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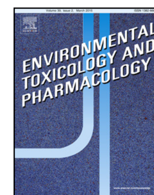

The contribution of keratinocytes in capecitabine-stimulated hand-foot-syndrome

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ABSTRACT

Capecitabine, as the first-line treatment for multiple tumor types, has a serious drawback of hand-foot-syndrome (HFS) that limits its clinical use. However, the pathophysiology and mechanism of capecitabine-induced HFS is rarely known. Here we built the experimental mouse model of HFS induced by capecitabine at first and it was shown that 3 of 6 mice appeared HFS in the 5th day and 5 mice occurred HFS in the 30th day. The corneous layer was reduced in capecitabine-induced HFS in vivo. Moreover, we found that capecitabine could significantly induce keratinocytes cells death in vitro through activated apoptosis pathway and decreased mitochondrial membrane potential. In conclusion, these results suggested that HFS of capecitabine may be developed from reduction of corneous layer through stimulation of intracellular mitochondrial dysfunction following activation of caspase-dependent apoptosis pathway.
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1. Introduction

HFS is a frequently adverse effect of numerous anti-tumor drugs including chemotherapeutic agents and targeted multi-kinase inhibitors (MKIs) such as capecitabine, sorafenib and sunitinib (Boussemart et al., 2013; Lipworth et al., 2009; Zhang et al., 2011), which was first reported in 1974 (Fischer et al., 2013; Hofheinz et al., 2012a; Zuehlke, 1974). And patients taking capecitabine therapy are most likely to suffer from HFS that the continual incidence is up to 74%, with 17% of the patients are afflicted with a serious form (grade 3). HFS of capecitabine which is approved for the first-line treatment of many tumor types including gastric, rectal, breast and colorectal cancer(Babacan et al., 2015; Hofheinz et al., 2012b; Roh et al., 2009; Zhao et al., 2011), as one of the most widely used antineoplastic drugs, largely affects its long-term and safe clinical application(Hennessy, 2005). However, the mechanism of capecitabine-induced HFS remain unknown, and there is also no effective strategies to reverse the HFS effects of capecitabine.

Recent studies suggested that the abnormal changes of keratinocytes were responsible for many skin diseases, such as psoriasis and keratosis pilaris (Haake and Polakowska, 1993). We hypothesized that the alterant keratinocytes may be the cause of HFS induced by capecitabine. In addition, the assumption that apoptosis and mitochondrial-related signaling pathways were pivotal to skin diseases induced by keratinocytes have been supported by several studies (Bianchi et al., 1994; Gole et al., 2014; Miller et al., 2014; Wrone-Smith et al., 1995). Then we asked whether such signaling pathway was involved in capecitabine-induced HFS.

Here we showed that corneous layer was reduced in the HFS induced by capecitabine and S9-Capecitabine could obviously induce keratinocytes cells death. Moreover, the decrease of mitochondrial membrane potential and the up-regulation of apoptosis-related proteins identified that mitochondrial apoptosis was closely related to capecitabine-induced HFS. Taken together, we found that the HFS of capecitabine occurred through mitochondria dysfunction and caspase-dependent apoptosis that inducing the cells death of keratinocytes and leading to corneous layer reduction.

2. Materials and measurements

2.1. Cell culture

HaCaT and NHDF cells were purchased from Tongpai (Shanghai) Limited Company of Biology Science and Technology. HaCaT and

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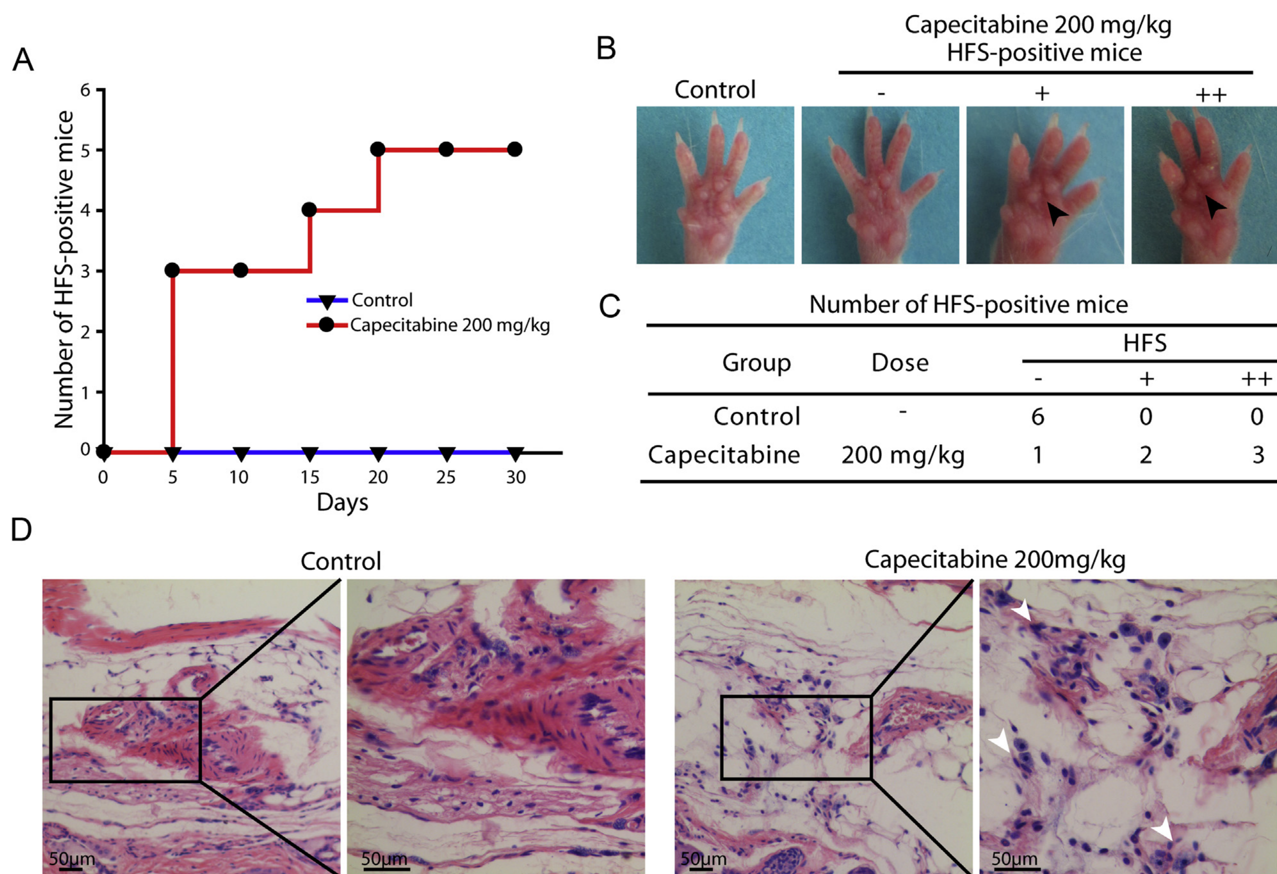

Fig. 1. Capecitabine induced hand-foot-syndrome in mice models.
Treatment scheme: Six weeks old mice were treated with capecitabine (200 mg/kg, i.g) or vehicle every day for 30 days. (A) The number of HFS-positive mice in control or capecitabine group (n = 6 for each condition) during treatment. (B) Representative images of limbs in control or capecitabine group. (C) The occurrence of HFS-posotive after the treatment for 30 days in control or capecitabine group (n = 6 for each condition). (D) Representative HE staining pictures in control or capecitabine group for pathological study. Scale bar: 50 μm.

NHDF Cells were cultured in DMEM medium containing 10% fetal bovine serum (Hyclone, Logan, UT, USA) and at 37 °C in a humidified atmosphere with 5% CO_2.

2.2. Reagents

Capecitabine (CAS: 154361-50-9) was purchased from Biochempartner. NADPH.4Na (n1630) was purchased from Roche., Sulforhodamine B (SRB, S1402-5G), DMSO, Tris-Base and Trichloroacetic acid were purchased from Sigma-Aldrich.

2.3. S9-Capecitabine incubation system

The S9 fraction was prepared from livers of rats and the protein concentration of same batch did not exceed 40 mg/ml. The S9 metabolic activation system (10 ml) consisted of 6 ml PBS (0.2 mol/L), 0.2 ml KCl (12.3%), 0.2 ml MgCl2 (8.1%), 1.0 ml G6-P (0.1 mol/L), 1.6 ml NADP (0.1 mol/L) and 1.0 ml S9 fraction. Capecitabine was dissolved in DMSO to 50 mM and added to the S9 metabolic activation system. After 48 h incubation at 37 °C in a humidified 5% CO2 incubator, the medium was gently activated and the supernatant was obtained for S9-Capecitabine. The control group contained only DMSO without capecitabine.

2.4. Antibodies

Antibodies cleaved-caspase-3 (#9661), cleaved-PARP (9544S) were obtained from Cell Signaling Technology (CST, USA), and antibodies Bcl-2 (sc-7382), Bax (sc-7480) GAPDH (sc-25778) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

2.5. Mice and treatments

Six-week-old male ICR mice were purchased from the Shanghai Laboratory Animal Center, Chinese Academy of Sciences and bred in specific pathogen-free (SPF) conditions that the animal facility were: a 12: 12 h light/dark cycle; a temperature of 18–29 °C; a relative humidity of 50 ± 20%; and ventilation changes of ≥10 per hour. Animals were supplied with an autoclaved pellet diet and ad libitum with reverse osmosis-filtered water. Animals were divided into two groups, vehicle-treated group (control, n = 6, vehicle was 0.5% CMC-Na, intragastrical i.g) and capecitabine-treated group (treatment, n = 6, 200 mg per kilogram of body weight, intragastrical i.g.), and daily treated for 30 days. Pictures were taken for morphological examination every five days. The animal experimental protocol has been reviewed and approved by the Animal Ethical and Welfare Committee (AEWC) and all of the animal experiments were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals.

2.6. Gene microarray

Limb tissues were isolated from mice that appeared HFS after treatment with capecitabine (200 mg/kg), and took the vehicle group as control. Total RNA was extracted using TRIzol reagent according to the manufacturer's instructions. Purified RNA sam-

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