THE FOLATE RECEPTOR: A POTENTIAL CANDIDATE FOR DRUG TARGETING TO Atherosclerotic Plaques

Felicia Antohe, Ph.D., Institute of Cellular Biology and Pathology “N. Simionescu,” 8, BP Hasdeu Street, Bucharest, Romania, Tel: 0040 723245489, E-mail: feliciaantohe@icbp.ro

Introduction

Heart disease and stroke, the main cardiovascular diseases (CVD), have become without doubt global epidemics of our days. High levels of LDL cholesterol and other abnormal lipids are among the main risk factors of atherosclerosis, the number one killer in the world; globally the number of deaths reached 16.7 million in 2002 and is on the rise according to World Health Organization statistics [1]. However, recent advances in CVD treatment together with improvements in surgical techniques increased the quality of life and reduced premature death rates and disabilities. Nevertheless, they still add a heavy burden to the rising global costs of health care. This commentary highlights not only the need for early recognition of the warning signs of a heart attack, but also the need for early biomarkers for prevention. Therefore, our groups focused on the development of safer and more effective treatments that can selectively target the cellular and molecular mediators of cardiovascular disease, i.e. atherosclerosis.

Atherosclerotic lesions have many features common to chronic inflammatory diseases [2]. These features include local enrichment of inflammatory cells, persistent cell proliferation, and fibrosis [3]. Located in the specific area of the arterial vessels wall, atherosclerosis is a multifaceted disease involving a progressive lipid disorder in which cells of the innate immune system ─ T lymphocytes and mainly macrophages ─ are involved in all stages [4]. The initial alterations of the endothelium, i.e. the increased permeability to oxidized lipoproteins, expression of adhesion proteins, and secretion of chemoattractant molecules, lead to the recruitment, binding and subsequent entry of monocytes into subendothelial spaces where they become activated macrophages [5]. The latter take up subendothelial accumulated modified and reassembled lipoproteins via their scavenger receptors to form macrophage-derived foam cells. In late stages of atherosclerosis due to the profound changes and the altered milieu even the endothelial cells take up lipids, generating endothelial cell-derived foam cells [6]. With time, increased lesion size, and local proliferation of smooth muscle cells can cause the plaque protrusion into the vascular
lumen. Weakening events, associated with the local inflammatory response, can lead to plaque rupture, thrombosis, and the ensuing myocardial infarction or stroke [7,8]. During this complex process, the macrophage is generally thought to play a major role in the pathology of the disease [9,10]. Foam cell-derived macrophages comprise the major volume of the early lesion or “fatty streak,” and they are even more numerous in late-stage lesions as well. Macrophages carry out several critical functions that are likely to impact the development and later on the instability of the plaque. They are involved both in the removal of dead cells and debris in the entire body and in helping to mobilize additional members of the immune system in case of infection. In addition, macrophages secrete cytokines and chemokines that direct and amplify the local immune response once it is triggered [11]. It is highly possible that altered or unregulated control of these processes in foam cells exerts a profound effect on the evolution and behavior of macrophages within atherosclerosis lesions.

Previous in vitro studies involving foam cell-derived macrophages have reported changes in gene expression and/or cellular responses that suggest that excess lipid loading can modulate macrophage inflammatory potential [12-15]. Reported data suggest that antibodies to oxidized LDL may have an atherogenic effect by enhancing the lipid accumulation in macrophages in atherosclerotic vessels. These antibodies can be considered to be markers of the pathogenic determinants of atherosclerosis [16,17]. In situ studies of human and animal atherosclerotic lesions have identified the increased expression of several chemokine, tissue-remodeling, and lipid metabolism genes [3,18-20]. Our recent studies on an animal hyperlipidemic model demonstrated increased uptake of folate conjugates by activated macrophages [21]. Interestingly, this property is shared by activated macrophages from human patients with rheumatoid arthritis [22], an autoimmune disease, and by a broad variety of human cancers cells due to over-expression of the high affinity folate receptor on their surfaces [for review see 23].

Since tumor tissue generally presents an inflammatory micro-environment, these data collectively suggest that expression of a functional folate receptor is likely to occur during macrophage activation [22].

**Folate and Folate Receptors**

Folates are pterin-based vitamins required by eukaryotic cells for one-carbon metabolism and de novo nucleotide biosynthesis. The folate receptor (FR) is a 38 kDa glycosyl-phosphatidylinositol-anchored protein that binds to the vitamin folic acid with high affinity ($K_D < 1nM$) [24,25]. Upon FR binding, the receptor-ligand complex is endocytosed and delivered to a low pH compartment where dissociation of the vitamin from its receptor is promoted [26]. The FR expression is restricted to only a few cell types: epithelial cells from kidney and placenta, hematopoietic cells of the myelomonocytic lineage and myelocytic leukemia, while other normal tissues express either a low or nondetectable levels of FR [27].

The simple covalent attachment of folic acid to virtually any molecule or macromolecular complex produces a conjugate that can be internalized into FR-bearing cells in a manner similar to the uptake of free folic acid [28]. The study of folate receptor regulation in activated cells in different pathological condition is highly relevant to a variety of developing therapies [29-31].

For example, by linking folic acid to a relevant hapten and administering the folate-hapten conjugate intravenously to a patient, the folate-receptor expressing cells become decorated with immunogenic haptns. Following this marking of FR-expressing cells with haptns, the same
cells become opsonized with autologus anti-hapten antibodies which are then presumed to mediate cell removal via antibody-dependent cellular cytotoxicity [22].

**Folate Receptors in Hyperlipidemia**

Recently, spectrofluorometric and fluorescence microscopic analyses has shown significantly greater uptake of folate-conjugates with fluorescent tags by peritoneal macrophages of hyperlipidemic hamsters compared with those of hamsters fed a normal or folate-deficient diet. Moreover, systemically administered folate-fluorescent conjugates were found to accumulate as bright spots in protrusions of atherosclerotic plaques populated by macrophages, whereas a low level of staining was detected uniformly dispersed across the lesions where the senescent cells were located.

To demonstrate that hyperlipidemic conditions induce the over-expression of folate receptors on activated macrophages, we designed experiments on Golden Syrian hamsters maintained on a hyperlipidemic diet containing 3% cholesterol and 15% butter until the serum level of cholesterol and triglyceride was significantly higher than in the control groups [21].

![Figure 1](image.png)

Figure 1. Uptake of folate conjugates in atherosclerotic lesions located in cardiac valves of hyperlipidemic hamster. a: cryosections stained with Oil Red O and toluidine blue showing the presence of lipid deposits. b: the same section examined by fluorescence microscopy revealed the uptake of folate-Texas Red in activated macrophages. c: comparable section stained with Oil Red O and Hoechst demonstrating the high density of lipid-loaded cells in the plaque. Bars: 50 μm.

When the hyperlipidemic conditions reached a chronic level, the animals were i.p. injected with folate-Texas Red conjugates (35 μg/100 g body weight) and sacrificed 4 hours later. Extensive atherosclerotic lesions were detected in sections of the aortic arch and cardiac valves that were packed with lipid deposits as revealed by staining with Oil Red O (Figure 1a). Adjacent sections examined by fluorescence microscopy showed intense Texas Red fluorescence in focal areas of the fatty lesions, indicating significantly higher uptake of the folate-TR conjugate (Figure 1b) compared to control probes. The double staining of similar sections (with Oil Red O for lipids and Hoechst for nucleic acids) also demonstrated that the intense fluorescent spots were associated with the few activated macrophages that took up the folate conjugate more aggressively than other more quiescent macrophages that had presumably become senescent (Figure 1c). This observation is consistent with the conclusions of Nakashima-Matsushita et al. [32] showing that activated but not resting macrophages express the high affinity FR-β.
Remarkably, macrophages harvested from the peritoneal cavity of folate-TR-treated hyperlipidemic hamsters were also found to be significantly more fluorescent and to take up 2.5 times more folate-conjugate than macrophages from control animals. This enhanced uptake of folate-TR by peritoneal macrophages is in agreement with data from others showing that macrophages become activated systemically in atherosclerosis [11].

Our data also suggest that increased uptake of folate is a common characteristic of activated macrophages, regardless of their tissue of origin or cause of activation. Thus, until now, FR-activated macrophages have only been demonstrated in rheumatoid arthritis [22,32,33]. However, with our observation that FR-activated macrophages are also present in animals with atherosclerotic lesions, it would seem that this cell type might participate in the pathologies of a variety of autoimmune and inflammatory diseases. Preliminary data on animal models of inflammatory bowel disease, systemic lupus erythematosus, and sepsis, in fact, confirm this expectation [unpublished data of B. Varghese and P.S. Low].

A wide variety of folate-drug conjugates have been found to enter FR-expressing cells by receptor-mediated endocytosis [31]. This remarkable property may be used in the clinic for the early detection of atherosclerotic lesions, by targeting the folate conjugates to activated macrophages contained within the plaques. Thus, folate conjugated to fluorescent dyes or radiochemicals will localize to and allow the visualization of atherosclerotic lesions by optical or radioimaging methodologies, respectively. The folate-linked imaging/contrast agents might also aid in the diagnosis/evaluation of some of these diseases. Since therapeutic compounds can be targeted as easily as imaging agents, a large variety of remedies can now be explored for all pathologies in which activated macrophages are involved.

References