Immunology Letters 186 (2017) 20–27

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Short-term clinical efficacy of percutaneous irreversible electroporation combined with allogeneic natural killer cell for treating metastatic pancreatic cancer

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ARTICLE INFO

Keywords:
Short-term
Clinical efficacy
Percutaneous irreversible electroporation
NK cell
Pancreatic cancer
Clinical trial

ABSTRACT

This study was to determine how the short-term clinical efficacy of irreversible electroporation (IRE) combined with allogeneic natural killer (NK) cell therapy for treating metastatic pancreatic cancer. Between March and December 2016, we enrolled 40 patients who met the enrollment criteria and assigned them to two groups: simple IRE (IRE group, $n = 20$) and IRE plus allogeneic NK cell therapy (IRE-NK, $n = 20$). We evaluated immune function changes, quality of life, clinical response, and other related indicators. Combining allogeneic NK cells with IRE had a synergistic effect, not only enhancing the immune function of the patients, but also reducing the expression of carbohydrate antigen 19-9 (CA19-9) and CA242 and significantly exhibiting good short-term outcome and improving the quality of life of the patients. This is the first clinical trial to combine allogeneic NK cells with IRE for treating metastatic pancreatic cancer, and proves the safety and efficacy of the treatment.

1. Introduction

Pancreatic cancer (PC) is highly malignant; over 80% of patients are initially diagnosed with unresectable disease [1]. Various ablation methods play important roles in the treatment of locally advanced PC (LAPC). Despite recent advances in surgery, chemotherapy, and radiotherapy, there has been little improvement in the survival of PC [2], and PC continues to be the fourth leading cause of cancer mortality in both men and women. The estimated rates of 1- and 5-year survival of PC are 24% and 4.3%, respectively [3]. Chemotherapy and radiotherapy have been adopted as basic postoperative treatment strategies in metastatic PC; however, their effect is limited and their efficiency appears to have plateaued in the past decade [4–6]. Therefore, identifying more effective therapies for patients with metastatic PC remains an important clinical challenge.

Irreversible electroporation (IRE) is an emerging, non-thermal, image-guided tumor ablation technique that has been proven feasible and safe for treating locally advanced pancreatic tumors [7–10]. IRE is a newly developed method that causes apoptosis without injuring the structural components of tissues. Fuda Cancer Hospital (Guangzhou, China) has been conducting a prospective study of percutaneous IRE ablation of LAPC since the approval of this method in China.

In recent years, many studies have confirmed that cancer formation and progression in patients with metastatic PC are influenced in particular by tumor immune responses [11–16]; current approaches for activating the immune system focus on vaccination, such as with dendritic cell (DC) vaccines. DC–cytokine-induced killer (DC-CIK) cell immunotherapy may have powerful therapeutic effects under low tumor load conditions [17,18]. However, cancer cells often escape from immune attack by downregulating the expression of major histocompatibility complex (MHC) molecules and costimulatory molecules [19]. A series of clinical studies have shown that adjuvant cellular immunotherapy, such as that involving natural killer (NK) cells, Natural killer (NK) cells are important components of the innate immune system and play a critical role in the early host defense against cancer[20,21]. With progress in the NK cell biology field and in understanding NK function, adoptive NK cell transfer has promising anti-tumor effects on various cancers [22–26], including pancreatic cancer [27–31].

In this study, we prospectively assessed the short-term clinical

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http://dx.doi.org/10.1016/j.imlet.2017.03.018
Received 7 March 2017; Received in revised form 31 March 2017; Accepted 31 March 2017
Available online 06 April 2017

efficacy of IRE combined with NK cell immunotherapy in patients with metastatic PC to provide a potential therapeutic pattern.

2. Materials and methods

2.1. Ethics

This clinical trial was registered with the US National Institutes of Health (NCT02718859; Ph1/Ph2) and approved by the Guangzhou Fuda Cancer Hospital ethics committee. In accordance with the Declaration of Helsinki, written informed consent was obtained from each participant.

2.2. Patients

This was a prospective study of the therapeutic effects of the combined treatment for patients with metastatic PC enrolled between March and December 2016. We enrolled 40 patients using the following criteria: (1) expected survival > 3 months; (2) age 30–80 years; (3) Karnofsky performance status (KPS) > 60; (4) the following parameters were normal: total T cells (603–2990/μL), cytotoxic T cells (125–1312/μL), helper T (Th) cells (441–2156/μL), platelets ≥ 80×10^9/L, white blood cells ≥ 3×10^9/L, neutrophils ≥ 2×10^9/L, hemoglobin ≥ 90 g/L, prothrombin time–international normalized ratio, (0.8–1.5), adequate hepatic function (bilirubin < 20 μM, aminotransferase < 60 U/L) and renal function (serum creatinine < 130 μM, serum urea < 10 mM); (5) metastatic PC confirmed by pathology or imaging; (6) absence of level 3 hypertension, severe coronary disease, myelosuppression, respiratory disease, acute or chronic infection, and autoimmune diseases. The contraindications for participation were T cell lymphoma, and ongoing organ transplant or within 7 days after systemic chemotherapy. The enrolled patients were allocated to two groups (n = 20) with parallel assignments.

2.3. IRE procedure

All patients underwent neuromuscular blockade and general anesthesia. Computed tomography (CT) and ultrasound were used to guide electrode insertion, and IRE was synchronized to deliver electrical pulses coordinating with the cardiac rhythm (Fig. 1). The distance between the electrodes was 1.5–2.0 cm. One or more pullbacks were performed if the target region was > 2 cm in diameter. After the ablation, the patient was transferred to the intensive care unit for overnight observation, and then transferred to the general ward after no acute complications were confirmed. Relevant treatment was administered if there were any complications. Two surgeons (L.Z.N. and L.Z.) with 4–8 years of experience in image-guided tumor ablation performed all procedures.

2.4. CT examination

The patients were required to undergo plain CT and enhanced CT at 1 week pre-treatment, and followed at 1 month and 2 months post-treatment. The maximum diameter and CT value were measured and compared pre-treatment and post-treatment.

2.5. NK cell therapy

NK cells were generated according to previously published protocols [32], 80 mL peripheral blood from allogenic donors was drawn 7 days before cryoablation and the immunotherapy was given 3–5 days after cryoablation (adoptive transfer of NK cells performed 2 times continuously). For donor selection, the killer cell immunoglobulin-like receptors (KIRs) genotyping should be mismatched to the human leukocyte antigen (HLA) class I molecules of the patient [32–36].

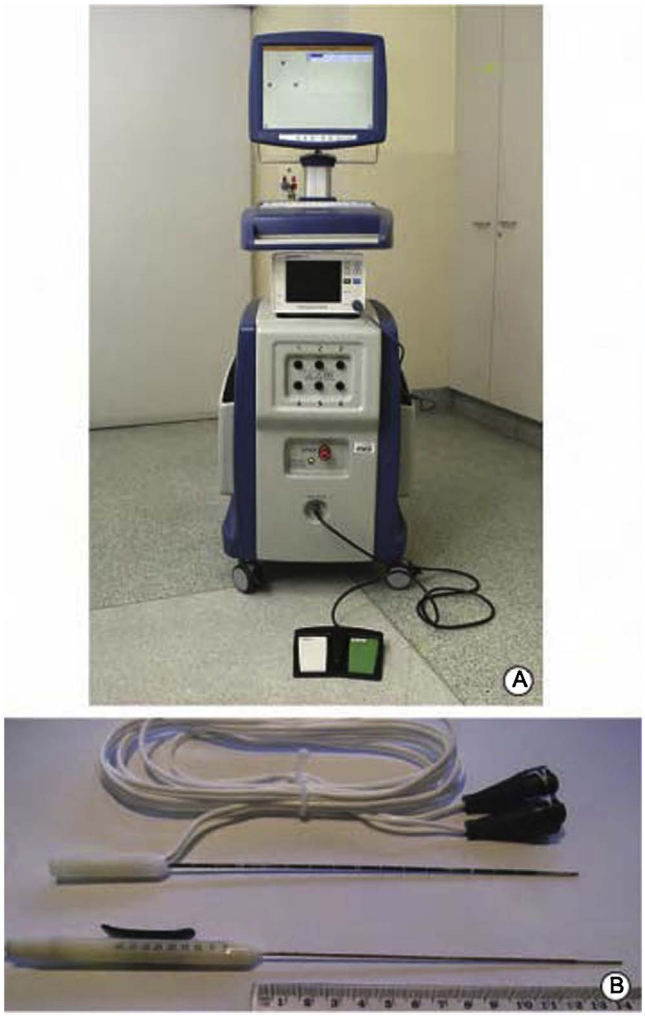

Fig. 1. Device of mobile IRE. A. Synchronous ecg recorder; B. Electrode needle.

Detected the peripheral blood from allogenic donors and the recipient by TIANamp Blood DNA Kit (Tiangen Biotech Co., Ltd, Beijing, China) and KIR/HLA-Cw Genotyping Low Resolution Kit (PCR-SSP) (Super Biotechnology Developing Co., Ltd, Tianjin, China).

For NK cells culture, using the Human HANK Cell In Vitro Preparation Kit (Hank Bioengineering Co., Ltd, Shenzhen, China), including the lethally radiated K562-mb15-41BBL (K562D2) stimulatory cells [37], plasma treatment fluid, lymphocyte culture fluid additives, serum-free medium additives and cell infusion additives. It is dedicated for the expansion and activation of NK cells in peripheral blood or umbilical cord blood mononuclear cells in vitro, the preparation of NK cells with higher quantity, purity and activity, namely HANK cells [32]. After culture, about 8–10 billion HANK cells can be harvested by using the NK Cell Serum-free Medium and culture bag (Haoyang Biological Manufacture Co., Ltd, Tianjin, China). The final cell count and quality control inspection were performed at day 9 of culture, and the qualified indicators included proportion of living cells ≥ 90%, proportion of CD56 + CD3- cells ≥ 85% (detection by flowcytometry was shown previously [32]), endotoxin content ≤ 1 EU/mL, cell viability ≥ 80% (K562 cells were used as target cells, cytotoxicity assay was shown previously [32]), Bacteria, fungi and mycoplasma culture negative. All cell preparation processes were performed by the same technician and assessed by another technician. After 12 days of cell culture, the NK cells were divided into three groups and intravenously infused into the patients from Day 13–15. Each patient must receive two cycles NK therapy continuously.