CLINICAL RESEARCH PROTOCOL

Ghrelin treatment of comatose patients after cardiac arrest:
A phase 2 clinical trial to measure safety and efficacy of
ghrelin to promote cerebral recovery

Ghrelin in Coma

PROTOCOL TITLE *'Ghrelin treatment of comatose patients after cardiac arrest: A phase 2 clinical trial to measure safety and efficacy of ghrelin to promote cerebral recovery'*

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CPC	**Cerebral Performance Categories scale**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
GCS	**Glasgow Coma Scale**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
LVAD	**Left Ventricular Assist Device**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
WBP	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Approximately half of all comatose patients after cardiac never regains consciousness because of severe postanoxic encephalopathy. The other half may be left with cognitive or motor disturbances. Currently, there is no treatment to promote cerebral recovery. Treatment with acyl-ghrelin improved functional recovery under experimental in vivo and in vitro conditions, and decreased histologically measured neuronal damage. Ghrelin has been tested in over one hundred human studies, including studies in healthy volunteers and patients with cardiopulmonary diseases, neuro-endocrine diseases, psychiatric diseases, and neurodegenerative diseases. Serious adverse events were extremely rare and difficult to attribute to ghrelin administration

Objective: First, we aim to measure safety and efficacy of intravenous treatment with acyl-ghrelin to promote cerebral recovery (functional outcome) in comatose patients after cardiac arrest. Second, we will study efficacy of ghrelin to modify case fatality, time to awaken, long term (cognitive) outcome, and cardiovascular outcomes, , treatment with vasoactive drugs, use of cardiac-assist devices, and cardiac biomarkers.

Study design: This will be a phase 2 multicenter, double blind, placebo controlled randomized clinical trial.

Study population: 160 comatose patients (GCS score of 8 or lower) after cardiac arrest and successful cardiopulmonary resuscitation, admitted to intensive care units of participating hospitals, will be included within 12 hours after resuscitation.

Intervention (if applicable): Intravenous treatment with acylated ghrelin 600micrg twice daily for 1 week vs. placebo.

Main study parameters/endpoints: The primary outcome measure will be functional outcome as expressed as the score of the cerebral performance category (CPC) at 6 months.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Ghrelin has been administered as an infusion or a bolus in a variety of doses to at least 1850 study participants. At the doses evaluated in the 66 published studies with adverse event reporting, ghrelin demonstrated an excellent short-term safety profile with few adverse effects. On the other hand, the population under study is vulnerable and exact effects of Ghrelin treatment in this specific population are unknown.

Taken together, we assume the risk of an increase of morbidity or mortality is small. The remaining small risk is acceptable, given the severity of brain damage in the population under study, the insecure prognosis, and the current lack of effective treatments to improve brain recovery and functional outcome.

1. INTRODUCTION AND RATIONALE

Comatose patients after cardiac arrest have an insecure prognosis

Anoxic brain damage after cardiac arrest is one of the most common causes of coma worldwide. In the Netherlands alone, approximately 5000 patients are admitted, yearly. Epidemiologic studies have predicted a rising incidence, because of an increasing prevalence of cardiovascular risk factors and the aging population. Postanoxic encephalopathy is the most common cause of death in patients that survive cardiac arrest to hospital admission. As opposed to increased survival of cardiopulmonary resuscitation, outcome of postanoxic coma has improved only little over the past years. Despite treatment on an intensive care unit, approximately half of all comatose patients never regain consciousness as a result of severe hypoxic-ischemic brain damage.[1,2] In the other half, there is a large probability of lasting brain damage with functional and cognitive impairments. There is limited knowledge of the pathophysiology of brain damage in postanoxic coma and hardly any insight into the severity of brain damage in individual patients.

Treatments are not available

Effective treatments to improve brain recovery in postanoxic coma are unavailable. Over the past years, there has been no substantial scientific progress. The only general treatment of presumed benefit has been cooling the brain to 32°C. This was based on evidence from two small trials in 2002. However, the benefit of cooling has become uncertain since the recent large Targeted Temperature Management trial, where cooling to 32°C was associated with the same outcome as cooling to 36°C.[3] Currently, most experts believe that prevention of hyperthermia (fever) is more important than induction of hypothermia. An important rationale behind all studied, but ineffective, neuroprotective strategies, including hypothermia, has been prevention of secondary damage by inhibition of neuronal activation. The presumption is that this should preserve the remaining energy in order to maintain basic cellular processes. However, we observed that hypoxia causes wide spread inhibition of neuronal activity in itself.[4] We established that insufficient neuronal activity during more than 24 hours is an independent predictor of absence of recovery to physiological activity patterns *in vitro*[5] and in patients with postanoxic coma.[6,7] This association was unrelated to the duration of the initial circulatory arrest or to the actual oxygen level. This suggests that, after the initial insult, it is not only the lack of energy, but also lack of neuronal activity, which may lead to secondary irreversible damage or recovery. None of the previously tested neuroprotective modalities by inhibition of activation were of proven benefit.[8]

Ghrelin was effective in animal models

Contrary to previous attempts, we now propose a treatment modality with mild neuronal activation. We found a massive increase of physiological neuronal activity and formation of new synapses by mild neuronal activation with Ghrelin in vitro.[9] Furthermore, Ghrelin prevented apoptosis in living rats after cardiac arrest, with improved neurological recovery.[10] In more than ten rat studies on focal cerebral ischemia and reperfusion (mainly by intraluminal vessel occlusion), ghrelin improved neuronal survival and functional recovery without exception, where in most studies it was assumed that ghrelin prevented apoptosis.[11]

Ghrelin is a naturally occurring hormone and mildly excitatory neurotransmitter. The presumed mechanism of action is slowing down of apoptosis.[12–15] Since hypoxia induced neuronal inactivity was independently associated with progression towards irreversible damage, both *in vitro*[16] and in patients,[6,7] we assume that the beneficial effects of ghrelin are mediated by mild neuronal activation, preventing ill-fated neuronal inactivity.[5] This is perpendicular to the classical view of neuroprotection by inhibition, which is currently applied in all comatose patients after cardiac arrest.

Ghrelin seems safe

Ghrelin has been administered as an infusion or a bolus in a variety of doses to at least 1850 study participants, including healthy participants and patients with obesity, prior gastrectomy, cancer, pituitary disease, diabetes mellitus, eating disorders, cardiovascular disease and neurodegenerative disease (for reviews please see[17,18]). Taken together: there is strong evidence that ghrelin stimulates appetite and increases circulating GH, ACTH, cortisol, prolactin, and glucose in various patient populations. There is a paucity of evidence regarding the effects of ghrelin on LH, FSH, TSH, insulin, lipolysis, body composition, cardiac function, pulmonary function, the vasculature, and sleep (review[17]).

At the doses evaluated in the 66 published studies with adverse event reporting, ghrelin demonstrated an excellent short-term safety profile with few adverse effects.[17] Serious adverse events such as pneumonia, enteritis, and lung cancer were extremely rare and difficult to attribute biologically to ghrelin administration. Most of the severe adverse events derived from 1 study of ghrelin v.s placebo administration in severely ill patients with pulmonary cachexia, a group that is vulnerable to developing additional medical problems.[19] Mild adverse events occurred in approximately 20% of participants receiving ghrelin. The most common effect was transient flushing, which occurred in 10% of volunteers, but resulted in discontinuation of study medication in only 3 of the 939 participants in whom adverse event collection was reported.[20–22] There was no difference in the percentage of participants experiencing flushing between bolus and infusion routes of administration.

Larger ghrelin doses may increase the risk of flushing, as indicated by the higher rate of flushing in the 2 ghrelin bolus studies that employed the largest tested dose (10µg/kg/dose). The most common gastrointestinal side effect was gastric rumbles, which occurred in 22 participants (2.3%) and was never severe enough to lead to ghrelin discontinuation. Gastrointestinal side effects and increased thirst were more common in volunteers who received continuous ghrelin infusions, perhaps due to the longer duration of exposure to ghrelin. Few participants developed neurocognitive effects including somnolence, fatigue, vertigo, or change in mood (26 subjects, 2.8%). These effects were more common in subjects who received ghrelin bolus, potentially due to the rapid ghrelin delivery.[17] For more details please see investigators brochure.

First effective neuroprotective treatment in cerebral ischemia?

We propose to study the effect of ghrelin on neurological recovery of comatose patients after cardiac arrest based on the large probability of a poor outcome in this patient group, lack of effective treatments to promote brain recovery, consistent beneficial effects of ghrelin under experimental *in vitro* and *in vivo* conditions, and substantial evidence of safety.

If mild stimulation of neurons with Ghrelin provides clinically relevant improvement of recovery after hypoxic-ischemic brain damage in postanoxic coma, this will be the first identified effective treatment. This will be of large relevance for patients, families, and society given the high incidence and large impact of the disease, and the large probability of a poor outcome without adjunctive treatment. Apart from the potential clinical value, the first effective neuroprotective treatment in hypoxic-ischemic brain damage will be of conceptual value, and may be translated to other patients with cerebral ischemia, such as patients with ischemic stroke.

We know of no patents or other initiatives aiming at testing effects of ghrelin or other modalities based on neuronal activation in postanoxic coma.

2. OBJECTIVES

Primary Objective

We aim to measure safety and efficacy of intravenous treatment with acyl-ghrelin to promote cerebral recovery in comatose patients after cardiac arrest. Safety will be monitored throughout hospitalization and during follow-up using all AEs reported, and by interim analyses by an independent DSMB. Efficacy will be measured by the primary outcome measure, i.e. functional recovery as measured by the Cerebral Performance Category (CPC) scale at six months after cardiac arrest.

Secondary Objective(s):

To measure safety and efficacy of ghrelin to modify

1. Case fatality
2. Time to awaken (time interval between resuscitation and Glasgow Coma Scale (GCS) score of 14)
3. Long term outcome: CPC and cognitive functioning at 12 months
4. Cardiovascular measures:

Mean arterial blood pressure day 1-7 (mean, highest, lowest)
Heart rate day 1-7 (mean, highest, lowest)
Arrhythmia day 1-7: yes / no. If yes: type of arrhythmia
Cumulative dose of vasopressive medication day 1-7
Cumulative dose of inotropic medication day 1-7
Sequential Organ Failure Assessment score day 1-7
Kidney function day expressed as GFR day 1-7
CVVH day 1-7: yes / no
Assist devices day 1-7: yes / no

5. Biomarkers

Cardiac: troponine and CK / CK-MB ratio at day 0, 1, 2 or 3
Neurological: NSE day 1, 2, 3
Endocrinological: cortisol, growth hormone, prolactine, ACTH, IGF-1 day 1, 2, 3
Highest glucose levels at day 1-7

6. Gastro-intestinal: gastric residual volume (day 1-7, during ICU admission)

3. STUDY DESIGN

This will be a phase 2 multicenter, double blind, placebo controlled randomized clinical trial.

4. STUDY POPULATION

4.1 Population (base)

Comatose patients (GCS score of 8 or lower) after cardiac arrest and successful cardiopulmonary resuscitation, admitted to intensive care units of participating hospitals, will be included within 12 hours after return of spontaneous circulation. Informed consent and randomization will have to be obtained within 12 hours after return of spontaneous circulation. The trial treatment will have to be started within three hours after randomization (please see procedure at 7.3).

4.2 Inclusion criteria

In order to be eligible to participate in this study a subject must meet the following criteria:

- Age ≥18 years
- Out of hospital cardiac arrest
- Successful cardiopulmonary resuscitation
- Return of spontaneous circulation ≤12 hours ago
- GCS score on admission ≤ 8 or suspected coma in patients who are sedated
- Admission to intensive care unit
- Hemodynamic and respiratory stability as determined by the treating intensive care physician, with the minimum requirement of mean arterial pressure > 65 mmHg. Treatment with inotropes, vasopressors or IABP is allowed.

Of note, cardiac arrhythmia is frequently observed in the patient group under study. Patients with cardiac arrhythmia can be included and with cardiac arrhythmia after inclusion, trial treatment can be continued, unless the treating intensive care physician decides otherwise.

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Age <18 years
- A known progressive neurological disease
- Expected death within 48 hours

4.4 Sample size calculation

This is a phase 2 trial aiming at estimation of safety and potential of efficacy. Power calculations are hampered by lack of any data on ghrelin effects in patients with postanoxic encephalopathy. An intended sample size of 160 patients is based on analysis of any shift in the direction of a better outcome on the CPC scale, a mean CPC sore without treatment at six months of 3.20, SD of 1.8 (based on our observations on natural history in 277 patients)[7], a mean difference between treated and untreated patients of 0.8 points on the CPC scale, alpha of 0.05 and power of 80%. The mean difference of 0.8 points on the CPC scale may be an overestimation of potential benefit. The consequent relatively small sample size is considered justified, given the need for larger future phase 3 trials if potential benefit is demonstrated. Interim analyses will only be directed at safety (comparison of SAEs between treatment groups), so no additional subjects need to be included to compensate for interim analyses.

5. TREATMENT OF SUBJECTS

The trial treatment will consist of intravenous treatment with acylated ghrelin 600micrg dissolved in 50cc normal saline by bolus (short term) infusion in at least 30 minutes twice daily, for 1 week vs. placebo (i.e. 50cc normal saline without ghrelin).[17] Treatment duration of one week is chosen because (i) ill-fated neuronal inactivity is mainly observed in the acute phase (first days) after cardiac arrest and (ii) previous phase 0 and 1 studies have proven safety with a treatment duration of one week.[17]

This trial treatment will be additional to current care. This indicates that all patients are treated according to national and local protocols for comatose patients after cardiac arrest. This includes targeted temperature treatment at 36ºC in all participating centers.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

Active treatment group: Acyl-ghrelin in normal saline
Placebo group: normal saline

6.2 Summary of findings from non-clinical studies

Hypoxic-ischemic brain damage in vitro: ghrelin treatment was associated with a massive increase of physiological neuronal activity and formation of new synapses in cultured neuronal networks exposed to hypoxia / ischemia.[9]

In vivo rat models of hypoxic ischemic brain damage after cardiac arrest: ghrelin prevented apoptosis in living rats after cardiac arrest, with less irreversible neuronal damage and better neurological recovery.[10]

In vivo rat models of hypoxic ischemic brain damage induced otherwise, mainly by intraluminal vessel occlusion: in more than ten rat studies on focal or global cerebral ischemia and reperfusion, ghrelin improved neuronal survival and functional recovery without exception, where in most studies it was assumed that ghrelin prevented apoptosis (For a review please see[11])

6.3 Summary of findings from clinical studies

Ghrelin has been administered as an infusion or a bolus in a variety of doses to at least 1850 study participants, including healthy participants and patients with obesity, prior gastrectomy, cancer, pituitary disease, diabetes mellitus, eating disorders, cardiovascular disease and neurodegenerative disease (for reviews please see[17,18]). In patients with neurological diseases, ghrelin treatment was found to be associated with neuroprotection.[18] In patients with cardiovascular diseases, ghrelin treatment was associated with improved cardiac functioning.[17] Ghrelin has not been administered to comatose patients after cardiac arrest, so far. Taken together: there is strong evidence that ghrelin stimulates appetite and increases circulating GH, ACTH, cortisol, prolactin, and glucose across varied patient populations. There is a paucity of evidence regarding the effects of ghrelin on LH, FSH, TSH, insulin, lipolysis, body composition, cardiac function, pulmonary function, the vasculature, and sleep.

6.4 Summary of known and potential risks and benefits

At the doses evaluated in the 66 published studies with adverse event reporting, ghrelin demonstrated an excellent short-term safety profile with few adverse effects.[17] Serious adverse events such as pneumonia, enteritis, and lung cancer were extremely rare and

difficult to attribute to ghrelin administration. Mild adverse events occurred in approximately 20% of participants receiving ghrelin. The most common effect was transient flushing, which occurred in 10% of volunteers, but resulted in discontinuation of study medication in only 3 of the 939 participants in whom adverse event collection was reported.[20–22] The most common gastrointestinal side effect was gastric rumbles, which occurred in 22 participants (2.3%) and was never severe enough to lead to ghrelin discontinuation. Few participants developed neurocognitive effects including temporary somnolence, fatigue, vertigo, or change in mood (26 subjects, 2.8%).[17]

6.5 Description and justification of route of administration and dosage

Justification of intravenous route: In all preclinical and clinical studies, intravenous administration was used, either as a bolus or as continuous infusion.[17]

Justification of bolus infusion (which in this study is not bolus infusion in a narrower sense, but rather short term infusion in at least 30 minutes): We chose for bolus (short term) infusion, because (1) bolus (short term) infusion suits with the natural evolution of endogenous ghrelin concentrations, which fluctuates over time, and (2) in human studies, effects of ghrelin were largest with bolus infusions as compared with continuous infusion.[17] There was no difference in the percentage of participants experiencing relevant adverse events between bolus and infusion routes of administration.[17] However, somnolence and fatigue occurred more often with bolus infusion. Therefore, to minimize the risk of relevant side effects, instead of bolus infusion in a narrower sense, we chose to apply short term infusion in with an infusion rate of max 10 µg/kg/30min, which indicates an infusion time of at least 30 minutes.

Justification of dosage: Starting point for the chosen regimen of 600µg/dose, two times daily, was optimization of the probability of treatment effect. The highest tested and safe dose regimen is 10µg/kg/dose for single doses. The highest tested regimen for treatment during one week or more is 3µg/kg/dose, two times daily, in human studies. In animal studies, intraperitoneal application of 10-200µg/kg/dose, two times daily during 3-8 weeks, was safe. Given these data, and with an elimination half-life of 27 to 31 minutes,[23] we chose for max 10µg/kg/dose, infused in at least 30 minutes. In close deliberation with the collaborating intensive care physicians, we chose a fixed quantity of 600µg/dose for pragmatic reasons. This is equal to approximately 7.5-10µg/kg/dose. Larger ghrelin doses possibly increased the risk of flushing, as indicated by the higher rate of flushing in the 2 ghrelin bolus studies that employed the largest tested dose (10µg/kg).[17] However, there was no association between dosage and serious adverse events.

Justification of treatment duration: We chose for a treatment duration of one week based on our previous studies, from which we know that the most robust changes of brain functioning (either recovery or secondary deterioration) take place in the first week after cardiac arrest. We justify this treatment duration of one week with previous human studies, where numbers of AEs in studies with treatments during 7, 10, or 14 days were equal to those in studies with shorter treatment durations[9].

6.6 Dosages, dosage modifications and method of administration

Active treatment group:

Acyl ghrelin 600µg in 50cc normal saline, intravenously, twice daily during one week.

The dosage will be modified in patients of less than 60kg to 10µg/kg.

Placebo group:

50cc normal saline

6.7 Preparation and labelling of Investigational Medicinal Product

Preparation and labelling of the investigational product will be done according to the GMP guidelines. Please also see the attached pharmacy manual and label text.

6.8 Drug accountability

Please see Pharmacy Manual.

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Main study parameter/endpoint

The primary outcome measure will be functional outcome as expressed as the score on the cerebral performance category (CPC) at 6 months.

7.1.2 Secondary study parameters/endpoints

Secondary outcomes include case fatality at one week and 6 months, time to awaken (time interval between resuscitation and GCS score of 14), EMV score and estimated CPC score at 1 week, CPC score at 3 and 12 months, and cognitive functioning at 12 months.

Cardiovascular secondary outcome measures are:

Mean arterial blood pressure day 1-7 (mean, highest, lowest)
Heart rate day 1-7 (mean, highest, lowest)
Arrhythmia day 1-7: yes / no. If yes: type of arrhythmia
Cumulative dose of vasopressive medication day 1-7
Cumulative dose of inotropic medication day 1-7
Sequential Organ Failure Assessment score day 1-7
Kidney function day expressed as GFR day 1-7
CVVH day 1-7: yes / no
Assist devices day 1-7: yes / no

Biomarkers are:

Cardiac: troponine and CK / CK-MB ratio at day 0, 1, 2 or 3
Neurological: NSE day 1, 2, 3
Endocrinological: cortisol, growth hormone, prolactine, ACTH, IGF-1 day 1, 2, 3
Highest glucose levels at day 1-7

Gastro-intestinal outcome measure is: gastric residual volume (day 1-7, during ICU admission)

7.1.3 Other study parameters

We assume that potential effects of ghrelin are mediated by mild neuronal activation, preventing ill-fated neuronal inactivity. This is based on studies under experimental in vitro and in vivo conditions.[10,18] To study effects of ghrelin on neuronal activity, we consider brain activity as measured by the EEG. Continuous EEG measurements during day 0-3 after

cardiac arrest are performed in all participating hospitals in the context of current care. The following EEG measures will be extracted and studied in relation to ghrelin treatment: the temporal brain symmetry index (tBSI),[24] the cerebral recovery index (CRI)[25] and EEG background continuity.[26] All three measures strongly represent restoration of brain activity after an hypoxic insult and are strongly related to clinical recovery.

7.2 Randomisation, blinding and treatment allocation

Subjects after cardiac arrest and successful cardiopulmonary resuscitation that are admitted to an intensive care unit of a participating hospital can be considered for enrolment in the study. Randomisation will be 1:1, in blocks of N=10, stratified by study site. Randomisation will be performed if the subject meets all inclusion/exclusion criteria and will be processed centrally by means of a web-based system that will provide the randomisation treatment arm (Ghrelin or placebo). The online system (Research Manager®), is constructed and validated for randomization and data management and has an audit trail. Contact persons of all participating centres can sign in and randomize their patients.

A legal representative or family member will be informed and patients will be included on the intensive care by their treating intensive care physician. Once informed consent is obtained, the collaborating hospital pharmacist will be notified by the intensive care physician. The pharmacist will sign in Research Manager, include the patient and randomize. The pharmacist will receive the treatment allocation through Research Manager, and thus be unblinded. He / she will prepare the treatment or placebo and provide it to the treating intensive care physician. The intensive care physician will remain blinded to the treatment allocation.

The investigator can decide to withdraw a subject from the study for urgent medical reasons. In this case the investigator can decide to ‘unblind’ the patient. For unblinding, the unblinded collaborating pharmacist will provide information on the treatment allocation to the treating intensive care physician.

7.3 Study procedures

All comatose patients after cardiac arrest will be admitted to intensive care units to receive treatment according to guidelines, as described in national and local ICU protocols.

A legal representative or family member of all eligible patients will be informed about the study as soon as possible after admission on the ICU by the ICU staff/research coordinators. Since any beneficial effect of the treatment under study is assumed to be larger with earlier

initiation, randomization and treatment should be initiated within 12 hours after cardiac arrest (ROSC). All participating centers have ample experience with inclusion of patients with acute cardiac arrest or cerebral ischemia in intervention studies.

Patients who are enrolled in this trial will receive intravenous treatment with acylated ghrelin 600micrg twice daily vs. placebo. The duration of the treatment is one week. This treatment duration of one week will be adhered to, both in patients that remain comatose, and in patients that awaken within a week. If patients are transferred from the ICU to a cardiac care unit or general ward within one week, treatment will be continued for a total of one week, as well. Intravenous treatment will be administered through infusion systems that are already in situ in all of these patients. There will be no additional punctures.

Baseline data will be obtained at admission as part of regular patient care and include: clinical data, EEG data, medical history, and use of medication. In addition, a venous blood samples (5cc) will be taken to measure the baseline ghrelin concentration. This sample will be collected during routine blood sampling; there will be no additional punctures.

After randomization, daily physical, neurological, and additional examinations at the ICU will be part of routine patient care. In addition, there will be blood sampling for measurement of ghrelin concentrations at day 1 and 3, measures of neuronal damage (NSE at day 1-3), measures of cardiac damage (troponine and CK/CK-MB ratio at day 0-3), hormone concentrations (cortisol, growth hormone, prolactine, ACTH, IGF-1 at day 1-3), and highest glucose levels at day 1-7. All blood samples will be collected during routine blood sampling, if possible; preferably, there will be no additional punctures. However, blood sampling for measurement of ghrelin concentrations will be done at 30±15 minutes after treatment with ghrelin or placebo. For these measurements additional blood samples will be taken, if necessary.

Apart from the study treatment and the venous blood sampling, patients will not be subjected to additional procedures during admission.

Surviving patients will be asked informed consent for participation and additional follow-up on long term outcome. Follow up at 3 and 6 months in surviving patients will be done by telephone interview by a trained research nurse three and six months after admission. The nurse will collect CPC scores based on a standard interview. At twelve months, patients will be invited to the nearest hospital or visited at their place of residence for detailed neuropsychological examination (please see F1 testbatterij NPO for details of the test

battery). Separate informed consent will be asked for neuropsychological examination at twelve months.

Table. Overview of study procedures

	Baseline (Day 0)	**Day 0-6**	**Day 1-7**	**Day 7**	**3m**	**6m**	**12m**
Ghrelin/ placebo treatment		X					
Venous blood sampling (largely within current care)	X		X				
CPC scores				X	X	X	
Neuropsychological examination							X

M = months, CPC = Cerebral Performance Category, to be collected by a telephone interview

The total burden in hours for the patient is max 12 hours. This includes multiple transfusions of the study drug, venous blood sampling, and the follow-up procedures at 3, 6 and 12 months.

7.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences.

7.5 Replacement of individual subjects after withdrawal

Subjects will be replaced after withdrawal for any reason.

7.6 Follow-up of subjects withdrawn from treatment

Every attempt will be made to complete the primary follow-up in these patients.

7.7 Premature termination of the study

Planned interim analyses will be performed after 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, and 100 inclusions. The interim analyses will only be directed at safety. The trial will be stopped because of any safety issue of the treatment under study, defined as a higher occurrence of SAEs in the treatment than in the control group. There will be no interim analysis for efficacy in this trial, since it is very unlikely that either ‘futility’ or ‘proof beyond reasonable doubt’ will be achieved prematurely in this relatively small phase 2 clinical trial.

8. SAFETY REPORTING

8.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

8.2 AEs, SAEs and SUSARs

8.2.1 (Serious) Adverse events reporting

As this is a randomised controlled trial with a new drug all (serious) adverse events mentioned in paragraph 8.2.2 and 8.3.3 are required to be reported to the Sponsor and recorded in the electronic database. The sponsor is responsible to report the SAE to the accredited METC that approved the protocol.

Reporting of SAEs will be the responsibility of the study coordinator and the principle investigator. The local principle investigators will report on AEs, SAEs and SUSARs within the relevant time windows mentioned below. A dedicated study nurse will collect all AEs, SAEs, and SUSARs and discuss these with the principle investigator, every week.

Since case fatality in the patient population under study is known to be 50-60%, with most deaths occurring in the first week of ICU admission, line listing of all SAEs including deaths is requested, with reporting to the accredited METC once per two months. Exceptions will be made for (1) the first ten inclusions in the trial, (2) SAEs that are probably related to the trial, and (3) Suspected Unexpected Serious Adverse Reactions, for which expedited reporting will take place.

8.2.2 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the use of Ghrelin. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded. An adverse event should be reported within 10 working days to the Principle Investigator. A serious adverse event should be reported to the Principle Investigator within 24 hours of first knowledge. The following adverse events will always be reported:

- Flushing and stomach problems (mild anticipated adverse events);[17]
- Second cardiac arrest needing cardiopulmonary resuscitation (SAE);
- Death (SAE).

8.2.3 Serious adverse events (SAEs)

The sponsor will report the SAEs through the web portal ToetsingOnline to the accredited METC that approved the protocol once per 2 months.

A Serious Adverse Event (SAE) is any untoward medical occurrence or effect that:

- **Results in death;**
- **Is life threatening (at the time of the event):** An event that, in the opinion of the investigator, would have resulted in immediately fatality if medical intervention had not been taken. This does not include an event that would have been fatal if it had occurred in a more severe form;
- **Requires hospitalisation or prolongation of existing inpatients' hospitalisation:** An event that results in an admission to the hospital for any length of time or prolongs the subject's hospital stay. An elective hospital admission will not be considered as a SAE.
- **Results in persistent or significant disability or incapacity:** An event that results in an unexpected (by investigators opinion) persistent or significant disability or incapacity;
- **Is a congenital anomaly or birth defect:** An anomaly detected at or after birth, or any anomaly that results in fetal loss;
- **Any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.**

8.2.4 Deaths

For SAEs with the outcome of death, the date and cause of death will be recorded on the appropriate case report form. Deaths that occur during the protocol that are more likely related to disease progression will be considered as an expected AE.

8.2.5 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 8.2.3);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Investigational Medicinal Product Dossier (IMPD); Investigator's Brochure for an unauthorised medicinal product.

The Principle Investigator will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same Principle Investigator and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The Principle Investigator will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the Principle Investigator has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

With any SUSAR, treatment allocation will be 'unblinded'.

8.3 Annual safety report

In addition to the expedited reporting of SUSARs, the Principle Investigator will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

8.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

8.5 [Data Safety Monitoring Board (DSMB) / Safety Committee]

The trial will be monitored by an independent data monitoring committee. The data monitoring board (DSMB) will consist of Dr. van der Worp (neurologist UMCU), Dr. Slooter (intensivist UMCU), Dr. M. van Smeden (biostatistician LUMC), and dr. Wilms (clinical pharmacologist Apotheek Haagse Ziekenhuizen en Hagaziekenhuis). The first three members of this DSMB are also involved in the TELSTAR trial (www.telstartrial.nl) and know the vulnerable population under study very well. Details on the DSMB and rules on analysis and reporting are included in K5: “DSMB charter”.

The DSMB is established to perform interim analyses for safety. Safety analyses will be performed at an individual patient level for the first ten inclusions in the trial. For these first ten inclusions, the DSMB will receive by email unblinded reports of SAEs, AEs, SUSARs, poor outcomes, and deaths after each individual inclusion. Thereby, the DSMB will have access to individual patient files. The information will be sent to the DSMB at one week ± 3 days after inclusion (i.e. the duration of treatment). Analysis of safety in these first ten inclusions will be largely qualitative. The first ten patients will be included sequentially in one hospital (MST), where the next inclusion will only take place after a positive sign from the DSMB.

Thereafter, the DSMB will perform safety analyses after 15, 20, 25, 50, and 100 inclusions. At that time, the DSMB will review data on SAEs, SUSARs, poor outcomes, and deaths per centre. This information will be sent to the DSMB at one week ± 3 days after inclusion for the interim analyses at 15, 20, and 25 inclusions. For these interim analysis, the trial will only continue after a positive sign from the DSMB. For the interim analyses at 50 and 100 inclusions, the information will be sent after having reached the secondary outcome measure at three months. In addition, the DSMB will review general aspects of the trial, including patient recruitment, patient inclusion, and unexpected events. Because of the expected high proportion of patients with a poor outcome in the patient group under study, and the consequent overlap between safety endpoints and the primary endpoint, the evaluation of safety by the DSMB will be quantitative and qualitative. In addition, suspected unexpected serious adverse reactions (SUSARs) will be reported <48 hours to the METC.

The composition, tasks and responsibilities of the DSMB are described in K5: DSMB Charter.

The DSMB will advise to stop the trial at the following moments:

- A higher number of SAEs in the treatment than in the control group
- Any SUSARs
- Any other safety issues

The advice(s) of the DSMB will be sent to the Principle Investigator of the study and the METC. Should the Principle Investigator decide not to fully implement the advice of the DSMB, the Principle Investigator will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

9. STATISTICAL ANALYSIS

9.1 Primary study parameter(s)

The primary analysis will be a single comparison between the treatment groups of the primary outcome measure after six months. This analysis will be performed according to the intention-to-treat principle. To assess the effect of treatment with ghrelin, the whole range of the CPC will be taken into account. This indicates that no dichotomy will be used in the primary analysis. Instead of a dichotomy, any shift in the direction of a better

outcome on de CPC scale will be analyzed. This will be expressed as a common odds ratio for improvement.[27]

9.2 Secondary study parameter(s)

With regard to the range of secondary outcome parameters, we will use simple 2x2 tables, two-group t-tests, Mann-Whitney tests, or multivariable linear and logistic regression models, where appropriate. In all analyses, statistical uncertainty will be quantified by means of 95% confidence intervals. Subgroup analyses based on the EEG pattern at 24 hours will be carried out for the following subgroups: (1) suppressed patterns and suppressed patterns with synchronized periodic activity, (2) continuous patterns, and (3) other patterns. This is based on our previous studies on EEG based outcome prediction of comatose patients after cardiac arrest, where the first subgroup represents patterns that are strongly associated with a poor prognosis, the second subgroup with a good prognosis, and the third subgroup with an intermediate prognosis.[refs] Although the size of this study will not allow for precise estimates of treatment effect in subgroups, we will assess heterogeneity of effects, and analyze consistency of effects on secondary outcomes.

9.3 Other study parameters

Baseline characteristics will be summarized by means of simple descriptive statistics.

9.4 Interim analysis (if applicable)

Please also see chapter 7: safety Reporting / DSMB. The trial will be monitored by an independent DSMB. Details on the composition of the DSMB and rules on analysis and reporting are included in K5: “DSMB charter”.

The trial will be stopped because of any safety issue of the treatment under study, defined as a higher occurrence of SAEs in the treatment than in the control group, any SUSAR, or any other safety concern. There will be no interim analysis for efficacy in this trial, since we consider it very unlikely that either ‘futility’ or ‘proof beyond reasonable doubt’ will be achieved prematurely in this relatively small phase 2 clinical trial.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (7th revision, Fortaleza, 2013) and in accordance with the Dutch Medical Research Involving Human Subjects Act (WMO) and local guidelines.

10.2 Recruitment and consent

Patients will be recruited at intensive care units at the participating hospitals. The treating intensivist / neurologist / clinical neurophysiologist will check the in- and exclusion criteria, inform a patient's legal representative, and ask for informed consent. Since any beneficial effect of the treatment under study is assumed to be larger with earlier initiation, treatment should be initiated within 12h after cardiac arrest, and as soon as possible after ICU admission. All participating centers have ample experience with inclusion of patients with acute cardiac arrest or cerebral ischemia in intervention studies. Survivors will be asked for informed consent for long term follow up (6 and 12 months) separately.

Written informed consent, documented on the informed consent form in accordance with GCP standards and study centre regulations, shall be obtained from each patient or its legal representative. First, a short version of the informed consent will be used to start the treatment within 12 hours after resuscitation. The more extensive patient information will be obtained within 48 hours after the start of trial treatment. The legal representative should sign this informed consent form as well. The original signed form will be kept in the Investigator Site File. The PI will retain a copy of the signed informed consent form in each patient's study record, and provide a copy to the patient or its legal representative.

10.3 Benefits and risks assessment, group relatedness

This study is conducted in comatose patients after cardiac arrest. This group of patients is is by definition (at least temporarily) incapacitated.

Severe postanoxic encephalopathy after cardiac arrest is one of the most common causes of coma worldwide. In the Netherlands alone, 3000-5000 patients are admitted, yearly. Half of all patients never awaken and die as a result of severe postanoxic encephalopathy. In the other half, there is a large probability of lasting brain damage with functional and cognitive impairments. Impact on patients and their families is huge. Specific treatments to improve brain recovery are unavailable and urgently needed. Over

the past years, there has been no substantial scientific progress. The only general treatment of presumed benefit is cooling the brain to 32°C, although its gain has become uncertain since the recent Targeted Temperature Management trial, and mechanisms of action are unclear.[3] Any new effective treatment to improve cerebral recovery and functional outcome will be of large relevance for patients, families, and society given the high incidence and large impact of the disease, and the large probability of a poor outcome without treatment.

Ghrelin is a naturally occurring 28 amino acid peptide functioning as a hormone (stimulating segregation of growth hormone) and mildly excitatory neurotransmitter. It is mainly produced in the stomach. The bioactive form, acylated ghrelin, represents 10% of the total amount of circulating ghrelin. A primary function is signaling nutrient availability from the gastrointestinal tract to the brain. Ghrelin is present in the healthy brain, where it influences mood, sleep-wake rhythm, learning, memory, and neurogenesis.[18] Ghrelin has been tested in over one hundred human studies, including healthy volunteers, patients with (severe) cardiopulmonary diseases, and neurodegenerative diseases. Serious adverse events were extremely rare and difficult to attribute to ghrelin.[17]

In an *in vitro* model of postanoxic encephalopathy, treatment with Ghrelin restored activity levels and had a large beneficial effect on neuronal survival and synapse formation.[9] In a state of the art rat model of cardiac arrest, ghrelin treatment attenuated histologically proven hypoxic brain injury, and improved functional recovery.[10] In another established model of global forebrain ischemia, ghrelin treatment improved neuronal survival as tested immunocytochemically.[15] In over ten rat studies on focal cerebral ischemia and reperfusion (mainly by intraluminal vessel occlusion), ghrelin improved neuronal survival and functional recovery without exception, where in most studies it was assumed that ghrelin prevented apoptosis.[12–15]

We propose to measure the effect of ghrelin on neurological recovery of comatose patients after cardiac arrest based on the large probability of a poor outcome in this patient group, lack of effective treatments to promote brain recovery, consistent beneficial effects of ghrelin under experimental *in vitro* and *in vivo* conditions, and substantial evidence of safety.

10.4 Compensation for injury

The Principle Investigator has a liability insurance which is in accordance with article 7 of the WMO.

The Principle Investigator (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within four years after the end of the study.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

For data collection and management, the Research Manager software will be used. All sites will receive a unique code and all patients will receive a study number by which all data will be coded. The principal investigator or trained and delegated member of the research team will have access to the coded source data, if necessary.

Several variables will be collected from hospital records for the purpose of this study as described in paragraph 6.3. The data required for the trial will be entered by the investigation sites into eCRFs in Research Manager. All site staff will be trained on correct eCRF completion. Only trained personnel will receive access and be able to enter data in the eCRF. The eCRF will not be considered as source data.

Study related correspondence, patient records, signed Informed Consent Forms and source documents are to be maintained by the study site for a minimum of 15 years after the end of this trial.

A patient code will consist of two parts. The first part refers to the hospital, the second part to the patient. The second part of the code starts with 001, 002, 003 … in each center.

Coded date will be stored in Research Manager, which fully validated and has an audit trail. Access to the data will only be provided to the researchers that are involved in the study. They will be delegated by the local PI by a delegation log. Research Manager distinguishes specific roles, for example, PI, nurse, pharmacist, monitor, DSMB. Data will be stored for 15 years.

Research Manager has a daily full back up to a dedicated backup server on a secure location (14 days storage). The backups are encrypted (AES 256).
Research Manager has a daily full back up to an external backup location on a secure location. The backups are encrypted (AES 256).
Research Manager has a weekly full back up to a dedicated backup server o a secure location. The backups are encrypted (AES 256).
Research Manager backup restore is tested periodically.

11.2 Monitoring and Quality Assurance

A site initiation visit will be performed after it has been verified that the site is prepared for the study and that the site requirements for study participation are met. During this training the study personnel will get all the information needed about the protocol, study procedures and the online database (Research Manager).

In accordance with Good Clinical Practice (GCP) guidelines, there will be a monitor system. Herewith it will be verified that
(a) the rights and well-being of the included patients are protected
(b) reported trial data are accurate, complete, and verifiable from source documents.
(c) the conduct of the trial is in compliance with the currently approved protocol, with GCP, and with applicable regulatory requirements.

Monitoring will be done by trained personnel and with adequate frequency to ensure that the investigator's obligations are being fulfilled. Frequency and timing of monitoring visits shall be determined by the Principle Investigator for each site based on enrolment rate and volume, study compliance and findings from previous visits. Details are described in the monitoring plan.
It will be verified whether signed and dated informed consent forms have been obtained from each subject before any study related procedures are undertaken. Also, compliance with the study protocol will be checked..

11.3 Annual progress report

The Principle Investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

11.4 Public disclosure and publication policy

This trial will be publicly registered in accordance with the Declaration of Helsinki on www.trialregister.nl.

Publications will be by the executive committee, in the name of the steering committee. Pre-defined sub-studies or post-hoc analysis by participating investigators are possible after consultation of the executive committee and only after publication of the primary results of the trial.

The executive committee consists of J. Hofmeijer, A. Beishuizen, M.J.A.M. van Putten, WM van de Bergh, and N. Foudraine and the study-coordinator (PhD student at the University of Twente). The steering committee consists of one or two additional local investigators from each participating center, and the executive committee. The steering committee will make decisions regarding continuation of the trial and protocol changes. Decisions will be prepared by the executive committee. The chairman of the steering committee (i.e. the principal investigator) will be advised by the independent data monitoring and safety committee. The study-coordinator is responsible for running the trial on a day-to-day basis, and will report to the executive committee.

12. STRUCTURED RISK ANALYSIS

12.1 Potential issues of concern

a. Level of knowledge about mechanism of action

Ghrelin is a naturally occurring 28 amino acid peptide functioning as a hormone (stimulating segregation of growth hormone) and mildly excitatory neurotransmitter. It is mainly produced in the stomach. The bioactive form, acylated ghrelin, represents 10% of the total amount of circulating ghrelin. A primary function is signaling nutrient availability from the gastrointestinal tract to the brain. Ghrelin is present in the healthy brain, where it influences mood, sleep-wake rhythm, learning, memory, and neurogenesis.[18]

Treatment with Ghrelin consistently improved functional recovery after cerebral ischemia in various living animal models.[10,12–14] Hereby, better functional recovery was associated with prevention of neuronal apoptosis. In an *in vitro* model of postanoxic encephalopathy, ghrelin prevented neuronal death by improved synapse recovery.[9] Taken together, it is assumed that the beneficial effects of ghrelin with regard to recovery of brain functioning after cardiac arrest are mediated by mild neuronal activation, preventing ill-fated neuronal inactivity, and thus preventing apoptosis.[5] This is perpendicular to the classical view of neuroprotection by inhibition.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

Ghrelin has been administered as an infusion or a bolus in a variety of doses to at least 1850 study participants, including healthy participants and patients with obesity, prior gastrectomy, cancer, pituitary disease, diabetes mellitus, eating disorders, cardiovascular disease and neurodegenerative diseaese.[17,18] There is strong evidence that ghrelin stimulates appetite and increases circulating GH, ACTH, cortisol, prolactin, and glucose across varied patient populations. There is a paucity of evidence regarding the effects of ghrelin on LH, FSH, TSH, insulin, lipolysis, body composition, cardiac function, pulmonary function, the vasculature, and sleep. Adverse effects occurred in 20% of participants, with a predominance of flushing and gastric rumbles and a mild degree of severity. The few serious adverse events occurred in patients with advanced illness and were not clearly attributable to ghrelin.[17]

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

Acyl ghrelin passes the blood-brain barrier after intravenous application.[28] Studies in a variety of animal models of neuronal injury have demonstrated neuroprotective effects.[29] In rat models of traumatic brain injury, ghrelin treatment increased survival and facilitated function recovery by suppressing inflammation and apoptosis.[30,31] In models of transient cerebral ischemia, ghrelin decreased infarct volumes and significantly reduced the expression of active cleavage products and apoptosis.[12–14] After global forebrain ischemia, ghrelin treatment was associated with less severe damage of hippocampal CA1 neurons.[15] In a state of the art model of coma after cardiac arrest, ghrelin treatment improved functional recovery and was associated with less apoptosis and less severe neuronal damage on histological examination.[10]

d. Selectivity of the mechanism to target tissue in animals and/or human beings

Although des-octanoyl (unacylated) ghrelin is found in higher serum concentrations, acylated ghrelin is the active form, responsible for effects in the brain. There are two variants of the central ghrelin receptor: GHS-R1a and GHS-R1b. The first has features of a typical G protein coupled receptor. The latter has been reported as an inactive form without signaling activity.[32] Following the discovery of ghrelin, various studies have demonstrated its importance in regulating food intake and body weight. These effects are largely driven by a high expression of GHS-R1a in the hypothalamus and pituitary. However, GHS-R1a is also expressed in the hippocampus, cortex, thalamus, raphe nuclei, ventral tegmental area, and substantia nigra,[33] mediating multiple physiological functions beyond those involved in metabolic activity. For example, ghrelin was able to regulate learning and memory, reward-seeking behavior, anxiety, and depression,[34,35] and had neuroprotective effects in neurodegenerative diseases.[36–39]

e. Analysis of potential effect

At the doses evaluated in the 66 published studies with adverse event reporting, ghrelin demonstrated an excellent short-term safety profile with few adverse effects.[17] Serious adverse events such as pneumonia, enteritis, and lung cancer were extremely rare and difficult to attribute biologically to ghrelin administration. Most of the severe adverse events derived from 1 study of ghrelin v.s placebo administration in severely ill patients with pulmonary cachexia, a group that is vulnerable to developing additional medical problems.[19] Mild adverse events occurred in approximately 20% of participants receiving ghrelin. The most common effect was transient flushing, which occurred in 10% of volunteers, but resulted in discontinuation of study medication in only 3 of the 939 participants in whom

adverse event collection was reported.[20–22] There was no difference in the percentage of participants experiencing flushing between bolus and infusion routes of administration. Larger ghrelin doses may increase the risk of flushing, as indicated by the higher rate of flushing in the 2 ghrelin bolus studies that employed the largest tested dose (10µg/kg). The most common gastrointestinal side effect was gastric rumbles, which occurred in 22 participants (2.3%) and was never severe enough to lead to ghrelin discontinuation. Gastrointestinal side effects and increased thirst were more common in volunteers who received continuous ghrelin infusions, perhaps due to the longer duration of exposure to ghrelin. Few participants developed neurocognitive effects including somnolence, fatigue, vertigo, or change in mood (26 subjects, 2.8%). These effects were more common in subjects who received ghrelin bolus, potentially due to the rapid ghrelin delivery.[17] To minimize risks, we chose to apply short term infusion in at least 30 minutes, rather than bolus infusion in a narrower sense.

f. Pharmacokinetic considerations

Intravenously administered ghrelin is rapidly cleared. Fifteen minutes after administration of a single 1 or 5micrg/kg bolus, total ghrelin plasma concentrations rose to 1059 and 6599fmol/l, from a mean baseline level of 169. Elimination half-life was 27 to 31 minutes.[36] A 61-fold increase in circulating total ghrelin has been reported 1 minute after iv injection of 10micrg/kg with an elimination half-life of 10 minutes.

g. Study population

Our study population consists of freshly resuscitated patients after cardiac arrest. These patients suffer from a life threatening disease and may be hemodynamically unstable. All patients are admitted to an intensive care unit. Our study population may be exceptionally vulnerable to cardiovascular adverse events.

In previous studies, cardiovascular effects of ghrelin were beneficial rather than detrimental. In healthy men, low-dose ghrelin infusion was associated with increased mean peak myocardial systolic velocity.[40] A large ghrelin iv bolus increased stroke volume and decreased systemic vascular resistance and mean arterial pressure (MAP) in healthy volunteers,[41] although a similar dose of sc ghrelin increased LVEF without change in MAP.[42] In participants with heart failure, higher and repeat dose infusions decreased MAP, pulmonary capillary wedge pressure, and systemic vascular resistance and increased cardiac index, stroke volume, and LVEF; these changes were associated with improved exercise capacity.[43,44] In these previous studies, cardiac effects of ghrelin were dose and route dependent, with greater potency from iv ghrelin compared to the sc route.

h. Interaction with other products

We know of no relevant pharmacokinetic or pharmacodynamics interactions between ghrelin and standard treatment modalities for patients after cardiac arrest.

i. Predictability of effect

There are no known biomarkers for effect of ghrelin on neurological or cardiovascular outcomes.

j. Can effects be managed?

There are no antidotes or antagonists available. Since all patients are initially admitted on intensive care units, access to adequate medical support is guaranteed.

12.2 Synthesis

Ghrelin has been administered as an infusion or a bolus in a variety of doses to at least 1850 study participants. At the doses evaluated in the 66 published studies with adverse event reporting, ghrelin demonstrated an excellent short-term safety profile with few adverse effects.[17] On the other hand, the population under study is vulnerable and exact effects of Ghrelin treatment in this specific population are unknown.

Taken together, we assume the risk of an increase of morbidity or mortality is small. The remaining small risk is acceptable, given the severity of brain damage in the population under study, the insecure prognosis, and the current lack of effective treatments to improve brain recovery and functional outcome.

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