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Research Paper

Spasmogenic Effects of the Proteasome Inhibitor Carfilzomib on Coronary Resistance, Vascular Tone and Reactivity

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ABSTRACT

Background: Carfilzomib (CFZ) is a new proteasome inhibitor used for the treatment of multiple myeloma. Besides heart failure, angina and myocardial ischemia occurred following administration of CFZ, which is not contraindicated in patients with recent myocardial infarction/unstable angina excluded from the safety trials.
Aim of Study: To test the effects of CFZ (10^{-9} to 10^{-7} mol/L) on vascular tone and reactivity in the isolated rabbit heart and aorta.
Methods and Results: CFZ administered by bolus injection to the isolated heart increased coronary perfusion pressure (CPP) at all tested concentrations and mildly raised left ventricular pressure and heart rate, only at the highest concentration. Addition of CFZ directly into the organ bath increased the basal tone of isolated aortic strips with contraction plateau reached after 10 min. This spasmogenic effect doubled following ablation of the endothelium. Pretreatment with CFZ amplified the vasospastic action exerted by KCl, noradrenaline (NA) and angiotensin II (A) on aortic strips, and impaired vasodilation following administration of nitroglycerin (NTG) and nifedipine (NFP) on the contraction plateau induced by KCl, NA and A. Aortic strips pretreated with CFZ exhibited impaired relaxation, as compared to untreated strips, following administration of acetylcholine (Ach), an endothelium-dependent vasodilating agent, on the plateau of NA contraction ($p < 0.05$).
Conclusions: CFZ increased CPP, resting vasoconstricting tone and the spasmogenic effect of different agents. Preincubation with CFZ decreased the anti-spasmogenic activity of NTG and NFP, as well as reduced by over 50% the vasodilating effect of Ach, suggesting that CFZ can impair vasodilation via an endothelium dependent mechanism. Further studies are warranted to establish its clinical safety in patients with known CAD and prior history of coronary spasm.

1. Introduction

Carfilzomib (CFZ) is a novel proteasome inhibitor given by intravenous infusion for the treatment of relapsed and/or refractory Multiple Myeloma (MM) (Neri et al., 2016). Chemically, it is a tetrapeptide epoxyketone derived from epoxomicin, a natural product that has been shown to inhibit the proteasome (Meng et al., 1999). CFZ exerts a potent and irreversible inhibition of the proteasomal chymotrypsin-like activity, by binding selectively the β5 subunit of the 20S proteolytic core particle, while it only has minimal affinity for the β1 and β2 subunits (at doses up to 100 nM) (Demo et al., 2007). The U.S. Food and Drug Administration (FDA) approved CFZ on 20 July 2012 for use in patients with MM, who have received at least two prior therapies [including treatment with bortezomib (BTZ) and an immunomodulatory agent] and who exhibited disease progression within 60 days of completion of the last therapy. More recently, CFZ has also been approved for combined use with lenalidomide and dexamethasone for the

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treatment of patients with relapsed MM (KYPROLIS (Carfilzomib) [Prescribing Information], 2015).

Proteasomes are protein complexes located in the nucleus and the cytoplasm of all eukaryotic cells. The main function of the proteasome is to degrade unneeded or damaged proteins by proteolysis (Dahlmann, 2016). Proteins are tagged for degradation with several residues of a small protein called ubiquitin, with final formation of a polyubiquitin chain, which is bound by the proteasome (Yao and Cohen, 2002). The ubiquitin-proteasome pathway (UPP) influences essential cellular functions including cell growth, differentiation, apoptosis, signal transduction, antigen processing and the inflammatory response. Proteasome inhibitors have effective anti-tumor activity. Selective inhibition in cancer cells of proteasome-mediated proteolysis results in a build-up of polyubiquinated proteins, which may cause cell cycle arrest, apoptosis, and inhibition of tumor growth (Grigoreva et al., 2015).

Chronic proteasome inhibition was associated with increased oxidative stress and early occurrence of atherosclerosis in a pig model of coronary artery disease (CAD) (Herrmann et al., 2007). In the same animal model, inhibition of the proteasome resulted in functional and structural alteration of the heart consistent with a hypertrophic-restrictive cardiomyopathy phenotype (Marfella et al., 2008). Sustained proteasome inhibition was also found to promote vascular cell senescence, thereby contributing to plaque progression, in asymptomatic elderly and adult patients undergoing carotid endarterectomy (Herrmann et al., 2013).

Although CFZ has a more favorable safety profile than other proteasome inhibitors, such as BTZ, mostly in regard to the lower incidence of peripheral neuropathy, analysis of the available clinical data seems to endorse the above experimental findings, confirming that treatment with CFZ may be associated with significant cardiovascular complications.

The safety profile of CFZ used as a single agent for the treatment of relapsed and/or refractory MM was analyzed in a total of 526 patients enrolled in the four phase II clinical trials based on which the U.S. FDA approval was granted. The most severe side effects reported include: sudden death (within 24 h from the infusion), pulmonary hypertension, heart failure (HF) (7.2%), myocardial ischemia and infusion reactions. Chest tightness of unknown mechanism has also been described and reported (Siegel et al., 2013).

Likewise, in the phase III, randomized, multicenter studies ASPIRE (Carfilzomib, Lenalidomide, and Dexamethasone versus Lenalidomide and Dexamethasone for the Treatment of Patients with Relapsed Multiple Myeloma) (Stewart et al., 2015) and ENDEAVOR (Phase 3 Study With Carfilzomib and Dexamethasone Versus Bortezomib and Dexamethasone for Relapsed Multiple Myeloma Patients) (Dimopoulos et al., 2015a; Dimopoulos et al., 2015b), the use of CFZ as part of combination regimens in patients with relapsed or refractory MM resulted in significantly higher rates of cardiac and cardiopulmonary adverse events including dyspnea, HF, ischemic heart disease and hypertension.

The goal of our study was to investigate whether CFZ exerts in vitro effects on vascular tone and reactivity in the isolated rabbit heart and thoracic aortic strips. The isolated heart was used to assess the effect of CFZ on myocardial contractility and coronary resistances. The thoracic aortic strips with and without endothelium were used to evaluate the effects of CFZ on vascular smooth muscle tone.

2. Methods

2.1. Rabbit Heart Preparations

All animal experiments were conducted ethically and approved by pertinent ethics committee. New Zealand albino rabbits of both sexes weighing 2–2.5 kg were used. The animals were sacrificed by cervical dislocation. The hearts were removed quickly and placed in an ice-cold Ringer-Locke solution, oxygenated with 100% O_2, and containing in millimoles per liter: NaCl, 136.9; KCl, 2.68; MgCl, 0.99; $CaCl_2$, 1.7; $NaHPO_4$, 0.42; $NaHCO_3$, 3.93; and glucose, 5.55 (pH 7.4) (Raddino et al., 1997).

Using a previously described procedure (Broadley, 1979), after removal of the pericardium and surrounding tissues, the hearts were perfused with Ringer-Locke solution according to the nonrecirculating Langendorff technique. The perfusion fluid was continuously gassed with 100% O_2, maintained at 37 degrees Celsius, and delivered to the aortic inflow cannula at a constant rate of 22–24 ml/min using a peristaltic pump (Gilson, Miniplus HP2HF). The perfusion pressure was measured by a Statham transducer connected to the sidearm of the perfusion cannula. Since retrograde flow (coronary flow) was kept constant during the experiment, coronary perfusion pressure (CPP) represented a direct measure of the coronary resistance. A fluid-filled balloon connected to a pressure transducer was inserted into the left ventricular cavity through an opening in the left atrium, thus obtaining an isovolumically beating preparation. The balloon was inflated to provide an end-diastolic pressure < 1.0 mm Hg (Ferrari et al., 1996). Both end systolic left ventricular pressure (LVP) and CPP were recorded simultaneously by using a polygraph (OTE Biomedica; C6B). Except for experiments evaluating chronotropic effects, the hearts were electrically paced to exclude LVP variations related to heart rate (HR) oscillations. Rectangular pulses (0.5 V @ 1.0 msec, up to threshold stimulation) were applied to the preparation via two platinum electrodes, one connected to the metal inflow cannula and the other implanted directly in the ventricular apex. The frequency of stimulation was 10% greater than the basal HR. The hearts were left to equilibrate for 30 min prior to drug administration. The maximal effects of the used agents were observed 5–15 min after addition to the perfusion fluid (Ferrari et al., 1996). CFZ was administered in the perfusion buffer or infused at three different concentrations (10^{-9}, 10^{-8} and 10^{-7} M) by bolus injection of 1 cc over 5 min, using a collateral arm of the perfusion cannula. The above concentrations were chosen based on prior in vitro work showing that carfilzomib doses ranging from 5 to 80 nmol/L resulted in a significant growth inhibition of mantle cell lymphoma (MCL) cells, harvested from peripheral blood samples or bone marrow aspirates obtained from patients with MCL (Zhang et al., 2013).

2.2. Aortic Preparations

After surgical isolation of the aortic segments, the media was separated from the adventitia and spirally cut into strips, according to Furchgott's technique (Furchgott and Zawadzki, 1980). The thoracic aortic strips (2 cm long and 3 mm wide) were then placed in 10 ml organ baths containing Krebs-Henseleit solution at 37 °C. Contractions were measured by means of an isometric transducer, connected to a pen writing recorder. An initial tension of 2 g was applied for 120 min before the administration of drugs. The effect of three different spasmogenic agents [potassium chloride (KCl, noradrenaline (NA), and angiotensin II (A))] was evaluated on aortic strips either untreated or precontracted for 60 min with CFZ. KCl (10^{-1} M), NA (10^{-6} M) and A2 (10^{-5} M) were administered directly into the organ bath. In a different set of experiments, following achievement of the plateau of contraction (usually 5–10 min after the administration) induced by CFZ, vasodilatory agents, such as nitroglycerin (NTG) and nifedipine (NFP) were added to the organ bath at different concentrations, ranging from 10^{-9} to 10^{-5} M. Some aortic strips were rubbed to eliminate the endothelial layer, whose absence was pharmacologically tested and confirmed by means of the acetylcholine test (Furchgott and Zawadzki, 1980). Endothelium-dependent vascular reactivity was also assessed by evaluating the antagonism induced by acetylcholine (Ach; 10^{-8} to 10^{-5} M) during NA-induced specimen contraction (10^{-5} M).

2.3. Data Analysis

Results were expressed as mean ± SEM of 6–8 experiments. The inhibitory response on LVP and CPP was calculated as a percentage

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