRA that issued the Clinical Trial Authorisation will be notified in writing if the trial has been concluded or terminated early.

Research governance and regulatory approvals
The trial will comply with the principles, requirements and standards set out in the Research Governance Framework and The Medicines for Human Use (Clinical Trials) Regulations 2004 and subsequent amendments.
Approval from a Research Ethics Committee and a Clinical Trial Authorisation is needed before the start of the trial.
The trial will registered with the International Standard Randomised Controlled Trial Number register.

Sponsorship
The Belfast Health and Social Care Trust (BHSCT) will act as sponsor.

Ethics
The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki.
This study will involve healthy volunteers. However, the research question cannot be answered without undertaking the study in these subjects.
Following detailed discussion of the study, written, informed consent will be obtained from the participant. Consenting processes are standardised, and are reinforced via training prior to study start-up.

Patient Confidentiality
Patient confidentiality will be maintained at every stage and compliance with the Data Protection Act (1998).

Good Clinical Practice
The trial will be carried out in accordance with the principles of the International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines (www.ich.org).

Trial Monitoring
Site monitoring will be directed by the sponsor according to the study risk assessment. Site visits will be performed on a regular basis to ensure that all regulatory requirements are met and to monitor the quality of the data collected. The CRF will be used for source data verification.

Indemnity
Queen's University Belfast will provide indemnity for the study.

Funding
UK Intensive Care Society

Safety and well being of study participants
Participant safety and well-being are protected by implementation of the sponsors SOPs as set out in the Research Governance Framework and The Medicines for Human Use (Clinical Trials) Regulations 2004. As sponsor the BHSCT requires all research to be managed through a dedicated Research Management System. Systems are in place to ensure that all investigators are able to demonstrate that they are qualified by education, training or experience to fulfil their roles and those systems and procedures are in place which can assure the quality of every aspect of the trial.
If new safety information becomes available, then study participants will be informed of this and asked if they wish to continue in the study. If the subjects wish to continue in the study they will be formally asked to sign a revised approved participant information sheet and consent form.

Safety of investigators
The University and the Trust have Health and Safety Policies applicable to all employees. All personnel should also ensure they adhere to any other Health and Safety regulations relating to their area of work. The Chief investigator will ensure that all personnel have been trained appropriately to undertake their specific tasks. As the study fits closely to standard practice, there are few risks identified which are hazardous to the investigators. The study team will complete GCP and consent training prior to start up.

Data Management

Data collection and recording
All data for individual subjects will be collected by the chief investigator or by a delegated investigator and recorded in the CRF. Due care will be taken to ensure data safety and compliance with the Data Protection Act 1998.

Data Storage
All essential documentation and trial records will be stored by the Chief Investigator in conformance with the applicable regulatory requirements and access to stored information will be restricted to authorised personnel.

Archiving
Trial documentation and data will be archived after completion of the trial in keeping with the applicable regulatory requirements.

Trial management
The Chief Investigator will take responsibility for the need to change the protocol for any reason, reviewing relevant information from other sources and considering recommendations from the DMEC. Day to day management will be undertaken via a trial management group composed of the Chief investigator and supporting staff. They will meet on a weekly basis to discuss study issues.

Data Monitoring and Ethics Committee (DMEC)
A DMEC will be appointed. The committee will be independent of the study team and will comprise 2 clinicians with experience in undertaking clinical trials.
The DMEC will meet to agree conduct and remit. The DMEC will meet after the first 3 subjects have been enrolled into the study and meet every 6 months thereafter. In the event of an occurrence of an unexpected severe adverse reaction an additional unplanned DMEC meeting may be convened. An interim analysis of efficacy is not planned although this issue can be discussed by the DMEC as required. The DMEC will function primarily as a check for safety, reviewing adverse events. They will report any issues pertaining to safety to the Chief Investigator. It will be the responsibility of the Chief Investigator to inform the sponsor who will take appropriate action to halt the trial if concerns exist about patient safety.

Trial schedule
Investigative site preparation: Aug 2012
Planned recruitment period: Nov 2012 - Feb 2014
Biochemical analysis: Feb 2013 – May 2014
Analysis and writing up of results: May 2014 – July 2014

Dissemination
The trial will be reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines (www.consort-statement.org).
Dissemination will be achieved in several ways: (1) the findings will be presented at national and international meetings with open access abstracts on-line e.g. the American Thoracic Society annual meeting; and (2) in accordance with the open access policies proposed by the leading research funding bodies we aim to publish the findings in high quality peer-reviewed open access (via Pubmed) journals. This will secure a searchable compendium of these publications and make the results readily accessible to the public, health care professionals and scientists.
Where appropriate, research details will also be posted on institutional websites available to the general public. In addition, the most significant results will be communicated to the public through press releases.

References

1. Brun-Buisson C, Minelli C, Bertolini G, Brazzi L, Pimentel J, Lewandowski K, et al. Epidemiology and outcome of acute lung injury in European intensive care units. Results from the ALIVE study. Intensive Care Med. 2004 Jan;30(1):51-61.
2. Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, et al. Incidence and outcomes of acute lung injury. N Engl J Med. 2005 Oct 20;353(16):1685-93.
3. Dowdy DW, Eid MP, Dennison CR, Mendez-Tellez PA, Herridge MS, Guallar E, et al. Quality of life after acute respiratory distress syndrome: a meta-analysis. Intensive Care Med. 2006 Aug;32(8):1115-24.
4. Rossi C, Simini B, Brazzi L, Rossi G, Radrizzani D, Iapichino G, et al. Variable costs of ICU patients: a multicenter prospective study. Intensive Care Med. 2006 Apr;32(4):545-52.
5. Cheung AM, Tansey CM, Tomlinson G, Diaz-Granados N, Matte A, Barr A, et al. Two-year outcomes, health care use, and costs of survivors of acute respiratory distress syndrome. Am J Respir Crit Care Med. 2006 Sep 1;174(5):538-44.
6. Perkins GD, Nathani N, McAuley DF, Gao F, Thickett DR. In vitro and in vivo effects of salbutamol on neutrophil function in acute lung injury. Thorax. 2007;62:36-42.
7. Frank JA, Wray CM, McAuley DF, Schwendener R, Matthay MA. Alveolar macrophages mediate epithelial barrier dysfunction in ventilator-induced lung injury. Am J Physiol Lung Cell Mol Physiol. 2006;291:L1191-8.
8. Ware LB, Matthay MA. The acute respiratory distress syndrome. N Engl J Med. 2000;342(18):1334-49.
9. Harrison P, Cramer EM. Platelet alpha-granules. Blood Rev. 1993 Mar;7(1):52-62.
10. Ruiz FA, Lea CR, Oldfield E, Docampo R. Human platelet dense granules contain polyphosphate and are similar to acidocalcisomes of bacteria and unicellular eukaryotes. J Biol Chem. 2004 Oct 22;279(43):44250-7.
11. Shyamsundar M, McKeown S, O'Kane C, Craig T, Brown V, Thickett DR, et al. Simvastatin decreases lipopolysaccharide-induced pulmonary inflammation in healthy volunteers. Am J Respir Crit Care Med. 2009;179:1107-14.
12. Sandstrom T, Bjermer L, Rylander R. Lipopolysaccharide (LPS) inhalation in healthy subjects increases neutrophils, lymphocytes and fibronectin levels in bronchoalveolar lavage fluid. Eur Respir J. 1992 September 1, 1992;5(8):992-6.
13. Jagielo P, Thorne P, Watt J, Frees K, Quinn T, Schwartz D. Grain dust and endotoxin inhalation challenges produce similar inflammatory responses in normal subjects. Chest. 1996 July 1, 1996;110(1):263-70.
14. Wesselius LJ, Nelson ME, Bailey K, O'brien-Ladner AR. Rapid lung cytokine accumulation and neutrophil recruitment after lipopolysaccharide inhalation by cigarette smokers and nonsmokers. Journal of Laboratory and Clinical Medicine. 1997 1997/01;129(1):106-14.
15. Maris NA, de Vos AF, Dessing MC, Spek CA, Lutter R, Jansen HM, et al. Antiinflammatory Effects of Salmeterol after Inhalation of Lipopolysaccharide by Healthy Volunteers. Am J Respir Crit Care Med. 2005 October 1, 2005;172(7):878-84.
16. Michel O, Nagy A, Schroeven M, Duchateau J, Neve J, Fondu P, et al. Dose response relationship to inhaled endotoxin in normal subjects. Am J Respir Crit Care Med. 1997;156:1157-64.
17. Thorn J. The inflammatory response in humans after inhalation of bacterial

endotoxin: a review. Inflammation Research. 2001 2001/05//;50(5):254-61.
18. Craig TR, Duffy MJ, Shyamsundar M, McDowell C, O'Kane CM, Elborn JS, et al. A randomized clinical trial of hydroxymethylglutaryl- coenzyme a reductase inhibition for acute lung injury (The HARP Study). Am J Respir Crit Care Med. 2011 Mar 1;183(5):620-6.
19. Bishop-Bailey D, Pepper JR, Larkin SW, Mitchell JA. Differential induction of cyclooxygenase-2 in human arterial and venous smooth muscle: role of endogenous prostanoids. Arterioscler Thromb Vasc Biol. 1998 Oct;18(10):1655-61.
20. Jones MK, Sasaki E, Halter F, Pai R, Nakamura T, Arakawa T, et al. HGF triggers activation of the COX-2 gene in rat gastric epithelial cells: action mediated through the ERK2 signaling pathway. FASEB J. 1999 Dec;13(15):2186-94.
21. Eliopoulos AG, Dumitru CD, Wang CC, Cho J, Tsichlis PN. Induction of COX-2 by LPS in macrophages is regulated by Tpl2-dependent CREB activation signals. EMBO J. 2002 Sep 16;21(18):4831-40.
22. Jozsef L, Zouki C, Petasis NA, Serhan CN, Filep JG. Lipoxin A4 and aspirin triggered 15-epi-lipoxin A4 inhibit peroxynitrite formation, NF-kappa B and AP-1 activation, and IL-8 gene expression in human leukocytes. Proc Natl Acad Sci U S A. 2002 Oct 1;99(20):13266-71.
23. Fukunaga K, Kohli P, Bonnans C, Fredenburgh LE, Levy BD. Cyclooxygenase 2 plays a pivotal role in the resolution of acute lung injury. J Immunol. 2005 Apr 15;174(8):5033-9.
24. Zarbock A, Singbartl K, Ley K. Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. J Clin Invest. 2006 Dec;116(12):3211-9.
25. Looney MR, Nguyen JX, Hu Y, Van Ziffle JA, Lowell CA, Matthay MA. Platelet depletion and aspirin treatment protect mice in a two-event model of transfusion related acute lung injury. J Clin Invest. 2009 Nov;119(11):3450-61.
26. El Kebir D, Jozsef L, Pan W, Wang L, Petasis NA, Serhan CN, et al. 15-epilipoxin A4 inhibits myeloperoxidase signaling and enhances resolution of acute lung injury. Am J Respir Crit Care Med. 2009 Aug 15;180(4):311-9.
27. Bates JJ, Watson RW, Glynn CM, O'Neill AJ, Fitzpatrick JM, Buggy DJ. Aspirin preserves neutrophil apoptosis after cardiopulmonary bypass. Shock. 2004 Jun;21(6):495-9.
28. Yoo CG, Lee S, Lee CT, Kim YW, Han SK, Shim YS. Effect of acetylsalicylic acid on endogenous I kappa B kinase activity in lung epithelial cells. Am J Physiol Lung Cell Mol Physiol. 2001 Jan;280(1):L3-9.
29. Erlich JM, Talmor DS, Cartin-Ceba R, Gajic O, Kor DJ. Prehospitalization antiplatelet therapy is associated with a reduced incidence of acute lung injury: a population-based cohort study. Chest. 2011 Feb;139(2):289-95.
30. Winning J, Neumann J, Kohl M, Claus RA, Reinhart K, Bauer M, et al. Antiplatelet drugs and outcome in mixed admissions to an intensive care unit. Crit Care Med. 2010 Jan;38(1):32-7.
31. Winning J, Reichel J, Eisenhut Y, Hamacher J, Kohl M, Deigner HP, et al. Anti-platelet drugs and outcome in severe infection: clinical impact and underlying mechanisms. Platelets. 2009 Feb;20(1):50-7.
32. Society BMAatRP. British National Formulary. 61 ed. London: Pharmaceutical Press; 2011.
33. Moore N, Le Parc JM, van Ganse E, Wall R, Schneid H, Cairns R. Tolerability of ibuprofen, aspirin and paracetamol for the treatment of cold and flu symptoms and sore throat pain. Int J Clin Pract. 2002 Dec;56(10):732-4.

34. Moore N, Charlesworth A, Van Ganse E, LeParc JM, Jones JK, Wall R, et al. Risk factors for adverse events in analgesic drug users: results from the PAIN study. Pharmacoepidemiol Drug Saf. 2003 Oct-Nov;12(7):601-10.
35. Buchanan MR, Brister SJ. Individual variation in the effects of ASA on platelet function: implications for the use of ASA clinically. Can J Cardiol. 1995 Mar;11(3):221-7.
36. Adhikari N, Burns KE, Meade MO. Pharmacologic therapies for adults with acute lung injury and acute respiratory distress syndrome. Cochrane Database Syst Rev. 2004(4):CD004477.
37. Matthay MA, Zimmerman GA, Esmon C, Bhattacharya J, Coller B, Doerschuk CM, et al. Future research directions in acute lung injury: summary of a National Heart, Lung, and Blood Institute working group. Am J Respir Crit Care Med. 2003 2003/04/01/;167(7):1027-35.
38. Donnelly SC, Strieter RM, Kunkel SL, Walz A, Robertson CR, Carter DC, et al. Interleukin-8 and development of adult respiratory distress syndrome in at-risk patient groups. 1993 1993/03/13/;341(8846):643-7.
39. Modelska K, Pittet JF, Folkesson HG, Courtney Broaddus V, Matthay MA. Acid-induced lung injury. Protective effect of anti-interleukin-8 pretreatment on alveolar epithelial barrier function in rabbits. American Journal of Respiratory and Critical Care Medicine. 1999;160(5 Pt 1):1450-6.
40. Parsons PE, Eisner MD, Thompson BT, Matthay MA, Ancukiewicz M, Bernard GR, et al. Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. Critical Care Medicine. 2005;33(1):1-6.
41. Haslam PL, Baughman RP. Report of ERS Task Force: guidelines for measurement of acellular components and standardization of BAL. Eur Respir J. 1999 Aug;14(2):245-8.
42. Goldstein SL, Leung JC, Silverstein DM. Pro- and anti-inflammatory cytokines in chronic pediatric dialysis patients: effect of aspirin. Clin J Am Soc Nephrol. 2006 Sep;1(5):979-86.