Principal Results of a Randomised Open Label Exploratory, Safety and Tolerability Study with Calmangafodipir in Patients Treated with a 12h Regimen of N-Acetylcysteine for Paracetamol Overdose (POP Trial)

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Principal results of a randomised open label exploratory, safety and tolerability study with calmangafodipir in patients treated with a 12 h regimen of N-acetylcysteine for paracetamol overdose (POP trial)

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1. Introduction

Paracetamol/acetaminophen (N-acetyl-p-aminophenol) is the most common drug taken in overdose in the United Kingdom (UK). Annually, overdose directly leads to around 60,000 hospital attendances in the UK with around half of these patients being admitted to hospital for emergency antidote treatment [1]. Paracetamol is directly responsible for the deaths of 100–150 people per year in the UK [2]. In the USA, paracetamol overdose accounts for >50,000 hospital attendances and around 450 deaths due to acute liver failure each year [3].

In overdose, the normal paracetamol detoxification pathways are overwhelmed which allows cellular injury to be produced by the
in more than half of recipients and anaphylactoid reactions in about a third [7]. (iii) Prolonged duration: The regimen is time consuming, taking at least 21 h, leading to significant hospital bed occupancy (around 47,000 bed days per year in England) [8].

To address the high incidence of ADRs and prolonged duration of the standard NAC regimen, a shorter 12 h intravenous regimen has been developed (the ‘SNAP’ regimen) [9]. In this regimen the initial loading dose (NAC 100 mg/kg in 200 mL) is given over 2 h, followed by a second dose (200 mg/kg in 1000 mL) infused over 10 h (total dose of NAC same as 21 h regimen). The SNAP regimen has been demonstrated to be effective at reducing the incidence of vomiting and anaphylactoid reactions, compared with the standard intravenous acetylcysteine schedule [9]. The SNAP regimen is as effective as the standard NAC regimen with regard to preventing liver injury after paracetamol overdose [10].

In this study we explored the safety and tolerability of combining the SNAP regimen with a new therapeutic agent. Mangafodipir was originally developed as a MRI contrast agent and approved for that indication by the USA Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Mangafodipir has been demonstrated to prevent paracetamol-induced liver injury in mice by acting as a superoxide dismutase (SOD) mimetic which prevents mitochondrial injury during the oxidative phase of toxicity [11]. Consistent with this mechanism of action, in mice, this protection is at a time point when NAC is no longer active [11]. Calmangafodipir ($Ca_2Mn(DPDP)_5$), is derived from mangafodipir, with 80% of the manganese in calmangafodipir being replaced with calcium. Based on the similarities between calmangafodipir and mangafodipir, it is anticipated that calmangafodipir would also exhibit SOD-mimetic pharmacologic actions similar to those of mangafodipir [12]. Calmangafodipir has been studied in a Phase 2 safety and efficacy study of chemotherapy-induced peripheral neuropathy in patients with advanced metastatic colorectal cancer (PLUNIT Trial). As is the case with paracetamol toxicity, a central mechanism of this neuropathy is oxidative stress. In the PLUNIT Trial calmangafodipir was well tolerated across three doses (2, 5 and 10 μmol/kg) and prevented the development of chemotherapy-induced peripheral neuropathy [13].

The primary objective of the POP Trial was to assess the adverse events (AEs) and serious adverse events (SAEs) associated with calmangafodipir co-treatment with the SNAP NAC treatment regime in patients with paracetamol overdose. A secondary study objective was the measurement of clinical and exploratory biomarkers of acute liver injury.

2. Methods

2.1. Trial design

This study, EudraCT number 2017-000246-21, ClinicalTrials.gov Identifier NCT03177395, was funded by the Sponsor, PledPharma AB, Stockholm, Sweden and was approved by the UK medicines regulator, MHRA (25th April 2017) and West of Scotland Research Ethics Committee 1, Glasgow, UK (11th April 2017). The trial rationale and full protocol have been published [14]. The following is a summary of the study methods as per the CONSORT Guidelines. The study was an open label, randomised, exploratory, rising dose design, NAC controlled, phase 1 safety and tolerability study in patients treated with NAC following paracetamol overdose. There were no significant changes to the protocol after trial commencement. This was a single centre study.

2.2. Participants

The inclusion criteria were: 1. Any patient with capacity admitted to hospital within 24 h of either a single acute paracetamol overdose or more than one dose of paracetamol (staggered) and deemed to require treatment with NAC (as per contemporaneous UK guidelines provided by the National Poisons Information Service via the Toxbase website).
2. Provision of written informed consent. 3. Males and females of at least 16 years of age. The exclusion criteria were: 1. Patients that do not have the capacity to consent to participate in the study. 2. Patients detained under the Mental Health Act or deemed unfit by the Investigator to participate due to mental health. 3. Patients with known permanent cognitive impairment. 4. Patients who are pregnant or nursing. 5. Patients who have previously participated in this trial. 6. Unreliable history of overdose. 7. Patients presenting >24 h after overdose. 8. Patients who take anticoagulants (e.g. warfarin) therapeutically or have taken an overdose of anticoagulants. 9. Patients who, in the opinion of the responsible clinician/nurse, are unlikely to complete the full course of NAC e.g. expressing wish to self-discharge. 10. Prisoners. 11. Non-English speaking patients. Patients who took a mixed overdose that included medications in addition to paracetamol were included in the trial.

2.3. Interventions

Study participants were randomly assigned to receive NAC and a single dose of calmangafodipir, or NAC alone. This was performed in 3 sequential cohorts of 8 patients within which patients received NAC + calmangafodipir (n = 6) or NAC alone (n = 2). The dose of calmangafodipir used within each of the 3 cohorts was 2, 5, or 10 μmol/kg [13]. Overall, 24 patients were planned to be allocated as follows: NAC alone (n = 6), NAC and calmangafodipir (2 μmol/kg) (n = 6), NAC and calmangafodipir (5 μmol/kg) (n = 6), NAC and calmangafodipir (10 μmol/kg) (n = 6).

Treatment started with a NAC IV infusion of 100 mg/kg in 200 mL saline or 5% dextrose over 2 h. After this infusion was complete, calmangafodipir was administered as a bolus IV infusion over 5 min at the dose specified by the dosing cohort. Those patients randomised to the NAC alone group had no intravenous injection. In all patients the NAC regimen continued with 200 mg/kg NAC diluted in 1000 mL delivered IV over 10 h. As per local clinical practice, there is one blood sample taken 2 h before the end of the second NAC bag (the 10 h time-point) and a second blood sample taken 10 h later (the 20 h time-point). NAC (at 200 mg/kg in 1000 mL IV over a further 10 h) was continued if any of the following criteria were reached: ALT activity had more than doubled since the admission measurement, OR ALT activity was two times the upper limit of normal (100 U/L) or more, OR international normalised ratio (INR) was >1.3 OR paracetamol concentration >20 mg/L.

2.4. Outcomes

Our primary objective was to determine the safety and tolerability of calmangafodipir add-on treatment to the SNAP NAC treatment regimen in patients treated for overdose. Therefore, the primary outcome was the occurrence of any AEs or SAEs. Patients were followed using their NHS Lothian electronic records. Primary outcome data were collected 7, 30 and 90 days after randomisation, as were events of special interest: representation to hospital (any reason), representation with liver injury, repeat overdose, death and transfer to liver transplantation unit.

Secondary outcomes included clinical observations (pulse rate, blood pressure, respiratory rate, pulse oximetry, temperature) and haematological and clinical biochemistry parameters. Liver injury was quantified by standard parameters (alanine transaminase (ALT) and the international normalised ratio (INR)) and also the following exploratory circulating biomarkers: keratin-18 (K18), caspase cleaved K18 (ccK18), microRNA-122 (miR-122) and glutamate dehydrogenase (GLDH). These outcomes were pre-defined in detail in our study protocol. There were no significant changes to the outcomes after trial commencement.

2.5. Sample size

There was no formal power calculation for this Phase 1 study. Six patients per group in this dose escalation study allowed initial exploration of potential dose limiting toxicity.

An independent safety data monitoring committee (SDMC) evaluated safety prior to each planned calmangafodipir dosing step increase and recommended the continuation or termination of the study. During recruitment, summary data of in-hospital mortality/morbidity and any other information available on major outcome events (including SAEs believed to be due to treatment) were supplied to the SDMC along with any other data that the committee requested. The stopping guidelines determined that all further patient enrolment would be paused pending advise if any of the following stopping rules were met: 1) Patient death, admission to a Critical Care Unit or admission to a Liver Transplantation Unit due to any reason, or 2) One suspected unexpected serious adverse reaction (SUSAR) that definitely or probably relates to either calmangafodipir or NAC or both.

2.6. Randomisation and blinding

The allocation sequence for each dosing cohort was created by an Edinburgh Clinical Trials Unit (ECTU) programmer (GM) using computer-generated random numbers, using blocking to ensure the required 6:2 ratio. The randomisation list was held centrally at ECTU in order to conceal treatment allocations until these were implemented via the secure web-based randomisation system. There was no blinding of participants or emergency department staff. The statistical analysis plan was written blinded to the treatment allocations.

2.7. Biomarker measurement

ALT and INR were measured as part of routine clinical care in the Biochemistry Laboratory at the Royal Infirmary of Edinburgh. Enzyme-linked immunosorbent assay (ELISA) was used to measure K18 – (Peviva M65 ELISA [classic]) and ccK18 (M30 Apoptosense ELISA, Bioaxxess, Tewkesbury, UK). miR-122 was measured by reverse transcription polymerase chain reaction (RT-PCR) as previously described [15]. The concentration of miR-122 was expressed as the Dct using spiked-in C. elegans miR-39 as an external normaliser and quantified as copy number per μL by generating a standard curve [15]. GLDH was measured by its oxoglutarate reduction activity (Alpha Laboratories Ltd., Eastleigh, UK). Biomarkers were measured in the Centre for Cardiovascular Science at the University of Edinburgh with the researcher blinded to the treatment allocation.

2.8. Statistical analysis

A detailed statistical analysis plan was finalised before locking of the trial database. This is detailed in the published protocol [14]. This initial exploration of safety and tolerability of calmangafodipir did not apply formal hypothesis tests; instead, 95% confidence intervals are presented where appropriate to indicate plausible effect sizes. The primary outcome analysis reported the number and percentage of patients experiencing an AE/SAE by randomised group and overall. Clinical observations and haematology and clinical biochemistry parameters were summarised by measurement time point and change from baseline. The ECG results were summarised by treatment group and overall at 2.5, 10 and 20 h. Conventional and exploratory biomarkers were summarised descriptively by treatment group and overall at baseline, 10 h and 20 h. Change from baseline was also summarised. Biomarkers were analysed by treatment group and overall at each time point using the mean and 95% confidence interval. Biomarkers were compared between each calmangafodipir dose and the combined NAC alone group using the difference in means and its 95% confidence interval.
required, measures were log-transformed and reported by geometric means and their ratio.

2.9. Role of the funder

Two investigators in this study were employed by PledPharma AB (study funder). Their input was in study design (DH) and monitoring (MB). The funder of the study had no role in data collection, data analysis, data interpretation, or writing of the paper. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. The corresponding author wrote the paper.

3. Results

From 5th June 2017 to 10th May 2018 304 patients presenting with acute poisoning were screened in the Emergency Department of the Royal Infirmary of Edinburgh (Fig. 1). Patients screened had toxicology presentations that included, but were not exclusively, paracetamol overdose. The main reasons for not being included were failure to meet inclusion criteria (n = 216, predominately because patient was not a paracetamol overdose that needed treatment) or due to meeting an exclusion criteria (n = 39). Twenty-four patients were randomised and all 24 received the full dosing of their allocated treatment. The 90 day follow up of the final participant was completed on 8th August 2018 which was the protocol-defined end-of-trial date. No patients were lost to follow up.

The baseline demographics of the 4 treatment groups are presented in Table 1. By chance, the NAC alone group reported ingestion of less paracetamol (normalised to body weight) than the calmangafodipir treated groups and had a lower median presentation paracetamol concentration. By chance, the NAC alone group presented to hospital, and started NAC treatment, an average of about 3 h later after overdose than the calmangafodipir treated groups. In each treatment group the following number of patients started treatment with NAC > 8 h after a single overdose: NAC alone: 3/6; NAC + calmangafodipir (2 μmol/kg): 2/6; NAC + calmangafodipir (5 μmol/kg): 2/6; NAC + calmangafodipir (10 μmol/kg): 1/6. Supplementary Fig. 1 represents graphically a post-hoc analysis of the relation between the paracetamol concentration and time from overdose for the single acute overdoses in this trial. The majority of the study participants had co-ingested other agents (Supplementary Table 1).

All randomised patients were analysed for the safety and tolerability primary outcomes (Table 2). During the 7 days after randomisation 23 out of 24 patients had at least one AE. Eleven patients had at least one SAE within the 90 day follow up period; 5 patients had at least one SAE within 7 days of randomisation. These SAEs were spread across the 4 treatment groups. Supplementary Table 2 presents the nature of the AEs and SAEs. There were no AEs or SAEs judged to be probably or definitely related to calmangafodipir. Seven patients experienced AEs judged definitely related to NAC. There were no anaphylactoid reactions to NAC. One death, that occurred 32 days after the start of NAC and calmangafodipir (5 μmol/kg) treatment, was judged to be unrelated to either NAC or calmangafodipir. There was one SUSAR reported for calmangafodipir in the 10 μmol/kg cohort. The SUSAR was hypokalaemia needing potassium replacement therapy that prolonged the patient’s hospital stay by 7 h. This was judged as probably related to NAC and possibly related to calmangafodipir. Secondary outcomes demonstrated no safety concerns for calmangafodipir in combination with NAC (Supplementary Table 2).

Liver injury was explored with conventional (Table 3) and exploratory biomarkers (Table 4). The confidence intervals for the fold change...
After the end of the SNAP regimen (1 from each of the following NAC alone group, 3 from 6 patients required additional NAC therapy groups reached this value (Supplementary Table 3). In total, in the need for NAC treatment to continue (and a secondary outcome pre-

In the NAC alone group 2/6 patients had an ALT activity calmangafodipir 1/18 had this ALT increase (Supplementary Table 3). 6 had an increase of 50% or more in ALT from baseline to 20 h. With 

0.92 (0.57 to 1.49), INR 1.10 (1.00 to 1.21)). In the NAC alone group 2/

8 h overdose to starting NAC, a clinically relevant value used to indicate 

In comparison to the K18 isoforms, mir-122 had similar mean and median relative changes when NAC + calmangafodipir treatment 

Table 1
Patient demographics. Patients were allocated to 4 treatment groups as described in the study protocol.

<table>
<thead>
<tr>
<th>Event</th>
<th>NAC alone (N = 6)</th>
<th>NAC + 2 μmol/kg calmangafodipir (N = 6)</th>
<th>NAC + 5 μmol/kg calmangafodipir (N = 6)</th>
<th>NAC + 10 μmol/kg calmangafodipir (N = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any adverse event</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>Any serious adverse event</td>
<td>2 (33%)</td>
<td>4 (67%)</td>
<td>2 (33%)</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>Adverse event starting after commencement of NAC treatment and within 7 days of consent</td>
<td>6 (100%)</td>
<td>5 (83%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>Serious adverse event starting after commencement of NAC treatment and within 7 days of consent</td>
<td>1 (17%)</td>
<td>1 (17%)</td>
<td>1 (17%)</td>
<td>2 (33%)</td>
</tr>
<tr>
<td>Adverse event unrelated to NAC</td>
<td>3 (50%)</td>
<td>5 (83%)</td>
<td>3 (50%)</td>
<td>5 (83%)</td>
</tr>
<tr>
<td>Adverse event possibly related to NAC</td>
<td>2 (33%)</td>
<td>2 (33%)</td>
<td>2 (33%)</td>
<td>2 (33%)</td>
</tr>
<tr>
<td>Adverse event definitely related to NAC</td>
<td>3 (50%)</td>
<td>2 (33%)</td>
<td>3 (50%)</td>
<td>2 (33%)</td>
</tr>
<tr>
<td>Adverse event unrelated to calmangafodipir</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>5 (83%)</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>Adverse event possibly related to calmangafodipir</td>
<td>0 (0%)</td>
<td>4 (67%)</td>
<td>2 (33%)</td>
<td>2 (33%)</td>
</tr>
<tr>
<td>Adverse event definitely related to calmangafodipir</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Adverse event definitely related to calmangafodipir</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>
groups were compared to the NAC alone group (Table 4). miR-122 increased in concentration from baseline to 20 h in patients treated with NAC alone (geometric mean of relative change (95%CI): 1.48 (0.24 to 0.33); 5 μmol/kg; 0.71 (0.06 to 8.52); 10 μmol/kg 0.76 (0.12 to 4.95)). However, miR-122 had greater variability than K18 isoforms and all confidence intervals included the null value (Supplementary Fig. 2).

GLDH was measured as pre-defined in our protocol. It was below the published lower limit of quantification (1 U/L) in 16 out of 72 samples. As per our trial protocol, the GLDH analysis is presented in Supplementary Table 4 [17]. There was no difference between groups.

### 4. Discussion

Paracetamol overdose is a common reason for emergency hospital admission. The only current treatment is NAC, but this antidote loses efficacy when treatment is delayed. In this study we demonstrate that calmanagafodipir, in addition to NAC, did not result in any safety issues.

Calmanagafodipir prevents cell injury by reducing oxidative stress – a central mechanism responsible for paracetamol-induced hepatocyte necrosis. It has been safely administered to patients with cancer and demonstrated to reduce the incidence of peripheral neuropathy. In this paper, we begin the process of exploring the clinical utility of calmanagafodipir in paracetamol overdose. As a starting point, it was important to determine whether there were any safety concerns when combined with NAC, an agent that commonly produces ADRs. Our data demonstrate no increase in AEs or SAEs at 3 ascending doses of calmanagafodipir when combined with the SNAP NAC regimen. An advantage of using the SNAP regimen in this trial, rather than the more widely used 21 h NAC regimen, is it produces substantially fewer ADRs. No patients in this trial had an anaphylactoid reaction yet these reactions occur in up to 30% of those treated with the 21 h regimen [9]. The improved safety profile of the SNAP regimen facilitated our analysis of any emergent calmanagafodipir toxicity. From this phase 1 study we conclude that there are no safety issues that preclude, or need special consideration in, future clinical trials.

Our pre-defined secondary objective was to explore conventional and exploratory biomarkers of liver injury. More patients in the NAC alone group required additional NAC therapy after completion of the SNAP regimen compared to the NAC + calmanagafodipir groups due to more patients having ALT increases. However, this phase 1 study is small and no firm conclusions can be drawn using ALT. In part, this is because early in the disease process - soon after overdose - ALT can remain small and no firm conclusions can be drawn using ALT.
The change from baseline to 20 h after starting NAC is presented as the relative change. A value of 1 indicates no change. SD = standard deviation. GSD = geometric standard deviation. The data for miR-122 are presented as the DCt using spiked-in mean and median as per study protocol. The change from baseline to 20 h is presented as the relative change. A value of 1 indicates no change. SD = standard deviation.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Initial</th>
<th>Geometric Mean (GSD)</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>K18 (U/L)</td>
<td>Baseline (2 h)</td>
<td>187 (2.30)</td>
<td>158</td>
<td>95,731</td>
<td>146,363</td>
</tr>
<tr>
<td>miR-122 (DCt)</td>
<td>Baseline (2 h)</td>
<td>1.85 (1.47)</td>
<td>1.71</td>
<td>1.18, 206</td>
<td>116,749</td>
</tr>
<tr>
<td>miR-122 (copies/mL)</td>
<td>Baseline (2 h)</td>
<td>1.22 (1.77)</td>
<td>1.85</td>
<td>1.48, 210</td>
<td>67,199</td>
</tr>
<tr>
<td>miR-122 (copies/mL)</td>
<td>10 h</td>
<td>5.58 (3.36)</td>
<td>5.17</td>
<td>3.83, 210</td>
<td>36,051</td>
</tr>
<tr>
<td>miR-122 (copies/mL)</td>
<td>20 h</td>
<td>6.70 (1.95)</td>
<td>5.41</td>
<td>7.12, 210</td>
<td>649</td>
</tr>
<tr>
<td>miR-122 (copies/mL)</td>
<td>Relative change from baseline to 20 h</td>
<td>1.48 (5.71)</td>
<td>2.23</td>
<td>0.07, 210</td>
<td>0.34</td>
</tr>
</tbody>
</table>

miR-122 that has been described in other studies [17]. All 6 patients in the NAC alone group had an increase in both K18 forms from baseline to 20 h. In the calmangafodipir-treated groups there was not an increase in the exploratory biomarkers. We note that GLDH was too low to be reliably quantified in this study. This is consistent with published evidence that demonstrates GLDH has reduced sensitivity when directly compared to K18 and miR-122 in patients with paracetamol overdose [14].

The data suggest that, with development, calmangafodipir may have value as an additional therapy to NAC in patients at increased risk of liver injury after paracetamol overdose. Such patients may include late presenters after overdose (greater than around 8 h) or those with evidence of liver injury at presentation. The exploratory biomarkers used in this study can sensitively identify liver injury before ALT is elevated. The exploratory biomarkers used in this study can sensitively identify liver injury before ALT is elevated.
Liver injury after overdose is rare if NAC starts within 8 h of overdose. The patients were not stratified at randomisation by their risk of developing liver injury. This, combined with the small patient numbers per treatment group, resulted in the NAC alone group having a higher proportion of patients who started NAC later than 8 h after overdose compared with the NAC and calmangafodipir groups. This difference across treatment groups should be considered when interpreting the effect of calmangafodipir. It should be noted, however, that the change in biomarkers was consistent in the NAC alone group regardless of overdose type. All the NAC alone patients had an increase in K18 and cK18 from baseline to 20 h including 2 early presenters (<8 h) and 1 supra-therapeutic overdose patient. In 5 out of these 6 patients the concentration of K18 at 20 h was greater than the published upper limit of normal of the healthy reference interval (151 U/L) [17]. With caution, we speculate that this may be consistent with K18 reporting liver injury that is too mild to cause an elevation in ALT. This hypothesis needs to be rigorously studied in subsequent trials that robustly determine whether calmangafodipir reduces paracetamol-induced liver injury biomarkers. These future trials would need to be large in size to demonstrate any impact on patient mortality should that be present. However, it should be feasible to demonstrate an effect on clinically and economically important outcomes such as the development of liver synthetic dysfunction and the length of hospital admission.

In conclusion, calmangafodipir was safe and tolerated in patients treated with NAC for paracetamol overdose and may reduce liver injury biomarkers. Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2019.07.013.

Contributions
The trial was designed by Dr. Dear and Dr. Henriksen. Trial set up and management was provided by ECTU. Patient recruitment and trial delivery was by EMERGE and Edinburgh Royal Infirmary. Sub-study biomarker analysis was by University of Edinburgh.

Declaration of Competing Interest
Dr. Dennis Henriksen and Ms. Marie Bengtson are employed by PledPharma AB. Dr. Dear is a member of the expert advisory group for the EU IMI funded TransBioLine Consortium.

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