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Assessment of atherosclerosis risk due to the homocysteine–asymmetric dimethylarginine–nitric oxide cascade in children taking antiepileptic drugs

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ABSTRACT

Purpose: The aim of this study was to assess the atherogenicity risk of antiepileptics in children by investigating the cascade, "hyperhomocysteinemia (HHcy) → asymmetric dimethylarginine (ADMA) increase → nitric oxide (NO) decrease", which is thought to contribute to the developmental process of atherosclerosis.
Methods: The participants included 53 epilepsy patients who received either valproic acid (VPA, $n = 26$) or oxcarbazepine (OXC, $n = 27$). Twenty-four healthy sex- and age-matched children served as controls. Fasting plasma total homocysteine (tHcy), ADMA and NO levels were measured.
Results: The differences in Hcy, ADMA, NO, vitamin B_{12} and folate levels between VPA, OXC and control groups were all insignificant ($p > 0.05$ for all). In the patient group (VPA and OXC groups), 22.6% of the children (12/53) had tHcy levels above the normal cutoff (13.1 μmol/l) for children and 17% of the children (9/53) had tHcy levels of greater than 15 μmol/l which is accepted as the critical value for an increased atherosclerosis risk ($p < 0.05$ for both). The difference in rate of HHcy between VPA and OXC groups was statistically insignificant ($p > 0.05$, for both cut off levels of HHCy). There was a positive correlation of tHcy levels and antiepileptic drug treatment duration in the patient group ($r = +0.276$, $p < 0.05$).
Conclusion: HHcy may develop in patients using OXC. Contrary to some previous publications, our data do not suggest that OXC is safer than VPA in terms of HHcy risk. Further prospective, large scale and longer term studies investigating all suggested pathways responsible for development of atherosclerosis due to HHcy should be conducted to define the exact mechanism responsible for AEDs related atherosclerosis.

1. Introduction

During the last few years, it has been shown that risk factors for atherosclerosis appear during childhood and adolescence and are already at that time associated with atherosclerotic changes in vessel walls.[1] As is well known, endothelial dysfunction is considered a precursor phenomenon. The concern is that acceleration of this atherosclerotic process due to a variety of unfavourable factors could lead to the emergence of cardiovascular diseases earlier than would otherwise be expected. An increased risk of fatal cardiovascular disease has been reported in patients with epilepsy[2]. However, any relationship to antiepileptic drugs (AEDs) remains unclear. Over the past two decades, high tHcy levels, an independent risk factor for atherosclerosis, ADMA, lipoprotein(a), and impaired lipid profiles, have been documented in several studies with children on AEDs.[3–9]

The mechanisms underlying the cardiovascular pathophysiology of HHcy are not yet fully understood. Numerous studies in animals and humans have demonstrated that HHcy leads to endothelial dysfunction due to decreased bioavailability of NO. Nitric oxide is a powerful vasodilator and anti-atherogenic agent that is produced in the endothelium from the amino acid L-arginine via the action of endothelial nitric oxide synthase (eNOS). One of the likely mechanisms of the decreased NO bioavailability in

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HHcy is the increased concentration of ADMA, an endogenous inhibitor of eNOS, which, in turn, is followed by reduced synthesis of NO.[10]

Asymmetric dimethylarginine is also considered to be a potential risk factor for the development of atherosclerosis.[11] It has been suggested that elevated levels of ADMA are associated with the severity of cardiovascular events.[12,13] Asymmetric dimethylarginine is produced from methylated proteins derived from homocysteine (Hcy) metabolism. It competes with L-arginine and causes a reduction in NO formation in the vascular wall. Plasma tHcy has been shown to increase endothelial cell generation of ADMA by inhibiting the activity of the enzyme dimethylarginine dimethylaminohydrolase (DDAH), which is responsible for ADMA metabolism.[14,15]

Valproic acid (VPA) and oxcarbazepine (OXC) are widely used AEDs in childhood epilepsy. In this study, we assessed whether children on these common antiepileptics had an increased risk of atherosclerotic diseases by investigating NO levels for the first time, in addition to tHcy and ADMA levels, and assessing possible associations among them.

2. Patients and methods

The study group consisted of 53 patients with epilepsy (26 girls, 27 boys) treated in the Department of Paediatric Neurology at Gazi University Faculty of Medicine (Table 1). All patients had received antiepileptic monotherapy (VPA, $n = 26$; OXC, $n = 27$) for at least 1 year. The presence of epilepsy due to cerebrovascular disease as well as mass lesions, history of cardiac or peripheral vascular disease, and renal, hepatic, or thyroid disease were exclusion criteria. Subjects using any other medication or vitamins were also excluded. All patients were judged to be in good health and free of a history of bleeding or thrombosis. A total of 24 healthy children served as the control group. This study was approved by the local ethics committee. Informed consent was obtained from parents.

Fasting venous blood samples were obtained from patients and controls to measure tHcy, NO, and ADMA levels. Serum and plasma were separated as soon as possible by centrifugation (5 min, $3000 \times g$). The samples were stored at $-80\,^{\circ}C$ until assayed. Plasma tHcy concentrations and ADMA levels were measured by high-performance liquid chromatography, and serum nitric oxide levels by an incubation assay method. Plasma tHcy levels $\geq$13.1 μmol/l were deemed to indicate hyperhomocysteinemia.[16]

Table 1
Characteristics of VPA, OXC, and control groups.

	Control group ($n = 24$)	VPA group ($n = 26$)	OXC group ($n = 27$)
Gender M/F (n)	13/11	16/10	11/16
Mean age (±SD, years)	12.1 ± 3.3	10.9 ± 3.1	10.8 ± 3.2
Mean duration of treatment (±SD, years)		3.4 ± 1.7	2.7 ± 1.4
Mean dose (±SD, mg/kg/d)		22.7 ± 7.0	22.5 ± 6.7

2.1. Statistical analyses

All statistical calculations were performed using the SPSS 11.5 software. Descriptive statistics were computed as means ± standard deviations and median (25–75th percentiles). Differences between groups were assessed using one-way analysis of variance, Pearson's chi-squared test, the Mann–Whitney U-test, and the Kruskal–Wallis test. The linear relationships between variables were evaluated using Spearman's rho coefficient. Statistical significance was set at $p < 0.05$.

3. Results

In total, 53 idiopathic epilepsy patients and 24 controls participated. Gender and mean age distributions did not differ significantly between the groups ($p > 0.05$ for both; Table 1). None of the differences in tHcy, ADMA, NO, vitamin B_{12}, and folate levels among the VPA, OXC, and control groups was significant (all $p > 0.05$; Table 2). No significant relationship was found among tHcy, NO, and ADMA levels ($p > 0.05$ for all). In the patient group, 22.6% (12/53) of the children had tHcy levels above the normal cutoff level (13.1 μmol/l), and 17% (9/53) of the children had tHcy levels greater than 15 μmol/l, the critical value for increased atherosclerosis risk. In contrast, no subject in the control group had tHcy levels above these values (Table 3). The difference in HHcy between the VPA and OXC groups was not statistically significant ($p > 0.05$ for both cutoff levels of HHcy). In the patient group, a statistically significant correlation was detected between the duration of treatment and tHcy level ($r = +0.276$, $p < 0.05$), whereas no such correlation was detected for the NO and ADMA levels ($p > 0.05$ for both). No statistically significant difference was found in ADMA and NO levels between patients with tHcy above

Table 2
Comparison of tHcy, ADMA, and NO levels.

	Control group[a] ($n = 24$)	VPA group[a] ($n = 26$)	OXC group[a] ($n = 27$)	p
tHcy (μmol/l)	9.2 (6.6–11.3)	9.2 (6.5–12.8)	9.3 (8.2–13.5)	>0.05[b]
ADMA (μmol/l)	2.1 (2.0–2.3)	2.1 (2.0–2.3)	2.2 (1.8–2.3)	>0.05[b]
NO (μmol/l)	1.8 (1.4–2.3)	1.8 (1.3–2.7)	2.3 (1.4–3.1)	>0.05[b]
Folic acid (ng/ml)	8.87 (7.27–10.66)	7.78 (6.08–9.40)	8.43 (6.45–10.67)	>0.05[b]
Vitamin B_{12} (pg/ml)	499.1 (385.6–678.3)	439.8 (319.7–544.3)	489.9 (378.5–640.9)	>0.05[b]

[a] Values are expressed as median and median (25–75th percentiles).
[b] p-Value, compared with control, VPA, and OXC groups.

Table 3
Comparison of the patients and control group for HHcy according to cutoff values.

	Control group ($n = 24$)	Patient group ($n = 53$)		p
		VPA group ($n = 26$)	OXC group ($n = 27$)	
tHcy > 13.1 (μmol/l), n (%)	–	6 (23.1)	6 (22.2)	0.014[a]
tHcy > 15.0 (μmol/l), n (%)	–	5 (19.2)	4 (14.8)	0.049[a]

[a] p-Value, compared between control and patient groups.