INTISARI

Latar belakang: Spinal muscular atrophy (SMA) merupakan penyakit neuromuskular degeneratif yang diketahui memiliki destabilisasi sitoskeleton aktin melalui disregulasi jalur sinyal RhoA/ROCK akibat defisiensi protein Survival Motor Neuron (SMN). Kurkumin diketahui memiliki mekanisme dalam memodulasi jalur persinyalan RhoA/ROCK1. Pentagamavunon-1 (PGV-1), sebagai analog sintetik kurkumin dengan stabilitas kimia yang lebih baik, berpotensi untuk memodulasi jalur tersebut, namun efek spesifiknya dalam konteks SMA masih terbatas untuk dieksplorasi.

Tujuan: Mengevaluasi efek pemberian kurkumin dan PGV-1 terhadap jalur RhoA/ROCK dan morfologi sitoskeleton aktin secara *concentration-dependent* pada lini sel fibroblas SMA.

Metode: Penelitian dilakukan secara *in vitro* pada lini sel fibroblas SMA tipe I, SMA tipe II. Kelompok perlakuan dibagi menjadi perlakuan kurkumin menggunakan tiga tingkatan konsentrasi (5 µM, 10 µM, dan 15 µM) dan perlakuan PGV-1 menggunakan tiga konsentrasi (0,75 µM, 1,5 µM dan 2,25 µM) dengan kontrol DMSO 0,1%. Viabilitas sel dievaluasi menggunakan uji MTT untuk menentukan konsentrasi. Analisis regresi nonlinear dilakukan untuk mendapatkan nilai IC_{50} dari senyawa. Uji protein RhoA dan ROCK1 menggunakan ELISA. Uji statistik menggunakan uji ANOVA satu arah dan uji *post hoc* Tukey. Morfologi sitoskeleton aktin divisualisasikan melalui pewarnaan fluoresen phalloidin dengan mikroskop fluoresen.

Hasil: Nilai IC_{50} PGV-1 pada fibroblas SMA tipe I, SMA tipe II, dan fibroblas primer non-SMA masing-masing sebesar 1,42 µM, 1,58 µM, dan 19,68 µM, lebih rendah dibandingkan kurkumin yang memiliki nilai IC_{50} sebesar 14,24 µM, 15,83 µM, dan 108,82 µM. Pemberian PGV-1 menunjukkan perbedaan kadar protein RhoA dan ROCK1 secara *concentration-dependent*, sedangkan kurkumin tidak menunjukkan perbedaan kadar kedua protein tersebut. Analisis mikroskopis memperlihatkan bahwa kedua senyawa menginduksi disorganisasi jaringan mikrofilamen aktin-F. Kurkumin menyebabkan hilangnya serat stres, sementara PGV-1 memicu penebalan serat stres serta perubahan morfologi sel berupa pembulatan sel yang disertai akumulasi aktin di area kortikal.

Kesimpulan: PGV-1 memiliki potensi sitotoksik lebih tinggi dibanding kurkumin, memengaruhi peningkatan kadar protein RhoA dan ROCK1 secara *concentration-dependent*, serta menginduksi perubahan morfologi dan disorganisasi mikrofilamen aktin-F, sedangkan kurkumin hanya menyebabkan hilangnya *stres fiber* tanpa efek pada protein total RhoA dan ROCK1 yang signifikan.

Kata kunci: Kurkumin, PGV-1, RhoA, ROCK1, spinal muscular atrophy, fibroblas

ABSTRACT

Background: Spinal muscular atrophy (SMA) is a degenerative neuromuscular disease known to have destabilization of the actin cytoskeleton through dysregulation of the RhoA/ROCK signaling pathway due to deficiency of the Survival Motor Neuron (SMN) protein. Curcumin is known to have a mechanism in modulating the RhoA/ROCK1 signaling pathway. Pentagamavunon-1 (PGV-1), a curcumin analog with improved chemical stability, has shown potential to modulate this pathway; however, its specific effects in the context of SMA remain poorly explored.

Objective: To evaluate the effects of curcumin and PGV-1 on the RhoA/ROCK signaling pathway and actin cytoskeleton morphology in a concentration-dependent manner using SMA fibroblast cell lines.

Methods: This *in vitro* study was conducted using fibroblast cell lines derived from SMA type I and SMA type II patients. Cells were treated with curcumin at three concentrations (5 µM, 10 µM, and 15 µM) and PGV-1 at three concentrations (0.75 µM, 1.5 µM, and 2.25 µM), with 0.1% DMSO as the control. Cell viability was assessed using the MTT assay to determine appropriate concentrations, and IC_{50} values were calculated using nonlinear regression analysis. Protein levels of RhoA and ROCK1 were quantified using ELISA. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Actin cytoskeleton morphology was visualized by phalloidin fluorescent staining using fluorescence microscopy.

Results: The IC_{50} values of PGV-1 in SMA type I, SMA type II, and primary non-SMA fibroblasts were 1.42 µM, 1.58 µM, and 19.68 µM, respectively, which were lower than those observed for curcumin (14.24 µM, 15.83 µM, and 108.82 µM). PGV-1 treatment resulted in concentration-dependent alterations in RhoA and ROCK1 protein levels, whereas curcumin treatment did not produce significant changes in either protein. Microscopic analysis demonstrated that both compounds induced disorganization of actin microfilaments. Curcumin led to the loss of stress fibers, while PGV-1 induced stress fiber thickening and morphological changes characterized by cell rounding accompanied by cortical actin accumulation.

Conclusion: PGV-1 exhibits higher cytotoxic potency than curcumin, modulates RhoA and ROCK1 protein levels in a concentration-dependent manner, and induces marked alterations in actin cytoskeleton organization. In contrast, curcumin primarily causes stress fiber loss without significant effects on RhoA/ROCK1 protein levels.

Keywords: Curcumin; PGV-1; RhoA; ROCK1; spinal muscular atrophy; fibroblasts