ATHEROSCLEROTIC VASCULAR DISEASE: WILL FOLATE OR GENE THERAPY BE USEFUL?

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INTRODUCTION

Modern technology gives the hope for novel approaches for prevention and treatment of atherosclerosis. In this paper, we will consider two extremely different potential approaches for treatment of atherosclerotic vascular disease, vitamins and gene therapy.

One potential treatment for vascular disease is to treat hyperhomocysteinemia with B vitamins. There is strong evidence that hyperhomocysteinemia is associated with an increased risk of cardiovascular disease. Furthermore, it is clearly established that administration of high doses of folate, vitamin B6 and vitamin B12 substantially reduce plasma concentrations of homocysteine in patients. We do not know, however, whether treatment of hyperhomocysteinemia will reduce the risk of cardiovascular events. The goal of our studies is to try to understand mechanisms by which hyperhomocysteinemia may predispose to cardiovascular disease and its complications.

A second potential treatment of atherosclerotic vascular disease involves gene transfer to blood vessels. There are many major obstacles that will need to be surmounted before gene therapy will be a useful therapeutic option. One of the obstacles is that it is difficult to achieve gene transfer with the vectors that are currently used to transfer genes, unless blood flow is stopped for several minutes. In this paper, we will describe some new approaches that we have developed to deliver adenoviral vectors to atherosclerotic blood vessels to allow gene transfer without stopping blood flow.

B Vitamins and Hyperhomocysteinemia

Severe hyperhomocysteinemia, with fasting plasma homocysteine concentrations greater than 100 μM, occurs in patients with heredi-

tary hyperhomocysteinuria due to homozygous cystathionine beta synthase (CBS) deficiency. This disorder predisposes to arterial and venous thrombosis, with about 50% of patients developing clinically significant vascular disease before the age of 30 (1). Predisposition to thrombosis appears to result from elevated concentrations of homocysteine, because other rare metabolic disorders that produce severe hyperhomocysteinemia are also associated with thrombosis (2).

Moderate hyperhomocysteinemia, with fasting plasma homocysteine concentrations of 10-40 μM, occurs in patients with inherited enzyme deficiencies (including heterozygous CBS deficiency, homozygous thermolabile methylene tetrahydrofolate reductase [MTHFR], and other deficiencies of folate utilization) and commonly as the result of dietary deficiencies of folic acid, vitamin B6, or vitamin B12 (2). Several recent studies have suggested that moderate hyperhomocysteinemia may be a risk factor for vascular proliferation (Figure 1) (3), stroke, peripheral vascular disease, and myocardial infarction (4). In contrast to hypercholesterolemia, however, it is not clear that hyperhomocysteinemia alone is a sufficient stimulus for development of atherosclerosis. It appears instead to predispose to the complications and perhaps progression of atherosclerosis.

In humans, plasma homocysteine levels can be decreased substantially by supplementation with folic acid (5), which suggests that hyperhomocysteinemia may represent an important treatable risk factor for vascular disease. Thus, hyperhomocysteinemia may be an important risk factor for complications of atherosclerotic vascular disease, and it is generally quite easily treated.

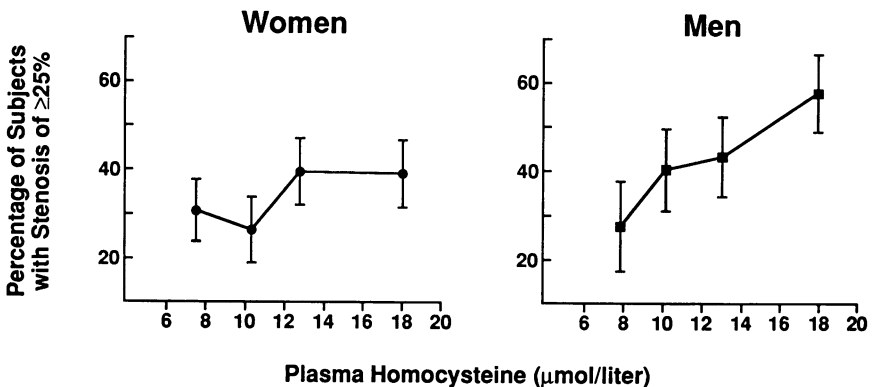Evidence from several laboratories suggests that homocysteine may

FIG. 1. Association of plasma homocysteine concentration with percentage of subjects with stenosis of the carotid artery. Women and men were divided into quartiles based on plasma homocysteine levels. Data are summarized from Ref. 3.

predispose to atherosclerosis or its complications by producing endothelial dysfunction. Intravenous administration of very high doses of homocysteine has been reported to produce endothelial cell injury in baboons (6), and high concentrations of homocysteine also produce injury to endothelium in tissue culture (7,8). Homocysteine in tissue culture decreases production of nitric oxide (9) by endothelial cells.

We and others have demonstrated that homocysteine alters hemostatic functions of endothelial cells in vitro by decreasing thrombomodulin-dependent activation of protein C (10,11) and altering the processing and secretion of von Willebrand factor (12).

These data in vitro suggest that elevated homocysteine levels may alter regulatory properties of endothelium. A limitation of these studies is that they used concentrations of homocysteine that are far higher than those found in plasma. In a recent study, we examined endothelial antithrombotic and vasomotor function in monkeys with moderate hyperhomocysteinemia (13). When monkeys were fed a normal diet, their plasma homocysteine level was approximately 4 μM. When the same monkeys were fed a diet that was enriched in methionine, relatively depleted in folic acid, and free of choline, the monkeys developed moderate levels of hyperhomocysteinemia (about 11 μM). The monkeys were studied in vivo, and we observed that activation of platelets by collagen produced profound decreases in blood flow to the leg, in contrast to modest reductions in flow in monkeys with normal levels of homocysteine. We attributed the altered vascular responses to endothelial dysfunction.

We also examined vasomotor responses of the carotid artery in these monkeys in vitro (13). Relaxation to acetylcholine, which reflects endothelial function, was impaired in monkeys with hyperhomocysteinemia (Figure 2). Thrombomodulin anticoagulant activity was also im-
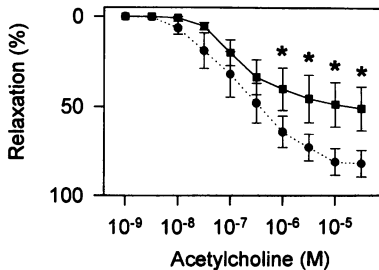

FIG. 2. Vasomotor responses of the carotid artery to acetylcholine in vitro. Responses to acetylcholine of hyperhomocysteinemic monkeys (squares) are impaired in comparison with responses of normal monkeys (circles). Values are mean ± SE, n = 7, *p < .05 vs. normal diet (13).

paired by hyperhomocysteinemia, which implies that resistance to thrombosis is impaired during hyperhomocysteinemia. The latter conclusion is based on the concept that thrombomodulin activates protein C, a potent anticoagulant, and that impairment of thrombomodulin activity by hyperhomocysteinemia would predispose to thrombosis.

Because plasma homocysteine concentration can be reduced rapidly by dietary supplementation with folic acid and other B vitamins, it will be important to determine whether treatment of hyperhomocysteinemia with B vitamins restores endothelial function and reduces the incidence of stroke and myocardial infarction (5).

Gene Therapy for Atherosclerotic Vascular Disease

A promising new approach for treatment of atherosclerotic vascular disease is gene therapy. The therapeutic approach involves transfer of cDNA to a blood vessel to encode a substance that affects vascular growth or function.

A promising method for gene transfer to blood vessels is to use replication-deficient adenovirus as a vector (14). Gene transfer to blood vessels in vivo generally has been accomplished by intraluminal administration of adenovirus or other vectors. There are major limitations to this approach. First, to achieve significant infection and then transduction of cells in the vessel wall, blood flow usually is stopped, often for many minutes. Second, in previous studies, intraluminal administration of vectors has generally been performed only after endothelium is denuded or severely damaged by balloon injury. Thus, it is difficult to use this approach to study vascular reactivity, because intact endothelium is important for normal vascular function.

These limitations led us to seek other methods for delivery of vectors, including perivascular application of adenoviral vectors. We stumbled on this approach by accident. We were injecting adenoviral vectors into the lumen of the carotid artery with a needle, and generally observed very poor transgene expression in the vessel. In one artery, however, we observed profound transgene expression (Figure 3A). It was not clear to us whether we might have either injected the virus directly into the vessel wall, or accidentally dripped the virus on the outside of the vessel. Nevertheless, based on this initial observation, we began to inject the virus into the sheath around either the femoral or carotid artery (Figure 3B). We found that when adenovirus is injected into the femoral or carotid sheath, there is excellent transfection of cells in the adventitia (15).

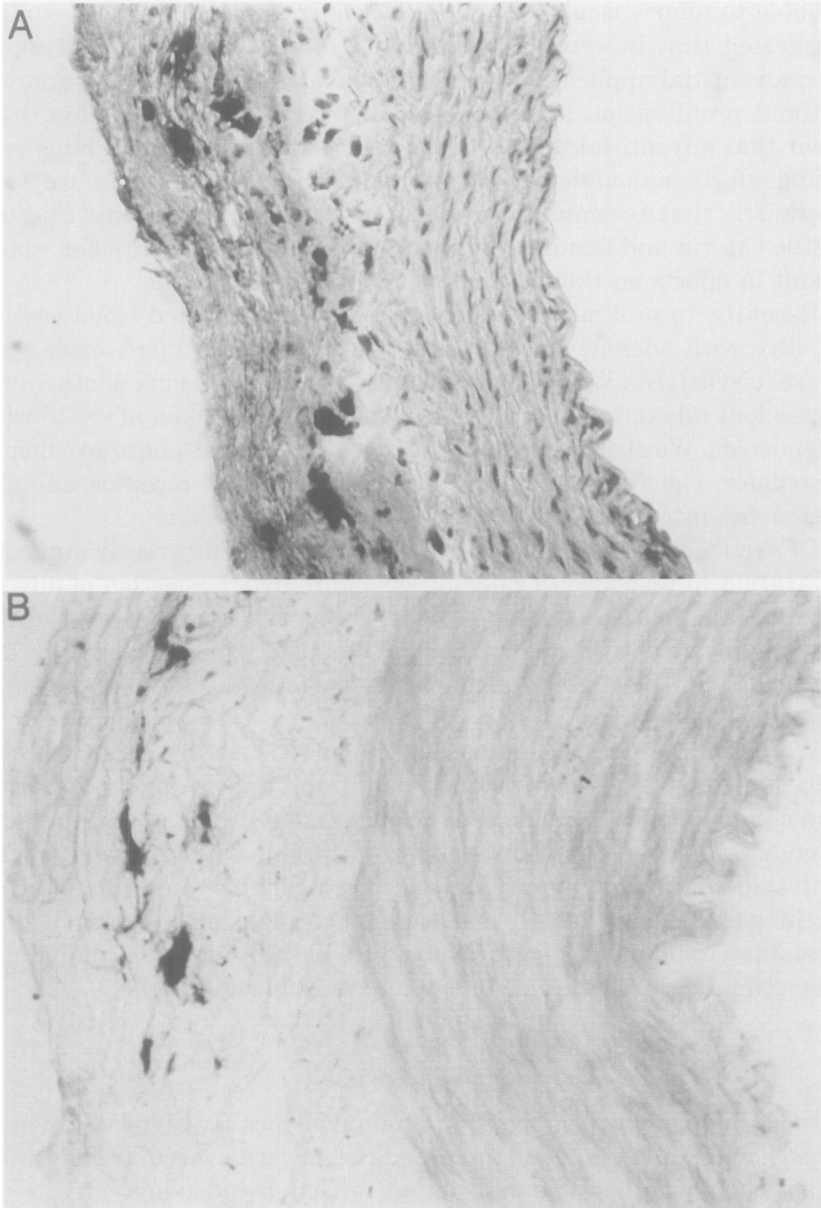We were concerned whether transduction of cells in adventitia would

FIG. 3A. Gene transfer to carotid artery of normal rabbit. During injection in the lumen of the vessel, recombinant adenovirus that expresses β-galactosidase (Ad/CMVβgal) was dripped on the outside of the vessel. There was far more expression of the transgene (with large dark-stained nuclei) than observed in other vessels after intraluminal injection. Fig. 3B. Gene transfer to femoral artery of normal monkey after injection of Ad/CMVβgal in the arterial sheath (15). Gene transfer was observed in adventitia.

be able to alter vascular function. However, several previous studies suggested that it would be possible to alter function. For example, periadventitial application of antisense oligonucleotides can suppress intimal proliferation after endothelial injury (16). In addition, it is clear that adventitial nerves which release nitric oxide can have profound effects in modulation of vasomotor tone (17). Therefore, we were optimistic that recombinant replication-deficient adenovirus injected in the carotid and femoral sheaths to accomplish gene transfer would result in effects on the media underlying the adventitia.

Recently, in preliminary studies, we have transfected blood vessels in vitro with adenovirus that expresses endothelial nitric oxide synthase (eNOS) (18). We have observed modest increases in endothelium-dependent relaxation to acetylcholine after gene transfer of eNOS with adenovirus. We observed augmented relaxation in response to calcium ionophore. Calcium ionophore does not require a receptor and, by increasing intracellular calcium, it releases nitric oxide.

Of particular interest was the finding that we could greatly augment relaxation in response to calcium ionophore by transfection of adventitial cells alone. This finding provides direct evidence that gene transfer to the adventitia, as we can accomplish with perivascular approaches, can be used to alter function of the underlying vascular muscle.

We speculate that this approach might be useful therapeutically (19). For example, it may be possible to inhibit vascular proliferation and occlusion after placement of arteriovenous shunts, by dripping an adenoviral vector that expresses an antiproliferative enzyme on the shunt at the time of surgery. It is also possible that injection of virus in the pericardium (20), with expression of angiogenic factor (such as vascular endothelial growth factor–VEGF) (21) in pericardial fluid, may stimulate growth of coronary collateral blood vessels.

CONCLUSION

In closing, there is intriguing, strong evidence for an association of hyperhomocysteinemia and increased risk of cardiovascular disease. It is not clear whether this association reflects a cause-and-effect relationship. An intervention study, with reduction of hyperhomocysteinemia with B vitamins, will be required to answer this question.

Our research in relation to homocysteine focuses on mechanisms by which hyperhomocysteinemia produces vascular dysfunction. Our studies suggest that hyperhomocysteinemia interferes with antithrom-

botic mechanisms, which may explain, in part, the clinical consequences of hyperhomocysteinemia.

In relation to the possibility that gene therapy may be useful in treatment of atherosclerotic vascular disease, there are many obstacles to gene therapy which will need to be overcome. We have made some headway in relation to gene transfer to blood vessels. Perivascular administration of an adenoviral vector can be used to deliver a reporter gene to blood vessels. It is also possible to alter vasomotor function by gene transfer to the adventitia.

Thus, the physician of the future may have a broad range of therapeutic options in treatment of atherosclerotic vascular disease. We speculate that it may be possible to reduce the risk of atherosclerotic disease, including reduction in the risk of thrombosis, by using B vitamins to reduce plasma homocysteine levels. On the other hand, it may be possible to transfer therapeutic genes to treat atherosclerotic vascular disease.

ACKNOWLEDGMENTS

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DISCUSSION

Alexander, Atlanta: I want to go back to the issue of the homocysteine. Could you speculate on the mechanisms by which homocysteine affects the arterial wall? I think your answer will probably involve oxidants, anti-oxidants, and others mechanisms. I wonder if you might elaborate on some of the notions of the general metabolic changes in the arterial wall related to oxidative stress, the notions you are alluding to in the arterial wall that may be fundamental to a new approach to developing new therapeutic approaches to the disease.

Heistad: There are studies in tissue culture which indicate that homocysteine produces endothelial damage, perhaps by generation of hydrogen peroxide. It looks as though it is protected by catalase. As you suggested, it may involve oxidative stress. Oxidative stress may be important in a variety of ways, as Dan Steinberg would say. Perhaps in this mechanism, lots of approaches to reduce oxidative stress, perhaps with other vitamins, may be possible. It is a very active, hot topic, Wayne.

Criley, Torrance: Knowing as I do, or thinking as I do, that the media of the artery

contains smooth muscle, how does your nitric oxide contributor get into the media from the adventitia?

Heistad: We thought it probably would because, for example, there are nerves in the adventitia of some vessels that release NO which diffuses into the media. NO is very diffusible, so there was good rationale to think that it would happen. I think what happens is that the enzyme is expressed in the adventitia, releases NO and diffuses into the media.

Quesenberry, Worcester: I was interested in the duration of your transvection and whether or not you get immune reactivity to your transvected cells?

Heistad: We use a CMV promoter which is fast and short. The expression is about 2/3 maximal by one day. We haven't looked very systematically at the duration, but by a week in a normal animal, expression is gone. The RSV promoter is a little slower and lasts longer. As to inflammation, inflammation is very tissue-specific with adenoviral vectors. In the brain there is very little in the parenchyma itself. When we put the virus around a vessel, we don't see inflammation. This was a surprise, but I understand that another group has found the same thing. When we put the virus in the CSF, we definitely see inflammation. That is a topic that lots of people and companies are trying to address: How can the inflammatory response to adenoviral vectors be minimized?