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Sunitinib impairs the proliferation and function of human peripheral T cell and prevents T-cell-mediated immune response in mice ☆

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Abstract Sunitinib (sunitinib malate; SU11248; SUTENT) is a novel multi-targeted receptor tyrosine kinase inhibitor currently approved for the treatment of metastatic renal cell carcinoma. To analyze the possible use of this compound in combination with immunotherapeutic approaches, we investigated the effects of sunitinib on the human peripheral T cells and the induction of primary immune responses in mice. Sunitinib inhibited the proliferation of primary human T cells from normal healthy volunteers as well as from renal cell carcinoma (RCC) and other cancer patients. The inhibition was recoverable after drug withdrawal because sunitinib did not induce T-cell apoptosis even at 0.8 μM. In addition, sunitinib led to accumulation in G_0/G_1 phase of the cell cycle, inhibition of cytokine production, downregulation of activation markers expression and blockade of Zap-70 signaling in the T cells. Sunitinib significantly reduced the ear swelling induced by picryl chloride in mice. In light of these findings, the effects of sunitinib on the immune system should be emphasized for the therapy of metastatic renal cell carcinoma patients to avoid the impairment of T lymphocytes.

Introduction

Renal cell carcinoma (RCC) accounts for 2% to 3% of all malignant tumors in adults [1]. Patients with untreated metastatic RCC (mRCC) have an overall median survival of no more than 12 months and a 5-year survival of less than 10% [2,3]. Metastatic RCC is usually considered to be resistant to conventional chemotherapy and radiation, and cytokine-based immunotherapy using interferon-α (IFN-α) and interleukin-2 (IL-2) was the only effective treatment in the past 15 years [4,5]. Until recently, two novel tyrosine kinase inhibitors, sorafenib and sunitinib, were introduced in the treatment of RCC patients.

Sunitinib (marketed as Sutent, and previously known as SU11248) is an oral, small-molecule, multi-targeted receptor tyrosine kinase (RTK) inhibitor that was approved by the FDA

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for the treatment of RCC and imatinib-resistant gastrointestinal stromal tumor (GIST) on January 26, 2006 [3,6,7]. Sunitinib has become the standard of care for both of these cancers and is currently being studied for the treatment of many others [8–10]. These effects were associated with the blockade of receptor tyrosine kinase signaling by vascular endothelial growth factor receptors (VEGFR-1,VEGFR-2, VEGFR-3), platelet-derived growth factor receptor (PDGFRα, PDGFRβ), c-KIT, Fms-like tyrosine kinase-3 receptor (FLT3) and the receptor encoded by the ret proto-oncogene (RET) [7,11].

It was found that sorafenib, another outstanding agent for the treatment of mRCC, but not sunitinib, has a detrimental effect on DC phenotype and inhibits cytokine secretion, migration ability and T-cell stimulatory capacity [12]. Finke JH and his colleagues reported that sunitinib impaired regulatory T-cell function [13]. However, until now the effect of sunitinib on function of human peripheral T cells and immune responses have not been evaluated in detail. The aim of this study was to examine the effects of sunitinib on the function of T cells both *in vitro* and *in vivo*. We show here that sunitinib inhibits human peripheral blood T-cell proliferation, activation and cytokines production in vitro and reduces the T-cell-mediated immune response in mice.

Materials and methods

Animals

Female BALB/c and C57BL/6 mice (6–8 weeks old, 18–22 g) were supplied by the Model Animal Genetics Research Center (Nanjing, China). They were maintained in specific pathogen-free condition at 25 ± 2 °C and kept on a 12-h light–dark cycle. Animal welfare and experimental procedures were carried out strictly in accordance with the "Guide for the Care and Use of Laboratory Animals" (The Ministry of Science and Technology of China, 2006) and the related ethical regulations of our university. All efforts were made to minimize the animals' suffering and to reduce the number of animals used.

Cells and reagents

Peripheral blood mononuclear cells (PBMC), collected from normal healthy volunteer donors, RCC and other cancer patients, were purified by Ficoll density gradient centrifugation. Normal human T cells were enriched from PBMC by R&D Systems' Human T Cell Enrichment Columns (R&D, MN). The purification rate of $CD3^+$ T cells was 90 ± 5%. The cells were incubated in RPMI 1640 medium (Gibco, MD) supplemented with 10% New Born Calf Serum (NBS) in a humidified atmosphere of 5.0% CO_2 at 37 °C.

Sunitinib (Pfizer, NY) was dissolved in dimethyl sulfoxide (DMSO) to a concentration of 20 mM as stock solution, which was stored at −20 °C until use. Phytohemagglutinin (PHA), myelin oligodendrocyte protein (MOG) and staphylococcal enterotoxin B (SEB) were obtained from Sigma (MO).

Cell proliferation assay

Cell proliferation was determined by two methods. For H^3-TdR incorporation assay, the cells were incubated with graded concentrations of sunitinib for 60 h, pulsed with 1 μCi tritiated thymidine per well, and incubated for another 12 h before they were harvested, and incorporated radioactivity was then quantified. For carboxyfluorescein diacetate succinimidyl ester (CFSE) proliferation assay, purified human PBMCs were suspended in PBS containing 0.1% BSA and labeled with the vital dye CFSE at a final concentration of 2.5 μM for 10 min at 37 °C. After labeling, the cells were washed three times in NBS-rich medium and resuspended in PRMI 1640 medium (2.5×10^6 cells/ml). The CFSE-labeled cells were pre-incubated with sunitinib for 1 h as indicated and then cultured for 96 h in the presence of 5 μg/ml PHA or were stimulated with 10 μg/ml anti-CD3 and 1 μg/ml anti-CD28 for 72 h . In the case for examining the effect of sunitinib on the T lymphocytes activated by specific antigens, an experimental autoimmune encephalomyelitis (EAE) was induced in C57BL/6 mice as previously described [14], and the mice were monitored for clinical symptoms and graded from 1 to 4 as follows: 0, no clinical expression of disease; 1, floppy tail without hind limb weakness; 2, hind limb weakness with or without flaccid tail; 3, hind leg paralysis and floppy tail; and 4, hind leg paralysis accompanied by floppy tail and urinary or fecal incontinence. Animals that progressed to a clinical score of 4 were euthanized on day 14 and lymph nodes were collected. The cells from EAE mice were then stimulated by 2 μg/ml MOG with various concentrations of sunitinib for 96 h. The proliferation was evaluated using H^3-TdR incorporation assay.

Cell cycle analysis

Approximately 1×10^5 cells were collected at specified time points after culture, washed twice with PBS and fixed in ice-cold 70% ethanol for 30 min and then incubated with 10 μg/ml propidium iodide (PI) and 10 μg/ml RNase for 30 min. Thereafter, the cells were analyzed by a flow cytometer (BD, CA).

Detection of apoptosis

An annexin V-fluorescein isothiocyanate (FITC)/PI double-stain assay was performed in accordance with the manufacturer's protocol (Jingmei Biotech, Shenzhen, China). Briefly, the T cells (2×10^5) were collected and resuspended in 100 μl of binding buffer containing 1 μl of annexin V-FITC and 1 μl of PI then incubated for 15 min in the dark at room temperature. Analysis was immediately performed using a flow cytometer.

IL-2 assay

Human blood T cells (1×10^5 cells/well) were cultured in 96-well plates with or without sunitinib for 24 h, in the presence or absence of anti-CD3 and anti-CD28 antibodies. Interleukin 2 (IL-2) production was measured with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Biosource, SA) according to the manufacturer's instructions.

Cytokine analysis

PBMCs were stimulated with PHA in the presence or absence of sunitinib, as indicated in the text. Super-