ABSTRACT

Background: Transient global brain ischemic injury leads to oxidative stress, complement activation, blood brain barrier dysfunction and leukocyte infiltration, resulting in endothelial injury and endothelial adherent junction disturbance. These events cause the reduction of endothelial cell markers such as CD31 and a decrease in lumen area. Vitamin D is an antioxidant and has vasoprotective properties, however its effect is not yet fully understood.

Aim: To evaluate the effect of vitamin D treatment towards the mRNA expression of CD31 and lumen area in the hippocampus of Wistar rats with transient global cerebral ischemia.

Methods: Male Wistar rats (age 2-3 months, body weight 150-300 grams) were divided into 4 groups (n=6): sham operation (SO), bilateral common carotid artery occlusion (BCCAO) without vitamin D treatment, BCCAO + 0.125 µg/kgBW/day vitamin D (VD1) and BCCAO + 0.5 µg/kgBW/day vitamin D (VD2). Transient global cerebral ischemia was induced via BCCAO for 20 minutes. Rats received daily intraperitoneal injections of Vitamin D (VD1 and VD2) or 0.2% ethanol (SO and BCCAO) for 10 days, after which termination and extraction of total RNA from the hippocampus was conducted. Reverse transcription-polymerase chain reaction (RT-PCR) was conducted to measure CD31 mRNA expression. Hematoxylin-eosin (HE) staining was performed to quantify lumen area. Statistical analysis included one-way ANOVA followed by LSD post-hoc multiple comparison analysis, with $p<0.05$ considered to be statistically significant.

Results: Vitamin D treatment with dosages 0.125µg/kgBW/day and 0.5µg/kgBW/day led to significantly higher mRNA expression of CD31 in the hippocampus of transient global cerebral ischemia rat models as compared to BCCAO group ($p=0.008$ and $p<0.001$ respectively). The higher vitamin D dosage also resulted in significantly higher CD31 mRNA expression compared to the lower dosage ($p=0.015$). Lumen area was significantly higher in both groups treated 0.125µg/kgBW/day and of 0.5µg/kgBW/day of vitamin D compared to BCCAO ($p=0.027$ and $p=0.023$ respectively).

Conclusion: Vitamin D treatment leads to higher mRNA expression of CD31 and lumen area in the hippocampus of rats with transient global cerebral ischemia.

Keywords: Vitamin D, Transient Global Ischemic Brain Injury, CD31, Lumen area, Endothelial Injury

INTISARI

Latar Belakang: Cedera iskemia serebral global transien menyebabkan stres oksidatif, aktivasi komplemen, disfungsi *blood brain barrier* dan infiltrasi leukosit, yang mengakibatkan cedera endotel dan gangguan pada *endothelial adherent junction*. Peristiwa ini menyebabkan penurunan CD31 dan area lumen. Vitamin D memiliki sifat antioksidatif dan vasoprotektif, namun efeknya belum sepenuhnya dipahami.

Tujuan: Mempelajari efek pemberian vitamin D terhadap ekspresi mRNA CD31 dan *lumen area* pada hipokampus tikus Wistar model iskemia serebral global transien.

Metode: Tikus Wistar jantan (usia 2-3 bulan, berat badan 150-300 gram) dibagi menjadi 4 kelompok (n=6): *sham operation* (SO), *bilateral common carotid artery occlusion* (BCCAO) tanpa pemberian vitamin D, BCCAO + 0.125 µg/kgBB/hari vitamin D (VD1) dan BCCAO + 0.5 µg/kgBB/hari vitamin D (VD2). Iskemia serebral global transien diinduksi melalui BCCAO yang dilakukan selama 20 menit. Lalu, tikus disuntik dengan vitamin D (VD1 dan VD2) atau etanol 0.2% (SO dan BCCAO) secara intraperitoneal setiap hari selama 10 hari, lalu dilakukan terminasi dan ekstraksi RNA total dari hipokampus. *Reverse transcription-polymerase chain reaction* (RT-PCR) dilakukan untuk mengukur ekspresi mRNA CD31 dan pewarnaan *hematoxylin-eosin* (HE) dilakukan untuk mengkuantifikasi *lumen area*. Analisis statistik menggunakan *one-way ANOVA* diikuti *LSD post-hoc multiple comparison analysis*. Nilai $p<0.05$ dianggap signifikan secara statistik

Hasil: Pemberian vitamin D dengan dosis 0.125µg/kgBB/hari dan 0.5µg/kgBB/hari menghasilkan ekspresi mRNA CD31 yang lebih tinggi secara signifikan dibandingkan kelompok BCCAO ($p=0.008$ dan $p<0.001$ secara berturutan) pada hipokampus model tikus iskemia serebral global transien. Dosis vitamin D yang lebih tinggi juga menghasilkan ekspresi mRNA CD31 yang lebih tinggi secara signifikan dibandingkan dosis yang lebih rendah ($p=0.015$). *Lumen area* lebih tinggi secara signifikan pada kedua kelompok yang diberikan vitamin D 0.125µg/kgBB/hari ($p=0.027$) dan 0.5µg/kgBB/hari ($p=0.023$) dibandingkan kelompok BCCAO.

Kesimpulan: Pemberian vitamin D dapat meningkatkan ekspresi mRNA CD31 dan area lumen pada hipokampus tikus model iskemia serebral global transien.

Kata Kunci: Vitamin D, Cedera Otak Iskemik Global Transien, CD31, *Lumen area*, Cedera Endotel