Influence of primaquine and ritonavir interaction on CYP3A4 mRNA expression in HepG2 cell culture

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Abstrak

Latar Belakang: Pengobatan antimalaria dan anti-HIV secara bersamaan merupakan tantangan baru dalam penanganan koinfeksi malaria dan HIV. Primakuin merupakan substrat sekaligus inhibitor bagi CYP3A4, sedangkan ritonavir merupakan substrat, inhibitor, sekaligus inducer bagi CYP3A4. Tujuan penelitian ini adalah untuk mengukur ekspresi mRNA CYP3A4 pada kultur sel HepG2 yang diinduksi oleh pemberian primakuin dan ritonavir secara bersamaan. Metode: Pada penelitian pendahuluan, sel HepG2 diinkubasi dengan primakuin 30, 40, 50 uM; ritonavir 2, 10, 20 uM; DMSO <0,1 % sebagai kontrol negatif; atau rifampisin 20 uM sebagai kontrol positif. Adapun pada penelitian dengan perlakuan kombinasi obat, sel HepG2 diinkubasi dengan primakuin 40 uM+ritonavir 10 uM; DMSO <0,1 %; atau rifampisin 20 uM selama 72 jam. Sel dipanen menggunakan tripsin-EDTA dan RNA total diekstraksi menggunakan reagensia isolasi tripure. Setelah jumlah RNA total dikuantifikasi menggunakan alat spektrofotometer, ekspresi mRNA CYP3A4 diukur dengan real-time reverse transcription polymerase chain reaction (RT-PCR). Hasil: Terjadi peningkatan ekspresi mRNA CYP3A4 (1,22 kali lipat terhadap kontrol) pada sel HepG2 yang diinkubasi dengan primakuin dan ritonavir secara bersamaan. Hal ini menunjukkan bahwa efek induksi oleh ritonavir lebih dominan daripada efek inhibisi oleh primakuin. Kesimpulan: Pemberian primakuin dan ritonavir secara bersamaan meningkatkan ekspresi mRNA CYP3A4 in vitro.

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Abstract

Background: Concomitant treatment with antimalaria and antiretroviral drug is a new challenge in the management of malaria and HIV co-infection. Primaquine is a substrate and also an inhibitor of CYP3A4, while ritonavir is a substrate, an inhibitor, and also an inducer for CYP3A4. The objective of this study is to measure the CYP3A4 mRNA expression in HepG2 cell culture induced by primaquine and ritonavir co-treatment. Methods: For the initial study HepG2 cells were treated with 30, 40, 50 uM of primaquine; 2, 10, 20 uM ritonavir; DMSO ≤0.1 % for negative control; or 20 uM rifampicin for positive control. While for the co-treatment study the cells were treated with 40 uM primaquine+10 uM ritonavir; DMSO ≤0.1 %; or 20 uM rifampicin for 72 hours. The cells were harvested using trypsin?EDTA and total RNA was extracted using the Tripure isolation reagent. After determining the quantity of RNA spectrophotometrically, CYP3A4 mRNA expression was quantified using real-time reverse transcription polymerase chain reaction (RT-PCR). Results: The expression of CYP3A4 mRNA was up-regulated (1.22 fold over control) in HepG2 cells co-treated with primaquine and ritonavir. These data suggest that the induction effect of ritonavir was more dominant than the inhibitory effect of primaquine. Conclusion: Concomitant administration of primaquine and ritonavir result in up-regulation of CYP3A4 mRNA expression in vitro. (Med J Indones 2012;21:3-7) Keywords: CYP450 induction, CYP3A4, drug interaction, primaquine, ritonavir