Food and Chemical Toxicology 58 (2013) 394–400

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

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

Antitumor activity and toxicity relationship of annonaceous acetogenins

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ARTICLE INFO

Article history:
Received 19 February 2013
Accepted 16 May 2013
Available online 24 May 2013

Keywords:
Annonaceous acetogenin
Antitumor
Toxicity
In vivo

ABSTRACT

Annonaceous acetogenins (ACGs) are one of the most interesting classes of natural products appearing in the past two decades. Here, we studied the antitumor activity and toxicity relationship of ACGs including annosquamin B (1), bullatacin (2) and annosquatin B (3) *in vivo*. A single intraperitoneal (i.p.) injection of 100 μg/kg of annosquamin B, bullatacin and annosquatin B did not cause side effects in normal mice. Bullatacin treatment with five doses of 25 and 50 μg/kg in H_{22} hepatoma cells bearing mice resulted in about 61% reduction in tumor growth with hematologic parameters increased significantly in normal mice. Annosquamin B and annosquatin B treatments with 10 doses of 25, 50 and 100 μg/kg in the H_{22} hepatoma cells transplantation tumor model mice resulted in maximum 53.7% and 58.7% reduction in tumor growth, respectively, and did not cause severe side effects in normal mice. This study provided the evidence that adjacent bis-THF ACGs showed higher antitumor activity and toxicity than mono-THF and nonadjacent bis-THF ACGs *in vivo*. Furthermore, it was found that bullatacin led to liver and kidney toxicity via increasing calcium concentration, ROS production, and Bax expression and Bax/Bcl-2 ratio in rats with repeated treatment with bullatacin for 3 weeks.

1. Introduction

Annonaceous acetogenins (ACGs) constitute a series of natural products isolated exclusively from Annonaceae species, which show significant cytotoxicity in human cancer cell lines *in vitro* (Zafra-polo et al., 1996, 1998; Zeng et al., 1996; Alali et al., 1999; Kojima and Tanaka, 2009) and are a unique class of C_{35} or C_{37} secondary metabolites derived from the polyketide pathway (Liaw et al., 2010). Several oxygenated functions, such as hydroxyl, ketone, epoxide, tetrahydrofuran (THF) and tetrahydropyran (THP), or double or triple bonds may be present in their structures. Thus several types of ACGs have been characterized (Li et al., 2008). According to the number and location of the THF rings along the hydrocarbon chain, ACGs are classified into three main types: mono-THF, adjacent bis-THF and nonadjacent bis-THF ACGs (Bermejo et al., 2005).

ACGs are known to be very potent cytotoxic compounds, targeting the mitochondrial complex I (NADH: ubiquinone oxidoreductase) of the respiratory chain (Londershausen et al., 1991; Degli Esposti et al., 1994), whose architecture has been resolved recently (Efremov et al., 2010). The adjacent bis-THF ACGs are the most potent compounds in this family, and the nonadjacent bis-THF ACGs are, in general but not always, superior to the mono-THF ACGs, which, in turn, are more potent than non-THF ACGs *in vitro* tests. They have also been shown to overcome resistance in multidrug resistant (MDR) tumors (Oberlies et al., 1997). Mechanism of action studies have recently shown that ACGs inhibited HIF-1 activation by blocking the hypoxic induction of nuclear hypoxia inducible factor-1α (HIF-1α) protein (Coothankandaswamy et al., 2010).

Concentration–response profiles on panels of human cancer cell lines grown and treated in a monolayer or as a suspension have proven useful in identifying cytotoxic compounds for further studies. However, experiments with cells in monolayer or suspension do not provide sufficient information on the activity of compounds against clinically important solid tumors. These tumors contain gradients of nutrient concentrations and regional pH differences, which can cause regional variations in cell viability, metabolism, and sensitivity to treatment. Tumors become heterogeneous as they grow, developing subpopulations of different characteristics and activities (Casciari et al., 1994). Nowadays, all the scientific community are attempting to explore novel antitumor drugs, but the 'golden standard' of anticancerous effect in animal models is still the xenografting in immunodeficient host animals, mostly with subcutaneous grafting, and several tumor models *in vivo* have been established to rapidly and efficiently assess the antitumor activity of samples (Xu et al., 2007). H_{22} hepatoma cells transplantation tumor model is one of the most common tumor models employed in the antitumor research (Wu et al., 2010).

In vivo tests in mice showed the antitumor effects and even some possible adverse effects of ACGs (Cavé et al., 1997; Liaw

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http://dx.doi.org/10.1016/j.fct.2013.05.028

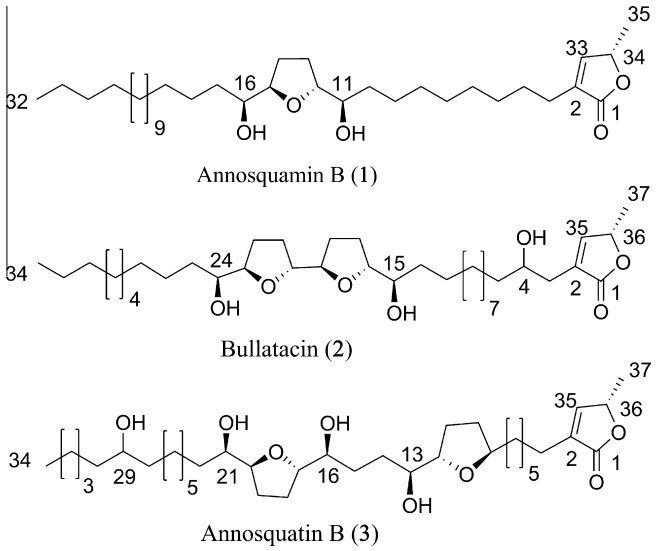

Fig. 1. Structure of annosquamins B (**1**), bullatacin (**2**) and annosquatin B (**3**).

et al., 2010). However, the antitumor activity and toxicity relationship of ACGs from three major structural classes is not clear so far.

In this study, the antitumor activity and toxicity of mono-THF ACG annosquamin B (**1**), adjacent bis-THF ACG bullatacin (**2**) and non-adjacent bis-THF ACG annosquatin B (**3**) (Fig. 1) were evaluated using the H_{22} hepatoma cells transplantation tumor model and normal mice, respectively. A 21-day repeated-dose toxicity experiment of bullatacin in rats was also carried out.

2. Materials and methods

2.1. Materials

Annosquamin B, bullatacin and annosquatin B were isolated from the seeds of *Annona squamosa* L. (Annonaceae) by our laboratory (Chen et al., 2011, 2012a,b) and the purity of each compound was determined to be over 98% by HPLC analysis and confirmed by LC-MS, NMR spectroscopy. 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) and cyclophosphamide (CTX) used as antitumor agents were purchased from Sigma (Shanghai, China). Fluo-3/acetoxy methyl (Fluo-3/AM) was purchased from Beyotime (Nantong, China). Other chemicals were all of analytical grade.

2.2. Animals

Male Kunming mice weighting 18–22 g were purchased from Slac Laboratory Animal Co., Ltd., (Shanghai, China). Sprague–Dawley rats (male, weighing 200 ± 20 g) were obtained from the Laboratory Animal Center of Nanjing Medical University (Nanjing, China). All the procedures were approved by Animal Ethical Council of Nanjing University of Chinese Medicine. The animals were maintained

Table 1
Toxicity findings during single-dose and repeated-dose toxicity of annosquamin B (**1**), bullatacin (**2**) and annosquatin B (**3**).

Sample (days)	Dose (μg/kg)	Start weight (g)	End weight (g)	Observation/comments
Control (7)	0	25.1 ± 1.5	33.4 ± 3.1	Normal[b]
1 (7)	Single 100	24.8 ± 1.9	32.5 ± 2.9	Normal[b]
2 (7)	Single 100	26.2 ± 2.0	34.2 ± 1.8	Normal[b]
3 (7)	Single 100	25.5 ± 2.0	33.5 ± 1.9	Normal[b]
Control (5)	0	25.2 ± 2.7	32.9 ± 3.9	Normal[b]
2 (5)	Repeated 25	23.1 ± 1.2	26.6 ± 2.5[a]	Weight loss
2 (5)	Repeated 50	25.4 ± 1.6	27.2 ± 0.4[a]	Weight loss
2 (5)	Repeated 100	25.5 ± 1.3	26.4 ± 0.5[a]	Weight loss and two mice died after 4–5 days
Control (10)	0	25.6 ± 1.7	35.5 ± 3.7	Normal[b]
1 (10)	Repeated 25	26.0 ± 0.8	33.7 ± 3.2	Normal[b]
1 (10)	Repeated 50	25.9 ± 0.8	34.2 ± 3.1	Normal[b]
1 (10)	Repeated 100	26.1 ± 1.1	34.6 ± 4.3	Normal[b]
3 (10)	Repeated 25	25.3 ± 2.6	34.5 ± 5.2	Normal[b]
3 (10)	Repeated 50	25.7 ± 2.0	35.6 ± 4.0	Normal[b]
3 (10)	Repeated 100	25.8 ± 1.1	35.0 ± 3.4	Normal[b]

The values are presented as mean ± S.D.
[a] $P < 0.05$ compared with control group (5).
[b] Incl hematology and pathology.

Table 2
Blood chemistry measurements after administration of annosquamin B (**1**), bullatacin (**2**) and annosquatin B (**3**).

Sample (days)	Dose (μg/kg)	ALT	AST	BUN	CR
Control (7)	–	23.0 ± 6.4	74.5 ± 14.3	6.6 ± 0.7	41.2 ± 8.5
1 (7)	Single 100	23.4 ± 7.8	75.3 ± 10.2	6.2 ± 0.4	40.8 ± 7.3
2 (7)	Single 100	22.0 ± 5.3	73.2 ± 11.5	6.8 ± 1.2	41.7 ± 8.9
3 (7)	Single 100	21.8 ± 8.3	78.4 ± 12.3	7.0 ± 0.8	42.1 ± 9.3
Control (5)	–	27.0 ± 3.3	76.0 ± 11.1	5.2 ± 0.4	31.0 ± 7.0
2 (5)	Repeated 25	37.9 ± 9.4[a]	94.4 ± 11.8[a]	7.0 ± 1.6[a]	26.2 ± 11.2
2 (5)	Repeated 50	52.2 ± 22.2[a]	88.0 ± 23.5	6.5 ± 1.0[a]	33.2 ± 9.8
2 (5)	Repeated 100	50.8 ± 22.9[a]	104.8 ± 19.1[a]	8.8 ± 2.9[a]	46.0 ± 8.4[b]
Control (10)	–	20.8 ± 5.7	84.4 ± 9.6	7.6 ± 0.7	48.8 ± 8.9
1 (10)	Repeated 25	21.3 ± 22.1	90.6 ± 22.1	7.3 ± 0.8	47.6 ± 11.6
1 (10)	Repeated 50	20.9 ± 7.0	110.7 ± 34.2	7.1 ± 1.5	46.9 ± 18.2
1 (10)	Repeated 100	27.6 ± 14.4	97.1 ± 22.9	7.3 ± 0.4	48.1 ± 16.1
3 (10)	Repeated 25	21.7 ± 9.6	78.4 ± 9.1	7.0 ± 1.2	46.1 ± 16.6
3 (10)	Repeated 50	24.9 ± 10.0	78.8 ± 16.3	6.8 ± 1.0	48.3 ± 16.9
3 (10)	Repeated 100	22.9 ± 10.6	74.6 ± 12.1	7.2 ± 0.9	49.7 ± 11.1

The values are presented as mean ± S.D.
[a] $P < 0.05$ compared with control (5).
[b] $P < 0.01$ compared with control (5).