Toxicology in Vitro 27 (2013) 745–751

Contents lists available at SciVerse ScienceDirect

Toxicology in Vitro

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

The effects of jaspamide on human cardiomyocyte function and cardiac ion channel activity

Karen Schweikart [a,*], Liang Guo [b], Zachary Shuler [b], Rory Abrams [b], Eric T. Chiao [b], Kyle L. Kolaja [b], Myrtle Davis [a]

[a] Developmental Therapeutics Program, National Cancer Institute, Bethesda, MD 20892, United States
[b] Global Early and Investigative Safety, Hoffmann-La Roche, Nutley, NJ 07110, United States

ARTICLE INFO

Article history:
Received 16 August 2012
Accepted 6 December 2012
Available online 20 December 2012

Keywords:
Stem cell-derived cardiomyocytes
Jaspamide
Cyclodepsipeptide
In vitro alternatives
Predictive toxicology
Cardiotoxicity

ABSTRACT

Jaspamide (jasplakinolide; NSC-613009) is a cyclodepsipeptide that has antitumor activity. A narrow margin of safety was observed between doses required for efficacy in mouse tumor models and doses that caused severe acute toxicity in rats and dogs. We explored the hypothesis that the observed toxicity was due to cardiotoxicity. Jaspamide was tested in a patch clamp assay to determine its effect on selected cardiac ion channels. Jaspamide (10 μM) inhibited Kv1.5 activity by 98.5%. Jaspamide also inhibited other channels including Cav1.2, Cav3.2, and HCN2; however, the Kv11.1 (hERG) channel was minimally affected. Using spontaneously contracting human cardiomyocytes derived from induced pluripotent stem cells, effects on cardiomyocyte contraction and viability were also examined. Jaspamide (30 nM to 30 μM) decreased cardiomyocyte cell indices and beat amplitude, putative measurements of cell viability and cardiac contractility, respectively. Concentration-dependent increases in rhythmic beating rate were noted at ⩽6 h of treatment, followed by dose-dependent decreases after 6 and 72 h exposure. The toxic effects of jaspamide were compared with that of the known cardiotoxicant mitoxantrone, and confirmed by multiparameter fluorescence imaging analysis. These results support the hypothesis that the toxicity observed in rats and dogs is due to toxic effects of jaspamide on cardiomyocytes.

Published by Elsevier Ltd.

1. Introduction

Jaspamide (jasplakinolide, NSC-613009), a cyclodepsipeptide isolated from the marine sponge *Jaspis johnstoni* (Crews et al., 1986) has been extensively investigated as a potential cancer therapeutic agent. Jaspamide exhibits antitumor activity in multiple in vitro tumor models for prostate and breast carcinomas and acute myeloid leukemia (Takeuchi et al., 1998; Bubley and Balk, 1996; Stingl et al., 1992; Fabian et al., 1995). Jaspamide inhibits the growth of prostate carcinoma PC-3 cells by disrupting the actin cytoskeleton (Senderowicz et al., 1995) and acts as a radiosensitizer against prostate and lung carcinoma cells in vitro (Takeuchi et al., 1998).

In vivo, a 7-day continuous subcutaneous infusion of 31.5 mg/m^2 jaspamide resulted in a 5-day tumor growth delay in mice bearing Lewis lung carcinoma xenografts (Takeuchi et al., 1998). However, in Fischer 344 rats given intravenous (iv) injections (0.8–4.0 mg/m^2/dose) every eight hours for three doses, a total dose of 4.5 mg/m^2/dose was lethal (Schindler-Horvat et al., 1998). A slightly lower dose was minimally toxic with signs of toxicity limited to hunched posture. Pulmonary edema and cardiac hemorrhage and congestion were present in rats that received lethal doses; however, jaspamide-related microscopic lesions were not noted in rats that survived to Day 15. In beagle dogs, a dose of 0.026 mg/kg/h (12.5 mg/m^2) given as a 24-h continuous iv infusion was not lethal, whereas 0.030 mg/kg/h (14.4 mg/m^2) given on the same route and schedule was a lethal dose. Pulmonary edema and cardiac hemorrhage and congestion were present in dogs that received lethal doses. Dogs that survived to the end of the study were not euthanized, therefore histopathology data are not available for sub-lethal doses (Schindler-Horvat et al., 1998). Based on the narrow therapeutic index observed in these studies, jaspamide was dropped from consideration for further development as an anticancer agent at the National Cancer Institute.

Given the observation of cardiotoxicity with jaspamide, mechanistic studies were undertaken to determine the effect of jaspamide on cardiac ion channel function and on the viability and contractile function in human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). Human iPSC-CMs have the complement of ionic currents and channel gating properties required for synchronized cardiomyocyte contractions (Ma et al., 2011). Action potentials from these cells have atrial-, nodal-, and

* Corresponding author. Address: Developmental Therapeutics Program, National Cancer Institute, Executive Plaza North Room 8038, Bethesda, MD 20892, United States. Tel.: +1 301 435 9165; fax: +1 301 480 4836.
E-mail address: schweikk@mail.nih.gov (K. Schweikart).

0887-2333/$ - see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.tiv.2012.12.005

ventricular-like properties, indicative of a heterogeneous cell population. Furthermore, the cells maintain synchronized contraction in a 96-well dish for more than 7 days, allowing high-throughput investigations of the putative cardiotoxic compounds in a functional human cardiomyocyte system (Ma et al., 2011; Guo et al., 2011). Herein, we report the results of mechanistic in vitro investigations of jaspamide on cardiomyocyte function and propose a mechanism of jaspamide induced cardiotoxicity.

2. Materials and methods

2.1. Compounds

Jaspamide (jasplakinolide, NSC-613009) was extracted from a sponge sample provided by the Coral Reef Research Foundation under a National Cancer Institute collection contract. Jaspamide was isolated and purified by the Natural Products Extraction Laboratory (SAIC-Frederick), and supplied as a solid powder in amber glass capsules. The capsules were capped with nitrogen and stored protected from light at −20 °C.

2.2. Patch clamp assay

The in vitro effects of jaspamide on cardiac ion channel activity were evaluated (Chantest Inc., Cleveland, OH) using an automated patch clamp system (PatchXpress 7000A, Molecular Devices, Sunnyvale, CA). Single mammalian cells (CHO or HEK293), each expressing one of 12 cardiac ion channels (calcium, potassium, or sodium, Table 1), were exposed to 10 μM jaspamide for 5 min at room temperature. Experiments were conducted in duplicate for each ion channel. The inhibition by jaspamide of the current flow through each ion channel was calculated as the mean percent inhibition ± the standard deviation. The sensitivity of each channel was confirmed using a positive control specific for each ion channel.

2.3. Cardiomyocyte cell culture

Cryopreserved human induced pluripotent stem cell-derived cardiomyocytes (iCell Cardiomyocytes®, Cellular Dynamics International, Madison, WI) were used in these studies. These cardiomyocytes exhibit the biochemical and electrophysiological characteristics of normal human heart cells, and form electrically connected layers that beat in synchrony (Anson et al., 2011). The cells were plated on a 0.1% gelatin-coated 6-well plate (2×10^6 cells/well) with plating medium (iCell Cardiomyocytes Plating Medium (iCPM); Cellular Dynamics International) and cultured at 37 °C with iCell Cardiomyocytes Maintenance Medium (iCMM; Cellular Dynamics International).

Cells were replated 1–3 weeks later to a tissue culture E-plate (ACEA Biosciences, San Diego, CA) at a concentration of approximately 5.0×10^4 cells/well. Plates were coated with 0.1% gelatin for approximately 3 h at 37 °C before cell plating. Cells were maintained in iCMM, which was changed every 2–3 days.

2.4. Assessment of cardiomyocyte viability

Jaspamide was dissolved in iCMM at a concentration of 60 μM. Half of the volume of maintenance medium in each well was replaced with the vehicle or jaspamide (final concentrations of 30, 100, 300 nM; and 1, 10, 30 μM). Each concentration was tested initially in triplicate for 72 h in one E-plate, and repeated in duplicate in an additional two E-plates seeded with cells from two different lots. The following positive controls were also tested: mitoxantrone (structural cardiotoxicant), E-4031 (hERG blocker), TTX (Na^+ channel blocker), nifedipine (L-type Ca^{2+} channel blocker) and S9947 (Kv1.5 blocker). Impedance technology (xCELLigence RTCA Cardio-96 System, ACEA/Roche) was used to monitor in real time the viability of cells treated with jaspamide or vehicle. An IC_{50} was calculated as cell index in treated wells vs. cell index in untreated (vehicle control) wells at selected time-points during 72 h exposure. The cell index is an impedance measurement whose increase reflects increases in the number of cells attached to the electrode of the well. The cell index is zero when there are no cells present or cells do not adhere to the electrodes. In addition, changes in cell morphology, cell adhesion, cell–cell interaction and/or cell viability can be reflected by a resultant change in cell index (Xi et al., 2008). The cell viability in each well of the E-plate was also assessed after 72 h exposure to jaspamide by ATP quantification using a CellTiter-Glo® Luminescent kit (Promega, Madison, WI).

The cardiotoxicity of jaspamide and mitoxantrone was further examined using a Cellomics® ArrayScan VTI HCS Reader with the Multiparameter Cytotoxicity 2 Kit (Cat. #8400202, Thermo Fisher Scientific, Pittsburgh, PA). In this experiment, cells from three different lots were plated on 6-well plates and cultured for one week, then replated into three 0.1% gelatin-coated 96-well back/clear plates as described above. Three days after replating the cells were treated with jaspamide or mitoxantrone for 72 h. Cells were then live-stained with nuclear (blue), membrane permeability (green) and mitochondrial membrane potential (red) dyes according to the kit instructions, and the fluorescent images were analyzed using the multiparameter cytotoxicity BioApplication module.

2.5. Assessment of cardiomyocyte function

As part of the cardiomyocyte studies described above, impedance was measured using the xCELLigence RTCA Cardio-96 System (Roche Applied Sciences, Indianapolis, IN). The change in beat amplitude (a measure of cardiac contractility) and beat rate (a measure of rate of rhythmic contraction of cardiomyocytes) was calculated as a percentage of the time-matched vehicle control

Table 1
Conditions for cardiac ion channels tested in the patch clamp assay.

Channel	Function	Expressed in cell type	Positive control
Cav1.2	$I_{Ca,L}$, high threshold calcium current	CHO	Nifedipine
Cav3.2	$I_{Ca,T}$, low threshold calcium current	HEK293	Nickel
HCN2	I_f, hyperpolarization-activated potassium current	CHO	Zatebradine
HCN4	I_f, hyperpolarization-activated potassium current	HEK293	Zatebradine
hERG	I_{Kr}	HEK293	E-4031
Kir2.1	I_{K1}, inwardly rectifying potassium current	HEK293	Barium
Kir3.1/3.4	I_{KACh}, inwardly rectifying potassium current	HEK293	Barium
Kir6.2/SUR2A	ATP-sensitive current, I_{KATP}	HEK293	Glybenclamide
Kv1.5	I_{kur}, ultra-rapid delayed rectifier potassium current	CHO	4-AP
Kv4.3	I_{to}, transient outward potassium current	HEK293	Flecainide
KvLQT/mink	I_{Ks}, slow delayed rectifier potassium current	CHO	Chromanol 293B