Immune enhancement in patients with predicted severe acute necrotising pancreatitis: a multicentre double-blind randomised controlled trial

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Take home message

Compared with placebo, immune-enhancing Thymosin α1 treatment did not reduce the incidence of infected pancreatic necrosis in patients with predicted severe acute necrotising pancreatitis. However, it might be effective in specific subgroups of acute necrotising pancreatitis, like patients with extended pancreatic necrosis.

Tweet

Immune enhancement with Thymosin α1 did not reduce the risk of infected pancreatic necrosis in acute necrotising pancreatitis patients.

ABSTRACT

Purpose

Infected pancreatic necrosis(IPN) is a highly morbid complication of acute necrotising pancreatitis(ANP). Since there is evidence of early-onset immunosuppression in acute pancreatitis, immune enhancement may be a therapeutic option. This trial aimed to evaluate whether early immune-enhancing Thymosin alpha 1 (Tα1) treatment reduces the incidence of IPN in patients with predicted severe ANP.

Methods

We conducted a multicentre, double-blind, randomised, placebo-controlled trial involving ANP patients with an APACHE II score≥8 and a CT severity score≥5 admitted within seven days of the advent of symptoms. Enrolled patients were assigned to receive a subcutaneous injection of Tα1 1.6 mg, every 12 hours for the first seven days and 1.6 mg once a day for the subsequent seven days or matching placebos(normal saline). The primary outcome was the development of IPN during the index admission.

Results

A total of 508 patients were randomised, of whom 254 were assigned to receive Tα1 and 254 placebo. The vast majority of the participants required ICU admission(479/508, 94.3%). During the index admission, 40/254(15.7%) patients in the Tα1 group developed IPN compared with 46/254 patients (18.1%) in the placebo group (difference -2.4% [95%CI -7.4% to 5.0%]; p=0.47). The results were similar across four predefined subgroups.There was no difference in other major complications, including new-onset organ failure (10.6% vs. 15.0%), bleeding (6.3% vs. 3.5%), and gastrointestinal fistula (2.0% vs. 2.4%).

Conclusion

The immune-enhancing Tα1 treatment of patients with predicted severe ANP did not reduce the incidence of IPN during the index admission.

Trial registration: Clinicaltrials.gov registry (NCT02473406).

Keywords: acute pancreatitis, immunosuppression, thymosin, pancreatic necrosis, infection

Introduction

The annual global incidence of acute pancreatitis (AP) is estimated to be 34 per 100,000 individuals [1]. A smaller subgroup of patients with AP(5-10%) develop acute necrotising pancreatitis (ANP) [2] and can experience a more prolonged disease course that commonly requires intensive care unit (ICU) admission, especially if infected pancreatic necrosis (IPN) develops[3, 4]. The bacteria responsible for IPN are often translocated from the gastrointestinal tract and reach the pancreas through several different transmission routes, including haematogenous, lymphatic, and transcoelomic [5, 6].

Attempts to reduce the risk of infection in ANP have included the use of prophylactic antibiotics [7] and enteral probiotics [8]. The former is no longer recommended because of issues like antibiotic resistance, methodological quality in previous studies, and fungal superinfection [9, 10]. The latter is controversial, as a prominent randomised controlled trial found an increased risk of gastrointestinal necrosis associated with probiotic treatment [8]. Given that there is evidence of immunosuppression in the early phase of AP and it is associated with infectious complications[11-14], a theoretical strategy to reduce the risk of IPN is to boost the host defence(immune enhancement) against bacterial infection [15].

Thymosin alpha 1 (Tα1), a polypeptide hormone isolated from the thymus, stimulates both innate and adaptive immunity[16]. In a pilot study of patients with AP, Tα1was effective in reducing the risk of developing IPN[17]. Based on this preliminary data, we conducted a multicentre randomised clinical trial to determine the effect of Tα1 treatment. We hypothesised that early immune enhancement with Tα1 may reduce the incidence of IPN in predicted severe ANP. The main results of this trial were presented at American Pancreatic Association (APA) 2021 Annual Meeting and published as an abstract[18].

Methods

Trial design and oversight

This is a multicentre, double-blind, randomised, placebo-controlled, parallel-group trial to assess the efficacy of Tα1 in addition to standard care on the development of IPN in patients with predicted severe ANP. The trial was approved by the local hospital ethics committees of all the participating sites and registered on the ClinicalTrials.gov Registry (NCT02473406) before enrolment commenced. The trial protocol was published in 2020 [19], and the full protocol and statistical analysis plan are available in **Supplementary Protocol**. This study was funded by the Science and Technology Project of Jiangsu Province of China (no. SBE2016750187) and partly supported by SciClone Pharmaceuticals Holding Limited, which provided trial drugs and support for meetings during the study period. The funders were not involved in the trial's design, data collection, interpretation, or manuscript preparation.

Study population

Patients diagnosed with AP aged 18 to 70 years and with an APACHE II score≥8 and CT severity score [20] ≥5 admitted to any of the participating sites within seven days of the onset of abdominal pain were eligible for inclusion. The diagnosis of AP was based on the Revised Atlanta Classification (RAC) criteria [2]. Patients were excluded if they were pregnant, had a history of chronic pancreatitis, had underlying malignancy, received intervention for pancreatic necrosis prior to enrolment, had a known history of severe cardiovascular, respiratory, renal, or hepatic diseases, or had pre-existing immune disorders such as AIDS. Detailed inclusion and exclusion criteria are provided in **Supplementary Protocol**.

At each site, informed consent was obtained from the patients or their next of kin before randomisation. Patients were enrolled from Mar 18, 2017, to Dec 10, 2020. Follow-up was completed on Mar 10, 2021.

Randomisation, masking and interventions

Each eligible participant was assigned randomly from a computer-generated sequence to either the Tα1 or placebo group in a 1:1 ratio, using a block size of 4 stratified by site. The random allocation sequence was generated by an independent statistician at the Jinling Hospital. Allocation concealment was achieved by using blinded medication packs. Patients were assigned to receive a subcutaneous injection of Tα1 (SciClone Pharmaceutical Co., Ltd, Hongkong) 1.6 mg, every 12 hours for the first 7 days and 1.6 mg once a day for the following 7 days or matching placebo (normal saline, Chengdu Tongde Pharmaceutical Co., Ltd, Chengdu) during the same period. The trial drug was administered for a maximum of 14 days, or until hospital discharge or death, whichever occurred first.

Participants, treating physicians, and investigators were blinded to the treatment allocation to minimise potential bias. The trial statistician was also blinded when developing the statistical programmes. Tα1 and placebo were supplied in identically labelled individual vials. All other aspects of the patients' care were provided based on the international guidelines[21]. Prophylactic antibiotics were not recommended accordingly. The details for the management of AP are in the **Supplementary Protocol**.

Data collection

A web-based database (Unimed Scientific Inc., Wuxi, China) was developed for data collection (accessed at capctg.medbit.cn). Before enrolment, a start-up meeting for data entry and storage training was organised at each participating site to ensure high-quality data collection.

Trial outcomes

The primary outcome was the development of IPN during the index admission. We define the term "index admission" as the first admission in a series of hospital admissions. The diagnosis of IPN was made when one or more of the following criteria were present: gas bubbles within pancreatic and/or peripancreatic necrosis on CT; a

positive culture from pancreatic and/or peripancreatic necrosis obtained by fine-needle aspiration, drainage, or necrosectomy[2]. All the positive cases were reviewed by a remote adjudication committee. Decisions of the remote adjudicating committee took precedence over clinicians. Secondary clinical outcomes include IPN at 90 days after randomisation and new-onset organ failure as defined by the Revised Atlanta Classification[2], as well as mortality, bleeding requiring intervention, gastrointestinal fistula requiring intervention, positive blood culture, and pancreatic fistula during the index admission. Secondary laboratory outcomes include C-reactive protein (CRP), monocyte human leukocyte antigen-DR (mHLA-DR), and lymphocyte count at day 7 and day 14 after randomisation and positive blood cultures. The details and definitions of all outcomes are provided in **Supplementary Protocol**.

Statistical analysis

The incidence of IPN during the index admission in our study population was approximately 25% from our previous studies [22, 23]. A sample size of 520 patients was estimated to provide 80% power at a 2-sided alpha of 5% to demonstrate an absolute risk reduction of 10% in IPN during the index admission (25% in the placebo group vs 15% in the Tα1 group) after accounting for 4% dropouts (PASS V.11, NCSS software, Kaysville, USA) [17]. The treatment effect was estimated based on our pilot study, which demonstrated an 80% relative reduction in the incidence of IPN(42% to 8%)[17].

Primary analyses were based on the intention-to-treat (ITT) population, and secondary sensitivity analyses were done on the per-protocol (PP) population for the primary outcome and key secondary outcomes. Continuous data are reported as means and standard deviations or as medians and interquartile ranges as appropriate, depending on their normality. Categorical data are expressed as numbers and percentages.

The generalised linear model (GLM) was employed to compare group differences in the primary outcome with site as a covariate, and the risk difference, together with its

95% confidence interval, were calculated. Adjusted analyses with prespecified covariates were also performed. The GLM was also employed for analyses of secondary outcomes with treatment as the single predictor. Kaplan-Meier curves were used to compare the cumulative incidence of IPN to 90 days after randomisation tested by log-rank test. Detailed descriptions for the analyses could be found in the **Supplementary statistical analysis plan**. Four subgroups were predefined for the evaluation of the incidence of IPN during the index admission and 90 days after randomisation: the severity of AP (severe and non-severe [2]), age (>60 and <60 years old), aetiologies of AP (biliary and non-biliary) and extent of pancreatic necrosis (>50% and ≤50%).

Analyses were conducted using SAS 9.4®. Statistical tests will be two-sided, and p values <0.05 will be deemed as significant. All authors had access to the study data and had reviewed and approved the final manuscript.

Results

Results of recruitment and baseline characteristics

During the study period, 3,569 AP patients were assessed for eligibility, of whom 508 were enrolled in the trial at 16 hospitals across China. The numbers of cases from each site are shown in **online supplemental Table S1**. Among those 508 randomised patients, 254 were assigned to receive Tα1 and 254 placebo. The most common reasons for exclusion were admission >7 days before evaluation and APACHE score <8. Ten patients(2%) received the study drug on the randomisation day, while the others on the day after the randomisation day. Eleven patients in the Tα1 group and eight patients in the placebo group withdrew consent during treatment but did not refuse follow-up and data usage (**Figure 1**). Three patients in the placebo group stopped research intervention midway due to adverse reactions.

Baseline demographics and characteristics were not significantly different between the Tα1 and placebo groups (**Table 1**). In both groups, hypertriglyceridemia was the leading cause of AP, accounting for approximately half of the cases (48.8% vs. 50%).

The vast majority of the trial participants required ICU admission(479/508, 94.3%). The numbers of patients who received the trial agent on each trial day are shown in **online supplemental Table S2**.

Primary outcome and secondary outcomes

During the index admission, 40/254 (15.7%) patients in the Tα1 group developed IPN compared with 46/254 patients (18.1%) patients in the placebo group (difference -2.4% [95%CI -7.4% to 5.0%]; p=0.47). Of the 86 IPN patients, 74 were diagnosed according to microbiological results, and 12 were based on radiological findings alone. At 90 days after randomisation, 57/254 (22.4%) patients in the Tα1 group developed IPN compared with 65/254 patients (25.6%) in the placebo group (difference -3.3% [95%CI -9.2% to 4.8%]; p=0.39). There was no difference in mortality between groups either within the index admission or at 90 days after randomisation (**Table 2**). The Kaplan-Meier curves for the cumulative incidence of IPN until 90 days after randomisation are shown in **Figure 2**. There was no significant difference in the probability of developing IPN between the Tα1 and placebo groups (Log-Rank P=0.39). The results of per-protocol analysis of the primary outcome and key secondary outcomes are shown in **online supplemental Table S3**.

There was no difference in other major complications, including new-onset organ failure (10.6% vs. 15.0%; difference -4.3% [95%CI -8.2% to 1.9%]; p=0.15), bleeding (6.3% vs. 3.5%; difference 2.8 [95%CI -0.7 to 10.5]; p=0.15), and gastrointestinal fistula (2.0% vs. 2.4%; difference -0.4% [95%CI -1.8% to 3.9%]; p=0.75) during the index admission. Moreover, there were no significant differences in length of ICU or hospital stay, the requirement for catheter drainage, minimally-invasive debridement, or open surgery (**Table 2**). For mHLA-DR, no difference was detected on day7 and day14 between groups(**online supplemental Table S4**). The additional secondary endpoints regarding organ failure and laboratory endpoints are shown in **online supplemental Table S4-5**.

Subgroup analyses

There was no significant heterogeneity in the effect of Tα1 on the incidence of IPN during the index admission and 90 days after randomisation in any of the four predefined subgroups (**Figure 3, online supplemental Table S6-7**). In a *posthoc* subgroup analysis, the effect of Tα1 is neutral in patients caused by hypertriglyceridemia or other etiologies(**online supplemental Table S6-7**).

Adverse events

Adverse events occurred in 21 patients in the Tα1 group and 19 in the placebo group (8.3 % vs. 7.5 %, P=0.742) (**online supplemental Table S8**). The most common adverse event was venous thrombosis which occurred in 6 patients (2.4%) in the Tα1 group vs. 5 (2.0%) in the placebo group. All adverse events are listed in **online supplemental Table S8**.

Discussion

In this multicentre, double-blind, randomised, placebo-controlled trial, immune enhancement using Tα1 did not significantly reduce the incidence of IPN during the index admission or within 90 days of randomisation in patients with ANP. Given the varied range of severity of AP [2], this study was designed to select more severe patients based on the APACHE II score at enrolment [24]. However, we failed to show a difference in the primary outcome.

Our results are not consistent with the results from an experimental animal study [17] and the pilot clinical study [25]. There are several possible explanations. First, current animal models can not recapitulate all aspects of human AP, especially for a complication like IPN, which often occurs several weeks after admission [26, 27]. Second, the pilot study recruited only 24 patients from a single centre, making its findings vulnerable to type I error. Third, the dose regimen in the present trial is different from

the pilot one with a longer duration of drug administration (one week in the pilot versus two weeks in the present) and lower initial dose (6.4mg per day in the pilot versus 3.2 mg per day in the present). There were two time-course considerations in designing the dose regimen: (1) infection mainly occurs beyond the second week after disease onset [3, 28], and a two-week regimen should be able to cover the period interval better when prevention is possible; (2) immunosuppression typically develops early in the first week and usually slowly recovers during the second week [12], which is the reason for prescribing half the dose during the second week of treatment. A similar step-wise dose reduction was used in a previous study testing Tα1 in sepsis [29], showing that Tα1 could reduce 28-day mortality. Moreover, since Tα1 has a short elimination half-life ranging from 1.7 to 2.1 hours[30], a long term period of administration may exert better effects. Last, the incidence of IPN during the index admission is lower than expected in the placebo group (18.1% vs. 25% for sample size estimation), which might make our trial underpowered. Before initiation of recruitment, we changed the time interval of the primary outcome from "28 days" to time until "index hospital discharge" due to concerns regarding loss of follow-ups and the possibility of incomplete data. During the trial, we followed up all the participants for 90 days after randomisation, and the incidence of IPN was 25.6% in the placebo group by then. Still, the Tα1 treatment did not result in a reduction of IPN by 90 days after randomisation.

There is evidence to support a shifting balance between the systemic pro-inflammatory response and the compensatory anti-inflammatory response over the early course of AP [13, 31]. It was considered that the pro-inflammatory response occurs in the first few days to weeks and that the compensatory anti-inflammatory response occurs later. However, analyses in patients with sepsis and AP suggest that these responses can also run in parallel and that there is an association between early-onset immunosuppression and poor outcomes in AP, including increased risk of infectious complications [32, 33]. In sepsis patients, immune-enhancing therapies had been repeatedly evaluated with agents like Tα1[29] and nivolumab[34]. In AP patients, previous trials

investigating immunomodulatory therapy to block the early pro-inflammatory response have not been convincing [35], and this includes drugs like lexipafant [36, 37] and octreotide [38]. In patients with severe COVID-19, observational studies showed that Tα1 attenuated lung injury and decreased mortality [39, 40]. Despite the theoretical benefits of immune enhancement with Tα1 and the encouraging results from the pilot study[17], Tα1 did not reduce the incidence of IPN or improve any of the clinical outcomes in this trial.

In the subgroup analyses, larger treatment effects were seen in patients with a greater extent of pancreatic necrosis (>50%) and those aged more than 60 years old, although not statistically significant. We should interpret all the subgroup results with caution. First, the power was not enough to detect the differences among treatments. Second, the definition of necrosis is relatively subject based on a single CT scan. Third, we excluded patients with advanced age because age≥70 years is an independent risk factor for mortality in severe AP[41], and the immune system becomes slower and less responsive over age[42]. However, excluding these patients makes the study subgroup for elderly patients even smaller.

In line with the excellent safety profile reported in previous studies, Tα1 showed satisfactory safety performance in this trial. Three patients discontinued treatment due to adverse reactions (one erythema and two unexplainable fever) but received the placebo. For the other secondary outcomes, although the incidence of bleeding did not differ from previous trials[43, 44] and was not significantly higher in the intervention group, we strongly recommend future studies regarding necrotising pancreatitis monitor this potentially lethal complication.

The study has several limitations. The first is that the incidence of IPN may have been affected by the use of antibiotics and the criterion for repeating a CT scan because they were not mandatory standardised but left to the clinical team to decide. The second is that there were problems (failed multisite lab standardisation) with the measurement of mHLA-DR, a validated cell-surface signature for risk stratification in critically ill patients[45]. We obtained mHLA-DR data from less than half of the study subjects, which

may explain why there is no difference between groups. The third is that APACHE II misclassifies the severity of AP in almost a third of patients, which could also have contributed to the negative results[46]. Moreover, the timing of treatment might have been too late. The current trial included patients up to one week after the advent of symptoms, which may increase the heterogeneity of the study population. Apart from the timing, the appropriate duration of therapy is unclear. Last, nearly 50% of the study patients had hypertriglyceridemia as etiology, significantly higher than results from an international registry[47]. The increase of hypertriglyceridemia-induced AP in Chinese cohorts might be attributed to changes in dietary habits[48] and genetic factors[49]. Although the effect of Tα1 did not vary across patients caused by hypertriglyceridemia or other etiologies, the distinct etiological distribution leaves the generalisability of the observed results in doubt.

In conclusion, the immune-enhancing Tα1 treatment of patients with predicted severe ANP (APACHE II ≥8 at enrolment) did not significantly reduce the incidence of IPN during the index admission compared with placebo. Future trials seeking to investigate this approach will need to determine the best way to select patients and decide on the most effective dose and duration of Tα1 treatment.

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Deidentified individual participant data are available indefinitely in the electronic database. Data can be accessed through capctg.medbit.cn with the approval of the authors. Request for data can be made to the corresponding author (ctgchina@medbit.cn) and will be discussed during a meeting of the Chinese Acute Pancreatitis Clinical Trials Group (CAPCTG).

Compliance with ethical standards

Conflicts of interest:

Dr. Weiqin Li reports consultancy fees and grants from SciClone Pharmaceuticals. Dr. Zhihui Tong reports speaker fees from SciClone Pharmaceuticals. Dr. Lu Ke reports

speaker fees frees from SciClone Pharmaceuticals. Dr. Vikesh Singh reports consultant fees and grants Abbvie, medical advisory board participant for Envara, and grants from Theraly and Orgenesis. The other authors have no relevant conflict of interest to declare.

Ethical approval:

The protocol was prospectively approved by the human research ethics committee of all participating institutions and reported prior to completion of the study. This study was approved by the ethics committee of Jinling Hospital. The ethical approval document ID is 2015NZKY-004-02. Site ethical approvals were obtained from ethics committees of the participating sites before commencement of recruitment.

Consent to participate:

Consent to participate was provided prospectively from all participants or their next of kin.

The signed consent forms for all participants included consent to publication of aggregate data. The authors all consent to publication of the manuscript.

Consent for publication:

Figure legends

Figure 1：Enrolment, randomisation, and follow-up of patients in the TRACE trial.

TRACE denotes Thymosin α1 in Prevention of Infected Pancreatic Necrosis Following Acute Necrotising Pancreatitis. APACHE II denotes acute physiology and chronic health evaluation II. CTSI denotes computed tomography severity index. ITT denotes intention to treat. Tα1 denotes Thymosin α1.

Figure 2: Time- to-infection by day 90.

The Kaplan-Meier curves for the cumulative incidence of infected pancreatic necrosis from randomisation to day 90 in the intention-to-treat population.

Figure3: Subgroup analysis of the risk of infected pancreatic necrosis by the index hospital discharge and day 90. Panel A shows the relative risk of infected pancreatic necrosis during the index admission between the two treatment groups. Panel B shows the relative risk of infected pancreatic necrosis up to 90 days after randomisation. A relative risk of less than 1.0 indicates better results for the Tα1 group.