Thrombosis Research (2007) **120**, 117–126

ELSEVIER

THROMBOSIS RESEARCH

intl.elsevierhealth.com/journals/thre

REGULAR ARTICLE

Pro-thrombotic and pro-oxidant effects of diet-induced hyperhomocysteinemia

Derrick L. Sauls, Erin K. Arnold, Charles W. Bell, Jonathan C. Allen, Maureane Hoffman *

Pathology and Lab Medicine Service, Durham Veterans Affairs Medical Center, Durham, Nutrition Program, Department Food Science, NC State University, Raleigh, NC, USA

Received 2 May 2006; received in revised form 28 July 2006; accepted 3 August 2006
Available online 18 September 2006

KEYWORDS
Fibrinogen;
Oxidant injury;
Thrombosis;
Homocysteine;
Folate

Abstract Elevated plasma homocysteine levels are associated with the risk of atherosclerosis and arterial and venous thrombosis. We have previously demonstrated that rabbits rendered hyperhomocysteinemic by parenteral administration of homocysteine develop a dysfibrinogenemia that is associated with the formation of fibrin clots that are abnormally resistant to fibrinolysis. We suggested that this acquired dysfibrinogenemia contributes to the thrombotic tendency in hyperhomocysteinemia. However, it was possible that the homocysteine-associated dysfibrinogenemia was an artifact of the parenteral administration model. Therefore, the goals of the current study were to develop a diet-induced model of homocysteinemia in rabbits and determine whether a dysfibrinogenemia and evidence of oxidative stress develop in this model as they do when homocysteine is injected. We found that rabbits fed a diet severely deficient in folate and mildly deficient in choline develop mild hyperhomocysteinemia: 14.8±4.0 μM in deficient rabbits compared to 9.0±1.7 μM in controls. The deficient rabbits also develop evidence of oxidant stress: increased lipid peroxidation in liver, impaired mitochondrial enzyme activities in liver and elevated caspase-3 levels in plasma. Most importantly, the deficient rabbits also develop a dysfibrinogenemia characterized by increased resistance to fibrinolysis. We believe that this dietary model of homocysteinemia is clinically relevant and reproduces many features associated with hyperhomocysteinemia in previous work using *in vitro* and *in vivo* models. Our findings suggest that an acquired dysfibrinogenemia could play a role in the increased risk of atherothrombotic disease in mildly hyperhomocysteinemic human subjects.

* Corresponding author. Laboratory Service (113), Durham Veterans Affairs Medical Center, 508 Fulton St, Durham, NC 27705, USA. Tel.: +1 919 286 6925; fax: +1 919 286 6828.
E-mail address: maureane@med.unc.edu (M. Hoffman).

0049-3848/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.thromres.2006.08.001

Introduction

Elevated plasma homocysteine is well recognized as a risk factor for atherosclerotic and thrombotic cardiovascular disease [1–3]. Mounting evidence also suggests that high homocysteine levels in adults are a predictor of osteoporotic fractures [4] and may also be associated with impaired cognitive function and dementia [5,6]. However, the precise mechanisms responsible for the deleterious effects of homocysteine in the vascular system and other sites are not clear. A great many studies have shown deleterious effects of homocysteine on cells that might play a role in development of atherosclerosis. It has been suggested that homocysteine-induced cell injury involves oxidative damage [7–9]. *In vitro* studies have shown that homocysteine damages endothelial cells by increasing H_2O_2 production [10], affecting antioxidant defense systems [11], and promoting lipid peroxidation [12,13], as well as triggering apoptosis via mitochondrial oxidant production [14]. Homocysteine also promotes production of reactive oxygen species by isolated monocytes [15] and platelets [16].

Much of the research on the effects of homocysteine has utilized cell culture models and short-term exposure to very high levels of homocysteine to try to mimic long-term exposure to lower levels of homocysteine *in vivo*. This has provided important information on the potential effects of homocysteine, but has not allowed a clear determination of which mechanisms actually operate *in vivo*. Thus, experimental work in animal models has an important role to play in understanding the pathology of hyperhomocysteinemia.

Most studies on the effects of homocysteine in animal models has focused on its effects on cellular functions, especially on endothelial cells and vascular reactivity [2]. In addition to its cellular effects, we have reported that hyperhomocysteinemic rabbits develop abnormalities in the key coagulation protein fibrinogen. This acquired dysfibrinogenemia is characterized by formation of clots composed of abnormally thin, tightly packed fibers with an increased resistance to fibrinolysis [17,18]. Congenital dysfibrinogenemias that produce a similar resistance to fibrinolysis are associated with a clinically significant thrombotic tendency [19]. We have recently demonstrated that the same abnormalities of fibrinogen function can be produced by reaction of purified human fibrinogen with homocysteine thiolactone [20]. Thus, our findings suggest that hyperhomocysteinemia might directly promote thrombosis by interfering with the normal process by which intravascular clots are removed.

Homocysteine is produced by metabolism of dietary methionine. The liver is the major site of homocysteine metabolism, with kidney also making a significant contribution. The metabolism of homocysteine is complex [21]. The cycle that interconverts homocysteine and methionine also produces *S*-adenosyl-methionine (SAM), a key intermediate in one-carbon metabolism. Homocysteine is converted back to methionine by addition of a methyl group, a process called transmethylation. The methyl group can come from 5-methyltetrahydrofolate in a reaction that depends on folate and B_{12}. Alternatively, betaine can serve as the methyl donor for the formation of methionine from homocysteine. Betaine is a metabolite of choline, an intermediate in lipid metabolism.

Homocysteine can be removed from the methionine cycle when it is converted to cystathionine by the trans-sulfuration pathway, which requires vitamin B_6 as a cofactor. Cystathionine is then subsequently converted to cysteine, which is the rate-limiting component for synthesis of glutathione, a key buffer of intracellular oxidation-reduction reactions. Thus, several metabolic pathways could have an impact on plasma homocysteine levels. Elevated levels of homocysteine in otherwise normal humans can result from deficiency of one or more of the vitamin cofactors, high dietary methionine intake or reduced activity of one of the enzymes involved in its metabolism. Age and gender influence homocysteine levels [22–24], but the mechanisms underlying these relationships are not known. Renal failure is also associated with elevated homocysteine. While many factors can influence plasma homocysteine, folate status appears to be a major determinant of fasting homocysteine levels in otherwise healthy human subjects [25] and in patients with kidney failure [26].

In our initial studies on the effects of hyperhomocysteinemia we injected rabbits with homocysteine twice daily for 8 weeks. We chose to administer exogenous homocysteine parenterally to ensure that all experimental animals received the same dose of the agent (on a weight basis). In addition, it potentially allowed separation of the effects of elevated plasma homocysteine levels from the effects of folate deficiency *per se*. While injecting homocysteine as a means of increasing plasma homocysteine levels has some advantages, it does not mimic all aspects of homocysteinemia in human subjects. Firstly, the exogenously administered homocysteine is a mixture of D- and L-isoforms, while metabolically generated homocysteine is the L-isoform only. Secondly, metabolically