

RESEARCH ARTICLE**Evaluation of Nail-fold Capillary Structures (Diameter and Tortuosity) in Carbamazepine-Treated Patients****Manat Pongchaidecha¹, Rutchapoom Muangkaew²**¹ *Faculty of Pharmaceutics (Clinical Pharmacy), Silpakorn University, Nakhon Pathom, Thailand*² *Department of Pharmacy, Chiangmai Neurological Hospital, Chiangmai, Thailand***Abstract**

Carbamazepine is an anti-epileptic drug and used for treatment of several diseases such as epilepsy, neuropathic pain (neuralgia) and bipolar affective disorders. Several reports have shown vascular adverse drug effects of anti-epileptic drugs, including carbamazepine. Very few reports have been involved with micro-vascular (capillary) structural changes, which may be related to potential adverse drug reactions in patients with long-term therapy. The aim of this observational study was to evaluate nail-fold capillary structural changes in carbamazepine-treated patients in relation to serum carbamazepine concentrations and its apparent adverse drugs reactions. Patients from Chiangmai Neurological Hospital (N=60) were recruited and enrolled according to specific inclusion and exclusion criteria. All patients were treated with monotherapy of carbamazepine. Calibrated nail-microscope Dino-Lite[®] was employed to measure nail-fold capillary structural changes (i.e. arterial and venous diameters and tortuous index) at the proximal nail-fold. The measurement was performed in 1st and 2nd month after starting the treatment. Serum carbamazepine concentrations were also measured by fluorescense polarization immunoassay (Abbot Axsym System[®]). Results showed that after one and two months of treatment the arterial loop diameters of the capillary were significantly decreased (9.05 ± 1.07 [baseline] vs 8.88 ± 1.10 and $8.78 \pm 1.15 \mu\text{m}$, $p < 0.001$, respectively). Tortuous index was also found to be increased from baseline after treatment for one and two months; $1.64 \pm 0.32 \mu\text{m}$ vs $1.86 \pm 0.38 \mu\text{m}$ vs $1.94 \pm 0.36 \mu\text{m}$, $p < 0.001$, respectively. Correlation between % arterial loop diameter changes of the capillary and serum carbamazepine concentrations existed ($r = -0.406$, $p < 0.05$), but not the turtuos index. Although frequency of adverse drug reactions was greater in patients whose serum carbamazepine concentrations $\geq 8 \mu\text{g/mL}$, there was no established association of both % arterial loop diameter changes of capillary and tortuous index with apparent short-term adverse drug reactions.

Keywords: nail-fold capillary, carbamazepine, adverse drug reaction

การประเมินโครงสร้าง (เส้นผ่านศูนย์กลางและความคด) ของหลอดเลือดฝอยที่ฐานเล็บในผู้ป่วยที่ได้รับยาคาร์บามาซีป็น

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บทคัดย่อ

คาร์บามาซีป็นเป็นยาต้านโรคลมชักที่ใช้ในการรักษาโรคหลายประเภทเช่น โรคลมชัก โรคปวดประสาท และโรคผิดปกติทางจิตแบบสองขั้ว รายงานหลายฉบับได้แสดงผลไม่พึงประสงค์ต่อระบบหลอดเลือดของยาต้านโรคลมชักซึ่งรวมทั้งยาคาร์บามาซีป็น มีรายงานน้อยมากเกี่ยวกับการเปลี่ยนแปลงโครงสร้างหลอดเลือดฝอย ซึ่งอาจจะเกี่ยวข้องกับอาการอื่นไม่พึงประสงค์ที่เป็นไปได้สูงในผู้ป่วยที่ได้รับการรักษาระยะยาว วัตถุประสงค์ของการศึกษาเชิงสังเกตนี้เพื่อประเมินการเปลี่ยนแปลงโครงสร้างหลอดเลือดฝอยที่ฐานเล็บที่สัมพันธ์กับความเข้มข้นของยาคาร์บามาซีป็นในซีรัมและอาการอื่นไม่พึงประสงค์ของยาที่ปรากฏในผู้ป่วยที่ได้รับยาคาร์บามาซีป็น ในการศึกษาครั้งนี้มีผู้ป่วยจำนวน 60 รายจากโรงพยาบาลประสาทเชียงใหม่ได้รับการเกณฑ์และคัดเลือกตามเกณฑ์การคัดเลือกเข้าและออกที่เจาะจง ผู้ป่วยทุกรายได้รับการรักษาด้วยยาคาร์บามาซีป็นแบบเดี่ยว การวัดการเปลี่ยนแปลงโครงสร้างหลอดเลือดฝอยที่ฐานเล็บใช้กล้องจุลทรรศน์ส่องเล็บที่ตรวจสอบแล้ว (Dino-Lite[®]) และดำเนินการวัดที่เดือนที่ 1 และ 2 หลังจากเริ่มการรักษา และความเข้มข้นของยาคาร์บามาซีป็นในซีรัมได้รับการตรวจโดยวิธี fluorescence polarization immunoassay (Abbot Axsym System[®]) จากการศึกษาพบว่าหลังจาก 1 และ 2 เดือนของการรักษาเส้นผ่านศูนย์กลางของ วงหลอดเลือดแดงฝอยมีค่าลดลงอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับเส้นผ่านศูนย์กลางก่อนการรักษา (9.05 ± 1.07 ไมโครเมตร [ค่าก่อนได้รับยา] เทียบกับ 8.88 ± 1.10 และ 8.78 ± 1.15 ไมโครเมตร ตามลำดับ, $p < 0.001$) ค่าดัชนีความคดมีค่าเพิ่มขึ้นทั้งเดือนที่ 1 และ 2 เมื่อเทียบกับก่อนได้รับยา (1.64 ± 0.32 [ก่อน] เทียบกับ 1.86 ± 0.38 [เดือนที่ 1] กับ 1.94 ± 0.36 [เดือนที่ 2] ไมโครเมตร, $p < 0.001$) เกิดความสัมพันธ์ระหว่างร้อยละการเปลี่ยนแปลงของเส้นผ่านศูนย์กลางวงหลอดเลือดแดงฝอยกับความเข้มข้นของยาคาร์บามาซีป็นในซีรัมโดยมีค่าสัมประสิทธิ์สหสัมพันธ์ $r = -0.406$ ($p < 0.05$) ไม่มีความสัมพันธ์ระหว่างดัชนีความคดกับความเข้มข้นของยาคาร์บามาซีป็นในซีรัม แม้ว่าความถี่ของการเกิดอาการไม่พึงประสงค์ของยาจะพบมากในผู้ป่วยที่ความเข้มข้นของยาคาร์บามาซีป็นในซีรัมมากกว่าหรือเท่ากับ 8 ไมโครกรัม/มิลลิลิตร แต่ไม่พบความสัมพันธ์ระหว่างร้อยละการเปลี่ยนแปลงของเส้นผ่านศูนย์กลางวงหลอดเลือดแดงฝอยและดัชนีความคดกับอาการไม่พึงประสงค์ที่ปรากฏ

คำสำคัญ: หลอดเลือดฝอยที่ฐานเล็บ, คาร์บามาซีป็น, อาการไม่พึงประสงค์

Introduction

Carbamazepine is an antiepileptic drug and used for the treatment of several diseases such as epilepsy, neuropathic pain (neuralgia) and bipolar affective disorders. Reports of adverse drug reactions can range from concentration-related CNS effects and hypersensitivity reactions.¹⁻³ Several reports have also shown vascular adverse drug effects of anti-epileptic drugs, including carbamazepine.^{4,5} Very few reports have been involved with micro-vascular (capillary) structural changes, which may be related to potential cardiovascular adverse drug reactions in patients with long-term therapy with carbamazepine. Gerstner et al. (2006) used capillary microscope and reported changes of nail-fold capillary structures and hemorrheology in pediatric patients with epilepsy who were treated with either carbamazepine or valproic acid.⁶ They found significant differences in capillary density, tortuous index of the capillaries, capillary diameter and flow rate of erythrocytes for both antiepileptic drugs. Additionally, there were changes in plasma-viscosity and the aggregation of erythrocytes.

The aim of this observational study was to investigate nail-fold capillary structural changes, i.e. arterial and venous loop diameters and tortuous index, in relation to serum carbamazepine concentrations and apparent adverse drug reactions.

Methods

Subjects

This prospective observational study was carried in patients, who were treated by monotherapy of carbamazepine, from Chiangmai Neurological Hospital (N=60). The patients were recruited and enrolled according to inclusion and exclusion criteria. The inclusion criteria were 1) male or female age between 18-60 years 2) monotherapy of carbamazepine 3) patients giving their consents. The exclusion criteria were 1) patients identified by doctors to be at risk if participating in the study 2) patients not giving their consents 3) patients whose treatment regimen was added with other antiepileptics 4) co-administration with other drugs such as erythromycin, clarithromycin, isoniazid, fluoxetine, fluvoxamine, verapamil, diltiazem, lamotrigine, ketoconazole and fluconazole 5) co-administration with drugs which affect vascular vessels, such as calcium channel blockers, nitrates, alpha-1 blocker, pseudoephedrine, phenylephrine 6) consumption of herbal or dietary supplement, such as pomegranate, ginkgo biloba, grape fruit, St.John's wort, evening primrose 7) patients who were diagnosed with congestive heart failure, COPD, Turner's syndrome, schizophrenia, systemic lupus erythematosus (SLE), Familial Mediterranean fever (FMF), scleroderma, dermatomyositis, polycythaemia, which can influence nail-fold capillary changes 9) patients having scar or wound or rough skin of the ring finger.

The study protocol was approved by the ethic committee of Chiangmai Neurological Hospital.

Instrument and data collection

The measurements were performed at baseline, the 1st and 2nd month after starting the treatment with monotherapy of carbamazepine. Compliance of medication for all enrolled patients was also tested for each visit.

Nailfold capillary measurement: Calibrated nail microscope Dino-Lite® (Chosen Technology, Thailand) was employed to measure the change in arterial and venous loop diameters of capillary at the proximal nail-fold. Patients were asked to take a rest for 20 minutes at room temperature of 25°C before the measurement. Microscopic photographs of 5 measurements from each patient were recorded for each visiting. Computer-aided software (DinoCapture) accompanied with the microscope was used to measure the arterial and venous loop diameters, together with estimation of tortuous index, the ratio between a widest part over a narrowest part of the capillary loop

Serum carbamazepine concentrations: Blood samples were collected 12 hrs from last dose at 1 and 2 months after treatment and stored at -20°C before analysis. Abbott AxSYM System (Automated Immunoassay analyzer) based on fluorescence polarization immunoassay was used for analysis of serum carbamazepine concentrations. The assay accuracy is 99.8% with sensitivity of 0.50 µg/mL to 80 µg/mL and specificity ≥90 %.

Adverse drug reactions: Detection of adverse drug reactions was carried out by Naranjo's algorithm

Statistical data analysis

Data were presented as mean±SD, otherwise stated. Continuous data with normal distribution were tested using parametric statistical method (i.e. repeated ANOVA), while categorical data or non-normal distributed data was tested with Chi-square test. Correlation test was performed by parametric Pearson's correlation test. SPSS 11.5 was used for statistical analysis.

Results

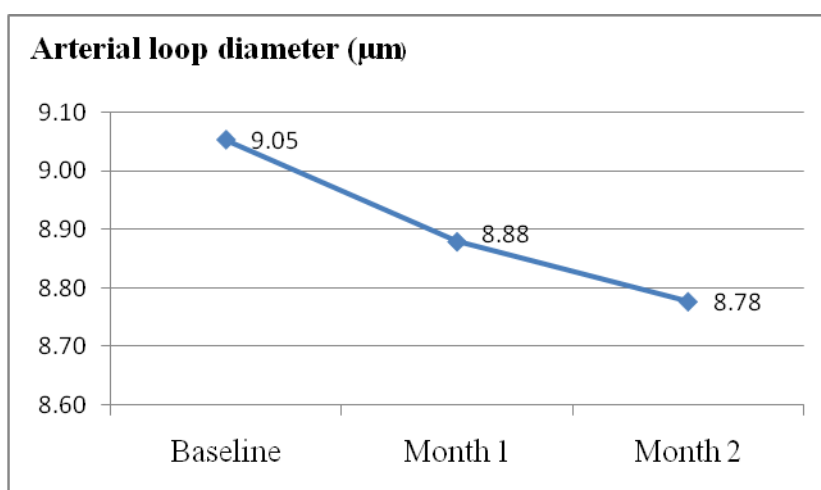
Table 1 shows characteristics of patients with monotherapy of carbamazepine. The majority of patients were female and all patients were age between 22-59 years. Their compliance was accounted for over 90% with average daily dose of 342.50±139.56 mg/day. The patients were diagnosed to have epilepsy or hemi facial spasm or trigeminal neuralgia (or neuralgia), whose average serum carbamazepine concentration was 4.88±2.43 (µg/mL).

Arterial, venous loop diameters and tortuous index

Significant decrease in arterial loop diameters was found after 2 months of treatment compared with those of baseline (9.05±1.07 µm [baseline] vs 8.88±1.10 and 8.78±1.15 µm, $p=0.001$) (Figure 1). In contrast to the arterial loop diameters, the venous loop diameters were not significantly changed after the same period of treatment (9.89.00±1.27 µm [baseline] vs 9.78±1.31 µm and 9.72±1.23µm, $p=0.16$) (Figure 2)

Table 1. Characteristics of patients with monotherapy of carbamazepine

Characteristics	N=60
Gender Male	22 (36.67%)
Female	38 (63.33%)
Age (yr) (range)	50.02±8.62 (22-59)
Weight (kg)	56.22±9.92
Height (cm)	160.57±8.71
BMI (kg/m ²)	21.68±2.49
Disease Epilepsy	7 (11.67%)
Hemi facial spasm	13 (21.67%)
Trigeminal neuralgia, neuralgia	40 (66.66%)
Compliance (%)	99.21±1.40
Dose/day (mg/day)	342.50±139.56
Dose/kg/day (mg/kg/day)	6.29±3.01
Serum carbamazepine concentrations (µg/ml)	4.88±2.43
Brand Tegretal CR [®] 200 mg	33 (55.00%)
Carmapine [®] 200 mg	27 (45.00%)

**Figure 1.** Change of arterial loop diameters after two months of the monotherapy of carbamazepine. Values are mean, N=60.

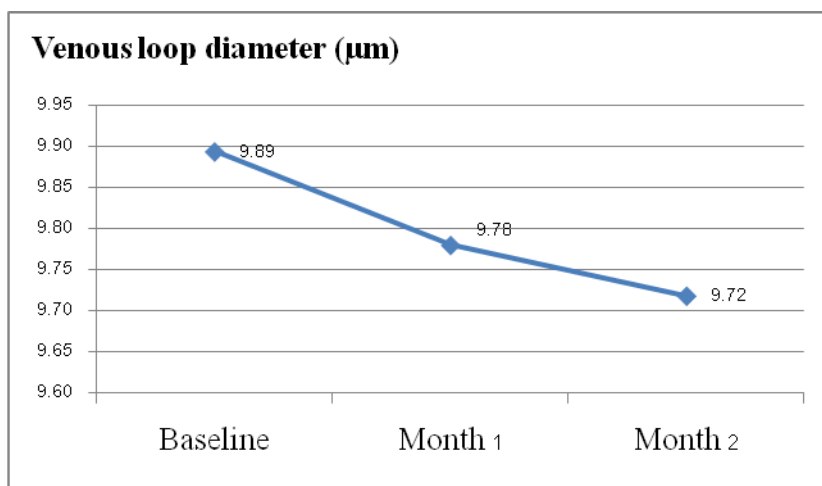


Figure 2. Change of venous loop diameters after two months of the monotherapy of carbamazepine. Values are mean, N=60.

Tortuos index, as the ratio between the widest part over the narrowest part of the capillary loop, was found to be 1.86 ± 0.38 and 1.94 ± 0.36 at the first and second month after treatment, respectively, and these values were significantly greater than that of baseline (1.64 ± 0.32) ($p < 0.001$) (Figure 3)

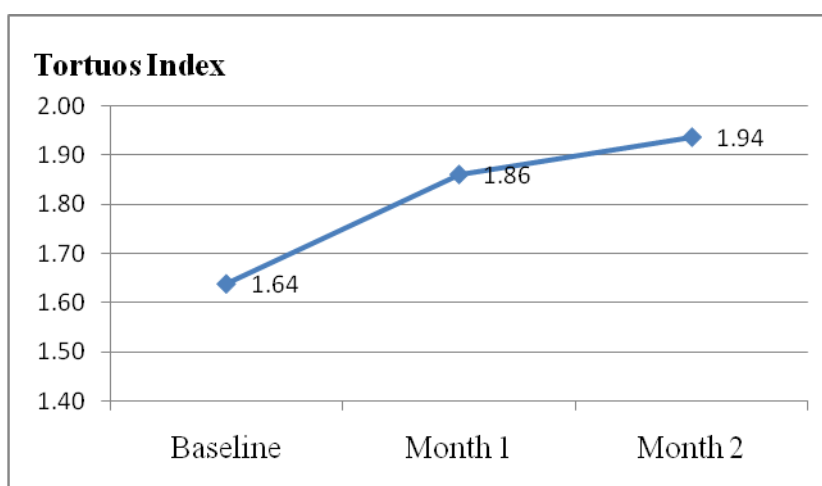


Figure 3. Tortuos index of the capillary loop after two months of the monotherapy of carbamazepine. Values are mean, N =60.

Relationship between serum carbamazepine concentrations and nailfold capillary structural change

Percentages of arterial loop diameter changes of the capillary, but not tortuous index, and serum carbamazepine concentrations were significantly correlated ($r=-0.406$, $p<0.05$)

Adverse drug reactions and their relationship with the nail-fold capillary changes

Possible adverse drug reactions, as detected by Naranjo's algorithm, were obtained from all 60 patients whose adverse drug reactions were recorded two times. Therefore, there were the total visits of 120. When using 8 $\mu\text{g/mL}$ as a cutoff concentration, the concentrations at which frequent adverse drug reactions usually found, 11 visits belonged to those with the concentrations ≥ 8 $\mu\text{g/mL}$ (10.66 ± 3.12 $\mu\text{g/mL}$) whereas 109 visits belonged to those with the concentrations < 8 $\mu\text{g/mL}$ (4.28 ± 1.88 $\mu\text{g/mL}$). Table 2 shows number of adverse drug reactions in relation to the cutoff concentrations. It is evident that about or greater than twice adverse drug reactions were found when the concentrations ≥ 8 $\mu\text{g/mL}$. There was no clear evidence of any correlation between these apparent adverse drug reactions with detected nail-fold capillary changes

Table 2. Adverse drug reactions in relation to serum carbamazepine concentrations at the cutoff concentrations of 8 $\mu\text{g/mL}$

Adverse Drug Reactions	< 8 $\mu\text{g/mL}$ (109 visits)	≥ 8 $\mu\text{g/mL}$ (11 visits)
1. Somnolence	53* (48.62%)	10 (90.91%)
2. Dizziness	48 (44.04%)	8 (72.73%)
3. Nausea	15 (13.76%)	5 (45.45%)
4. Impair cognition	9 (8.26%)	3 (27.27%)
5. Lost of appetite	3 (2.75%)	3 (27.27%)
6. Ataxia	0 (0%)	3 (27.27%)

*unit as visit

Discussion

This present study is the first of its kind to investigate changes in nail-fold capillary structure in adult patients with monotherapy of carbamazepine. The decrease in diameter of the arterial loop of nail-fold capillary was detected together with the increased change in tortuous index. The mechanism involved in the decreased diameter of the arterial loop can be explained by inhibition of sodium voltage gated channel which is found both in the neuron system and on cell membrane of endothelial cells. The endothelial cells are present at the arteries to the arterioles⁷, as well as they also exist at the vein.⁸ Inhibition of the sodium

voltage gated channel by carbamazepine at the capillary loop, especially the arterial loop, causes the vessel to dilate, and thus increase the diameter, and subsequently a drop in blood pressure for a short-term manner. The event is also enhanced by inhibition of the central nervous system via sympathetic pathway by the drug. However, auto-regulatory blood pressure via the renin-angiotensin system later plays a role in compensating the drop in blood pressure by stimulating the contraction of arterioles and thus reducing the diameter of the arterial loop as shown in this present study.⁹ On the contrary, for a long-term inhibition of sodium voltage gated channel remodeling of neural cells occurs and leads to increase more tetrodotoxin resistant sodium voltage gated channels. As a result, hyperexcitable neurons exists¹⁰ and promotes more gradual transmission of sympathetic pathway in the long run. This remodeling explains discrepancy found between this present study and that of Gerstner et al. (2006)⁶ showing that capillary diameters, especially the venous loop, were increased as the subjects were treated with carbamazepine for a longer period ranging from 9 months to 4.5 years.

For the tortuous index, results from this present study are in accordance with those of Gerstner et al.⁶ that the index increases together with elevated capillary density. Mechanisms that can possibly explain these changes may be involved with influence of catecholamine change in the sympathetic pathway. Catecholamine affects endothelial capillary via cAMP associated receptors through catecholamine-sensitive cyclase, which are found to be abundant at the endothelial capillary.¹¹ As a result, catecholamine then leads not only to capillary structural changes, but also their chemical composition changes as well.¹² Currently, catecholamine, but not its analogues, is found to stimulate endothelial cell via IFN-gamma, which activates dibutyryl-cAMP.¹³ Other possible mechanisms may be involved with elevated homocysteine by anti-epileptic drugs (AEDs).¹⁴⁻¹⁶ Homocysteine and homocysteine-related products has been shown to act as oxidants that are potential inducers of endothelial injury.^{17,18} Therefore, vascular damage, including tortuosity changes found with the nail-fold capillary, can possibly be observed after treatment by AEDs. Further research is needed to confirm these possible mechanisms.

This present study also show that at least percentage changes in arterial loop diameter are correlated with serum carbamazepine concentrations in a concentration-dependent manner. However, no established correlation between percentage changes in arterial loop diameter and concentration or dose-dependent apparent CNS adverse drug reactions. Further investigations concerning using and detecting appropriate outcomes of vascular adverse drug reactions in relation to nail-fold capillary structural changes are of interest.

Some limitations of this present study exist as follows: 1) This study was observational in nature, thus variation of doses could not be avoided, and the sample size was relatively small, other study designs with relative large sample size may be required to confirm these findings, 2) The disease effects on the findings could not clarify in this study due to not enough sample size for subgroup analysis, 3) Other outcomes such as flow rate and aggregation of erythrocytes, plasma-viscosity could not be measured due to limited capability of the nail microscope used. Higher capability of the instrument may be employed for further investigation, 4) Using only one operator without blinding to subject's visit is a

potential bias of this study. However, one can find practical use of this finding, particularly in the field of pharmacokinetics and therapeutic drug monitoring (TDM). For the pharmacokinetics field, the changes in arterial loop diameters can be used as a covariate for population pharmacokinetics in order to estimate more accurate population pharmacokinetic parameters such as a clearance of carbamazepine. For the TDM field, practitioners can apply the established correlation between the percentage changes in arterial loop diameters and serum carbamazepine concentrations to estimate the steady-state concentrations of the drug. At the moment, ongoing research is being carried on to investigate a possibility in the population pharmacokinetics field.

In conclusion, this study demonstrates that there are changes in the nail-fold capillary structures, i.e. the arterial loop diameter and tortuous index. The possible mechanisms are discussed and are warranted for further investigations. Although there are some limitations of the study, the findings can be applied for a practical use such as for TDM or for further research in population pharmacokinetic study.

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