REPORT

Synergism of sodium bicarbonate and baicalin against clinical *Candida albicans* isolates via broth microdilution method and checkerboard assay

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Abstract: Invasive fungal infections caused by *Candida albicans* constitute a prevalent worldwide health problem. Due to limited antifungal agents available, more efforts have been made towards searching the novel anti-candida drugs with low cytotoxicity. The present study was aimed to investigate the antifungal activities of baicalin and/or sodium bicarbonate (SB) against 29 *C. albicans* isolates including 27 clinical ones. By using broth microdilution method and checkerboard assay, it was observed that the minimum inhibitory concentrations (MICs) of baicalin and SB alone were ≥ 2048 μg/mL, and those of baicalin and SB in combination decreased 16-32 folds with fractional inhibitory concentration index (FICI) in a range of 0.094-0.375. The results presented the strong synergism between SB and baicalin in 27 clinical *C. albicans* isolates and provided an alternative choice against *C. albicans*.

Keywords: Anti-candida agent, synergism, clinical isolates, sodium bicarbonate, baicalin.

INTRODUCTION

Candida albicans has been recognized to be associated with the fast-growing invasive fungal infections (Whiteway and Oberholzer, 2004). The opportunistic fungi usually cause severe resistance owning to the overuse of conventional antifungals, making the search for novel antimycotic drugs with low cytotoxicity more urgent. To strengthen the activities of the conventional antifungal agent, drug combination has bene used to treat resistant clinical isolates (Tobudic *et al.*, 2012). Currently, many plant-originated chemicals (Zhang *et al.*, 2010) and "non-antibiotics" (Peters *et al.*, 2013) alone and in combination with traditional antifungal agents have been considered to be promising ways to combat fungal infections.

Baicalin is an extract from the traditional Chinese herbal medicine *Baikal skullcap* (*Scutellaria baicalensis* Georgi), and possess many biological activities including anticancer, antioxidative stress and anti-pathogenic fungi (Wei *et al.*, 2012; Wang *et al.*, 2015). Sodium bicarbonate (SB) is an ordinary chemical compound widely used as food additives which has been demonstrated to inhibit the growth of cutaneous fungal infection and onychomycosis (Letscher-Bru *et al.*, 2013). In a preliminary experiment, a synergism of baicalin with SB was observed in *C. albicans* SC5314.

This study was aimed to investigate the antifungal effect of baicalin and/or SB in 29 *C. albicans* isolates including 27 clinical ones by broth microdilution method and checkerboard assay.

MATERIALS AND METHODS

Strains and cultivation

C. albicans SC5314 and *C. albicans* ATCC10231 were kindly provided by Prof. Yuan Ying Jiang from College of Pharmacy, Second Military Medical University, Shanghai, China. The clinical *C. albicans* isolates were kindly provided by Prof. Huai Wei Lu, Clinical Laboratory, Anhui Provincial Hospital, Hefei, China. All strains were stored in YPD medium (1% yeast extract, 2% peptone, 2% dextrose; SHFENG, Shanghai, China) and 20% glycerol at -80°C. After sub culturing on Sabouraud dextrose broth (SDB, SHFENG, Shanghai, China) for 24 h at 37°C, the cells were harvested by centrifugation at 3000g and resuspended in RPMI-1640 medium (Invitrogen, CA, USA).

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Table 1: Interactions of baicalin and/or sodium bicarbonate ($NaHCO_3$) against clinical isolates of *Candida albicans*

C. albicans isolates		MIC_{90} alone (μg/mL)		MIC_{90} in combination (μg/mL)		FICI[b]	Interpretation
No.	Type [a]	Baicalin	$NaHCO_3$	Baicalin	$NaHCO_3$		
SC5314	S	2048	2048	128	128	0.125	SYN
ATCC10231	S	2048	2048	128	256	0.188	SYN
1	S	2048	2048	128	128	0.125	SYN
2	S	2048	2048	128	128	0.125	SYN
3	S	2048	4096	256	256	0.188	SYN
4	S	2048	2048	128	128	0.125	SYN
5	S	2048	4096	256	256	0.188	SYN
6	S	2048	2048	256	128	0.188	SYN
7	S	2048	2048	128	128	0.125	SYN
8	S	2048	2048	128	128	0.125	SYN
9	S	2048	4096	256	256	0.188	SYN
10	S	2048	2048	256	128	0.188	SYN
11	S	2048	4096	256	128	0.156	SYN
12	S	2048	2048	128	128	0.125	SYN
13	S	2048	4096	256	256	0.188	SYN
14	R	>2048	2048	128	128	<0.125	SYN
15	R	>2048	2048	128	128	<0.125	SYN
16	R	>2048	2048	256	256	<0.250	SYN
17	R	>2048	4096	256	128	<0.156	SYN
18	R	>2048	2048	256	128	<0.188	SYN
19	R	>2048	2048	256	256	<0.250	SYN
20	R	>2048	2048	256	128	<0.188	SYN
21	R	>2048	4096	128	128	<0.094	SYN
22	R	>2048	2048	256	256	<0.250	SYN
23	R	>2048	2048	256	128	<0.188	SYN
24	R	>2048	4096	256	256	<0.188	SYN
25	R	>2048	2048	256	512	<0.375	SYN
26	R	>2048	2048	256	256	<0.250	SYN
27	R	>2048	2048	128	128	<0.125	SYN

[a]S, sensitive, MIC of fluconazole < 64 μg/mL; R, resistant, MIC of fluconazole ≥ 64 μg/mL.
[b]FICI, fractional inhibitory concentration index.

Broth microdilution test

The minimum inhibitory concentrations (MICs) of baicalin and SB were determined based on CLSI M27-A3 (Clinical and Laboratory Standards Institute, 2008). The initial fungal cell concentrations were adjusted to 2×10^3 CFU/mL in RPMI-1640 medium, and then added into a 96-well flat-bottom polystyrene microtiter plate. The baicalin and SB were two-fold diluted ranging from 64-4096 μg/mL and 16-16384μg/mL, respectively. The MIC_{90} was defined as the lowest concentration of agents to cause 90% OD reduction at the wavelength of 490 nm by spectrophotometer (SpectraMax M2/M2e, USA) compared with the control. The control fungal cells were free of drugs.

Checkerboard assay

The final concentrations of baicalin and SB were serially two-fold diluted ranging from 16-1024 μg/mL and 4-4096 μg/mL, respectively. The fractional inhibitory concentration index (FICI) was equal to ($MIC_{baicalin}$ in combinatio/$MIC_{baicalin}$ alone) + (MIC_{SB} in combination /MIC_{SB} alone), in which synergism (SYN) was interpreted as FICI ≤0.5, indifference (IND) was defined as 0.5 <FICI ≤4.0, and antagonism (ANT) was FICI >4.0 (Odds, 2003).

RESULTS

Whether in fluconazole-sensitive or –resistant *C. albicans* isolates, the MICs of baicalin and SB alone were no less than 2048μg/mL, indicating that both antifungal activities were weak. However, their combined MICs decreased at least 16-32 folds with FICI ranging 0.094-0.375, inferring SB could facilitate the antifungal effect of baicalin against clinical *C. albicans* isolates (table 1).

DISCUSSION

Facing with the ever-increasing resistance to traditional antifungal agents, there have been at least two ways commonly used to improve the situation. One is to find

more drugs from the current "old ones" but with antifungal functions. The other is to strengthen the application of combination as the antifungal activities were quite weak in most of the "old drugs" (Shao *et al.*, 2016). Although SB and baicalin alone have been demonstrated to have a certain antifungal effects previously (Letscher-Bru *et al.*, 2013; Wang *et al.*, 2015), no reports were focused on their combination against clinical *C. albicans* isolates. For the first time, this work presented the strong synergism between SB and baicalin in 27 clinical *C. albicans* isolates. However, it could be observed that the combined MIC of baicalin/SB was still relative high. As they are clinically friendly, the combination of SB and baicalin is expected to perform better *in vivo*. Therefore, more works are needed and an alternative choice was provided against *C. albicans* in this study.

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