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Baicalin protects sertoli cells from heat stress-induced apoptosis via activation of the Fas/FasL pathway and Hsp72 expression

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ABSTRACT

Certain Chinese herbal medicines have antipyretic effects in both animal and human clinical practice. However, no report indicates their antipyretic effects on heat-stressed cells. The present study aimed to identify the protective effects of baicalin on the apoptosis of primary cultured bovine sertoli cells (SCs) subjected to heat stress (HS). The results demonstrated that HS induced apoptosis in the SCs exposed to 43 °C for 1 h as Fas/FasL was activated and caspase-3 was cleaved, the cells apoptotic rate was decreased. Moreover, the mRNA and protein levels of Hsp72 increased, whereas the cells apoptotic rate and expression of Fas, FasL, caspases 8 and 3 decreased in the SCs pretreated with various concentrations (0.1, 1, 10, 20 μg/mL) of baicalin prior to HS. In conclusion, baicalin ameliorates heat stress-induced cell apoptosis via the modulation of the cell survival rate through Fas/FasL pathway activation and the upregulation of Hsp72 expression in bovine SCs.

1. Introduction

Normally, testes are maintained 2–8 °C below the core body temperature [1] for optimal spermatogenesis. Mild hyperthermia disturbs their function and ultimately inhibits spermatogenesis [2]. A study on mice has demonstrated that the occurrence of transient heat stress (HS) leads to testicular weight loss, increase cell death, loss of reproductive cells and fertility [3]. Because of testicular sensitivity to an increase in temperature, the body has different mechanisms to maintain the proper temperature for normal orchic function [4]. Sertoli cells (SCs), which are considered nursing cells for developing sperm [5], are somatic cells in the testes and play an important roles in the process of sperm production. These cells are also easily affected by heat stress, which renders them unable to perform a supportive role for germ cell development, and they impairs male fertility [6]. Normally, the testis has been demonstrated to express the Fas ligand (FasL) at the highest level among tissues, and FasL expression is exclusively restricted to SCs [7], which regulates the number of spermatogenic cells by binding Fas receptors on the spermatogenic cells membrane.

The Fas/FasL system has been proposed as a key regulator of the activation of germ cell apoptosis [8]. Both FasL and Fas, which are expressed by SCs and germ cells, respectively, respond to environmental conditions and initiate germ cell death. It is advisable to evaluate the expression kinetics of both FasL and Fas after the induction of massive germ cell death in various testicular injury models. Evidence from previous studies suggests a role of DNA damage in the heat stress-induced apoptosis of SCs [9]. If SCs and germ cells are unbalanced, the sperm production and quality will decrease [10].

Heat shock proteins (HSPs), which were originally identified as stress-responsive proteins, belong to the most prominent group of proteins with functions involved in the folding and unfolding of other proteins [11]. The 70-kDa heat shock proteins (Hsp70s), which comprise the major class of proteins induced by increased temperatures, are chaperones that assist in the folding, assembling and disassembling of protein complexes [12]. The Hsp70 family includes a 73-kDa protein (Hsc73) which is constitutively expressed in healthy cells and a highly stress-inducible 72-kDa protein (Hsp72) which is normally typically expressed at low levels in healthy cells [13]. Untreated SCs do not normally express Hsp72; however, heat stress induces Hsp72 expression in the cell cytoplasm [14]. It has been demonstrated that heat stresses trigger apoptosis in somatic cells and induce Hsp72 gene expression, which limits apoptosis [15].

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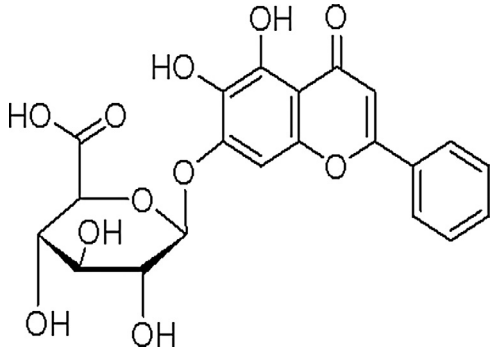

Fig. 1. Chemical structure of baicalin with a relative molecular weight of 446.35.

Baicalin, a type of flavonoid (Fig. 1), is extracted from the dried root of *Scutellaria baicalensis Georgi* [16], which is a traditional Chinese herbal medicine clinically used as an antipyretic, bacteriostatic and anti-inflammatory drug [17]. Studies have also demonstrated that baicalin can reduce the temperature of rats suffering with fever [18] and has pharmacological effects, including anti-oxidation activity [19] and the scavenging of oxygen-free radicals [20]. Baicalin effectively protects against concanavalin A-induced liver cell apoptosis [21]. In our previous study it was found that baicalin increased SCs survival rate under heat stress and improved expressions of both stem cell factors (SCF) and glial cell line-derived neurotrophic factor (GDNF) [22]. However, minimal information exists in the literature regarding the role of baicalin in SCs apoptosis induced by heat stress. This study aimed to address the hypothesis that baicalin can ameliorates heat stress-induced apoptosis through the Fas/FasL pathway and the regulation of Hsp72 expression in bovine SCs.

2. Materials and methods

2.1. Materials

Baicalin (concentration ≥ 98%) was bought from the National Pharmaceutical Engineering Center (Jiangxi, China). Dulbecco's Modified Eagle Media: Nutrient Mixture F-12 (DMEM/F-12), goat serum, penicillin and streptomycin for cell culture use were obtained from Life Technologies, Inc. (Grand Island, NY, USA). Collagenase Type IV, trypsin, dimethyl sulfoxide (DMSO) and CASP-3-C were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Goat polyclonal anti-Hsp72 (sc-1060) and GAPDH (sc-48166) and rabbit polyclonal anti-Fas (sc-7886) and FasL (sc-834) antibodies were all obtained from Santa Cruz Biotechnology, Inc. (USA). Rabbit polyclonal anti-caspase-8 (ab-4052) and caspase-3 (ab-90437) antibodies were obtained from Abcam (US), Inc. (UK). Secondary polyclonal anti-rabbit and anti-goat alkaline phosphatase-conjugated antibodies were purchased from Jackson ImmunoResearch Laboratories, Inc. (USA), Sigma.

2.2. Sertoli cells purification, culture and identification

Testes of bull calves were obtained from a calf serum-producing factory within 4 h after slaughter, the tunica albuginea was removed and seminiferous tubules were isolated from connective tissues under a stereomicroscope [23]. Collagenase IV (0.15%) was used to separated SCs from the tubules and remove the interstitial tissue in the supernatant; the remainder was subsequently digested in 0.25% trypsin. The suspension was filtered through a stainless steel 400-mesh sieve to remove undissociated tissues and debris; the filtrate was then pelleted by centrifugation at 1000 r/min for 10 min and washed twice.

Cell viability and density were determined by trypan blue exclusion using a hemocytometer [24]. The isolated cells were plated and cultured as dispersed single cells onto a tissue culture dish with a density of approximately 1×10^6 cells/mL in non-serum DMEM/F-12 medium and antibiotics at 34 °C under humidified atmosphere of 5% CO_2. After 5 h of incubation, majority of the cells spontaneously adhered to the surface of the flask. The non-adhering cells were removed, while the adhered cells were sequentially cultured with serum starvation for 48 h, digested with 0.25% trypsin and cultured with 10% fetal bovine serum (FBS) and 1% antibiotics DMEM/F-12 until the third generation for the experiment.

After purification, SCs were identified on the basis of their characteristic feature of bipolar corpuscle around the nucleus seen after Feulgen staining [25] and their unique expression of FasL via immunocytochemistry [26].

2.3. Cell treatment

Baicalin was dissolved in PBS and diluted with complete culture medium. In each experiment, the cells were incubated in the presence of baicalin (0.1, 1, 10 and 20 μg/mL, respectively) for the treated groups or the absence of baicalin (0 μg/mL) for the controls at 34 °C for 24 h prior to HS at 43 °C for 1 h and recovery at 34 °C for 6 h.

2.4. Identification of the cell apoptotic rate

SCs were washed with cold PBS and stained with propidium iodide (PI) and fluorescein isothiocyanate (FITC)-conjugated annexin V (FITC-AnxV) following the manufacturer's instructions. The cells were analyzed using BD FACS Calibur (USA).

Table 1
Primer sequences for the qPCR assay.

Gene	Primer sequences	PCR product size (bp)	GenBank [No.]
Hsp72	TGTCGCTGGGACTGGAGA GCTGGTTGTCCGAGTAGGTG	111	NM_203322.2
FasL	ACTACCGCCACCACCTCTGA GGCCACCAGAACCATGAAAA	85	NM_001098859.1
Fas	AAAAACTGGGGCTGCCCTTA CTTTGTGGGGGATGGAACAA	148	NM_174662.2
Caspase-8	CTGAGAGAAGAGGCCCGTGA CCCGGCTTAGGAACTTGAGG	173	DQ319070.1
Caspase-3	GAGCCTGTGAGCGTGCTTTT TGGTGCTGAGGATGACATGG	163	NM_001077840.1
GAPDH	ACGGCACAGTCAAGGCAGAG GTGATGGCGTGGACAGTGGT	376	NM_001034034.2