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Brain Research

Research Report

Severe hypertriglyceridemia does not protect from ischemic brain injury in gene-modified hypertriglyceridemic mice

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ARTICLE INFO

Article history:
Received 13 January 2016
Received in revised form
2 March 2016
Accepted 3 March 2016
Available online 10 March 2016

Keywords:
Ischemic stroke
Extreme hypertriglyceridemia
ApoCIII transgene
GPIHBP1 knockout, transient middle cerebral artery occlusion

ABSTRACT

Hypertriglyceridemia (HTG) is a weak risk factor in primary ischemic stroke prevention. However, clinical studies have found a counterintuitive association between a good prognosis after ischemic stroke and HTG. This "HTG paradox" requires confirmation and further explanation. The aim of this study was to experimentally assess this paradox relationship using the gene-modified mice model of extreme HTG. We first used the human Apolipoprotein CIII transgenic (Tg-ApoCIII) mice and non-transgenic (Non-Tg) littermates to examine the effect of HTG on stroke. To our surprise, infarct size, neurological deficits, brain edema, BBB permeability, neuron density and lipid peroxidation were the same in Tg-ApoCIII mice and Non-Tg mice after temporary middle cerebral artery occlusion (tMCAO). In the late phase (21 days after surgery), no differences were found in brain atrophy, neurological dysfunctions, weight and mortality between the two groups. To confirm the results in Tg-ApoCIII mice, Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1(GPIHBP1) knockout mice, another severe HTG mouse model, were used and yielded similar results. Our study demonstrates for the first time that extreme HTG does not affect ischemic brain injuries in the tMCAO mouse model, indicating that the association between HTG and good outcomes after ischemic stroke probably represents residual unmeasured confounding. Further clinical and prospective population-based studies are needed to explore variables that contribute to the paradox.

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http://dx.doi.org/10.1016/j.brainres.2016.03.005
0006-8993/

1. Introduction

Some risk factors for cardiovascular disease, include ischemic stroke, are also found to relate to outcomes, but in an opposite way. Smoking and obesity are reportedly associated with lower death rates or good functional outcomes, instead of higher death rates or bad functional outcomes (Akin and Nienaber, 2015; Ali et al., 2015; Brzecka and Ejma, 2015; Zhao et al., 2014). This phenomenon is termed "reverse epidemiology" or "risk factor paradox". This paradox phenomenon requires confirmation and further explanations. One of the explanations of this phenomenon is that unmeasured confounding factors may be contribute to this paradox. As a result, animal models without confounding factors are the best choice to verify and explain this paradox phenomenon.

As one of the main subtypes of hyperlipidemia, HTG is commonly observed in ischemic stroke patients, especially in China (Rother et al., 2008; Sacco et al., 2008; Zhao et al., 2005). However, the relationship between HTG and ischemic stroke has long been controversial. Epidemiological studies have been conflicting, partially because some have used fasting and others used nonfasting levels. Fasting triglyceride levels were not associated with ischemic stroke in the ARIC study (Shahar et al., 2003). Triglycerides did not predict the risk of ischemic stroke among healthy men enrolled in the Physicians' Health Study (Bowman et al., 2003). Similarly, in the Oslo study of healthy men, triglycerides were not related to the risk of stroke (Haheim et al., 1993). In the Emerging Risk Factors Collaboration meta-analysis, triglyceride levels were not associated with either ischemic or hemorrhagic stroke risk, and determination of fasting status did not appear to change the lack of association (Emerging Risk Factors et al., 2009). In contrast to the findings of the above studies, other studies have revealed a positive association between HTG and ischemic stroke. A meta-analysis of prospective studies conducted in the Asia-Pacific region found a 50% increased risk of ischemic stroke among those in the highest quintile of fasting triglycerides compared with those in the lowest quintile (Patel et al., 2004). Another meta-analysis of 64 randomized clinical trials that tested lipid-modifying drugs found an adjusted RR of stroke of 1.05 (95% CI, 1.03–1.07) for each 10-mg/dL increase in baseline triglycerides, although fasting status is not specified (Labreuche et al., 2010, 2009). The Copenhagen City Heart Study, a prospective, population based cohort study composed of approximately 14,000 persons, found that elevated nonfasting triglyceride levels increased the risk of ischemic stroke in both men and women (Freiberg et al., 2008). Similarly, the WHS found that in models adjusted for total and HDL cholesterol and measures of insulin resistance, nonfasting triglycerides, but not fasting triglycerides, were associated with cardiovascular events, including ischemic stroke (Bansal et al., 2007). Our animal model study also demonstrated that HTG accelerates atherosclerosis, which may further increase the risk of ischemic stroke (Zhang et al., 2008). Our result combined with the epidemiological studies indicate that HTG may be a weak risk factor for ischemic stroke and may be a post-prandial phenomenon.

The "risk factor paradox" is also observed in hypertriglyceridemic patients. Clinical and experimental outcome speculations indicate that HTG is associated with worse outcomes compared with normal TG patients. Nevertheless, previous studies have described the phenomenon of an "HTG paradox", reporting a protective effect of HTG with regard to functional outcome and mortality in ischemic stroke patients. Weir et al. (2003) first showed that higher triglyceride levels upon admission independently predict good outcomes after acute ischemic stroke. Moreover, on-admission HTG has been found to be independently associated with milder clinical symptoms at presentation (Dziedzic et al., 2004), lower on-admission infarct volumes (Pikija et al., 2006, 2009), and a lower all-cause and cardiovascular mortality over a 5-year period (Ryu et al., 2010). Regarding physical disability, higher triglyceride levels were independently associated with better outcomes 3 months after stroke in one study (Li et al., 2008) and less severe disability and reduced disability progression in another study over a 2.5-year follow-up period (Pikija et al., 2012). However, some studies contradict this relationship. One study implied that both hypertriglyceridemia and hypotriglyceridemia are risk factors for poor outcomes after ischemic stroke (Choi et al., 2012). Two other studies of small patient cohorts indicated that triglyceride levels do not affect disability 90 days after ischemic stroke (Cuadrado-Godia et al., 2009; Uyttenboogaart et al., 2008). Although most studies have proposed the HTG paradox phenomena, unanimous conclusions cannot be drawn from these clinical studies due to relative small number of patients, too many confounding factors and observation indexes that differed by study. Furthermore, clinical studies cannot explain the mechanisms underlying the HTG paradox. Consequently, the primary objective of the present study was to investigate the impact of HTG on brain I/R injury using the mouse models of inherited extreme hypertriglyceridemia.

Apolipoprotein CIII is a 79-amino acid glycoprotein and synthesized mainly by the liver. It is also synthesized by the small intestine, but to a lesser extent (Bobik, 2008). Apolipoprotein CIII can causes primary HTG by interacting with lipoprotein lipase (LPL) which hydrolyze triglyceride and interfering with the hepatic uptake of lipoproteins. Although HTG is induced by composite factors, including genetic and environmental aspects, clinical studies have revealed that elevated apolipoprotein CIII is the main feature of HTG patients. Furthermore, the plasma apolipoprotein CIII levels are strongly correlated with the fasting and post-prandial plasma triglyceride levels (Cohn et al., 2004a, 2004b; Zheng et al., 2010). In addition, transgenic apolipoprotein CIII mice (tg-ApoCIII mice) presenting extreme high triglyceride levels exhibit a normal

Table 1 – Physiological data for the Non-Tg and Tg-ApoCIII mice.

	Non-Tg	Tg-ApoCIII
Body weight (g)	25.3±0.5	24.5±0.4
Blood glucose (mg/dL)	168.1±10.2	172.6±9.7
Triglyceride level (mg/dL)	82.6±10.4	3142.6±270.5**
Total cholesterol (mg/dL)	80.5±4.7	459.1±30.5**

N=64 for Non-Tg and Tg-ApoCIII mice each. Data are expressed as the means±S.D.
** $P<0.01$ vs Non-Tg group.