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Statins Impair Glucose Uptake in Tumor Cells[1]

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Abstract

Statins, HMG-CoA reductase inhibitors, are used in the prevention and treatment of cardiovascular diseases owing to their lipid-lowering effects. Previous studies revealed that, by modulating membrane cholesterol content, statins could induce conformational changes in cluster of differentiation 20 (CD20) tetraspanin. The aim of the presented study was to investigate the influence of statins on glucose transporter 1 (GLUT1)–mediated glucose uptake in tumor cells. We observed a significant concentration- and time-dependent decrease in glucose analogs' uptake in several tumor cell lines incubated with statins. This effect was reversible with restitution of cholesterol synthesis pathway with mevalonic acid as well as with supplementation of plasma membrane with exogenous cholesterol.

Abbreviations: 2-DOG, 2-deoxyglucose; 6-NBDG, 6-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-6-deoxyglucose; CD, cluster of differentiation; CRAC, cholesterol recognition/interaction amino acid consensus; ^{18}F-FDG, [^{18}F]fluoro-2-deoxyglucose; FPP, farnesyl pyrophosphate; GLUT, glucose transporter; HA, hemagglutinin; MA, mevalonic acid; MβCD, methyl-β-cyclodextrin; PBMC, peripheral blood mononuclear cell; PET/CT, positron emission tomography/computed tomography; SUV, standardized uptake value

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Statins did not change overall GLUT1 expression at neither transcriptional nor protein levels. An exploratory clinical trial revealed that statin treatment decreased glucose uptake in peripheral blood leukocytes and lowered ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake by tumor masses in a mantle cell lymphoma patient. A bioinformatics analysis was used to predict the structure of human GLUT1 and to identify putative cholesterol-binding motifs in its juxtamembrane fragment. Altogether, the influence of statins on glucose uptake seems to be of clinical significance. By inhibiting ^{18}F-FDG uptake, statins can negatively affect the sensitivity of positron emission tomography, a diagnostic procedure frequently used in oncology.

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Introduction

Owing to the deregulated blood supply, rapidly growing tumor cells suffer from lack of oxygen and nutrients. An increased glucose metabolism seems to be an important mechanism of tumor cells adapting to hypoxia. Intriguingly, transformed cells tend to use anaerobic glycolysis as their major energy supply even under normoxic conditions such as during cell culture. Although glycolysis is less efficient in generating ATP than oxidative phosphorylation (tricarboxylic acid cycle), it is much faster and provides substrates to the synthesis of amino acids, nucleotides, and fatty acids as well as reduces equivalents to minimize toxic effects of reactive oxygen species [1,2]. The shift in ATP generation from oxidative phosphorylation to glycolysis even under normal oxygen conditions is called the Warburg effect. It seems that the first regulatory step in glycolysis, that is, the increased glucose uptake, is the biologic basis for the diagnostic procedure of [^{18}F] fluoro-2-deoxyglucose positron emission tomography (FDG PET) [3,4]. Although various mechanisms have been proposed to explain increased FDG uptake in growing tumors, a facilitative glucose transport by glucose transporters (GLUTs) seems to be the most important [5–7]. GLUT family includes 14 isoforms sharing common structural features including 12 transmembrane domains with both amino- and carboxy-terminal ends localized in the cytoplasm [8]. GLUT1 is frequently upregulated in tumor cells, which probably facilitates tumor growth beyond the size limited by their glycolytic capacity [9]. A number of studies indicate that increased GLUT1 expression correlates with higher tumor ^{18}F-FDG uptake [10–12].

We have previously demonstrated that statins, which are the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, can induce conformational changes in CD20 molecules. This results in impaired binding of anti-CD20 monoclonal antibodies and diminished ability to trigger complement-dependent cytotoxicity [13]. These effects turned out to be strictly cholesterol dependent because exogenous cholesterol could rapidly reverse the effects of statins. Atomic force microscopy, limited proteolysis, and functional data indicated that statins induced cholesterol-dependent conformational changes in CD20 tetraspanin. We hypothesized that statins could also induce conformational changes in other proteins with multiple membrane-spanning domains. Such possibility is also supported by a number of observations indicating that statins or membrane cholesterol depletion impairs the function of other proteins with multiple membrane-spanning domains including P-glycoprotein [14], P2X1-4 ATP receptors [15], voltage-gated chloride channel [16], G-protein–coupled cholecystokinin [17], or serotonin receptors [18]. Modeling of the serotonin$_{1A}$ receptor revealed that, in the presence of cholesterol, the receptor acquires a more compact structure, and its ligands exhibit higher binding energies when docked to the receptor in the presence of cholesterol [19]. A recent study that used small-angle neutron scattering revealed that cholesterol-rich micellar nanostructures determine transmembrane protein (GPCR) activity and that successful reconstitution of the receptor is dependent on cholesterol-protein interactions [20]. Moreover, cholesterol-binding motifs have recently been characterized in acetylcholine receptors, which contain five transmembrane domains [21]. Therefore, we decided to investigate the influence of statins on the glucose transport into tumor cells, which frequently overexpress GLUT proteins and require efficient glucose uptake for their increased metabolic demands.

Materials and Methods

Cell Culture

Human Burkitt lymphoma (Daudi, Raji, Ramos), human follicular lymphoma (DoHH2), human colon adenocarcinoma (LoVo), and human embryonic kidney (HEK293T) cell lines were purchased from the American Tissue Culture Collection (Manassas, VA). Cells (Daudi, Raji, Ramos, DoHH2, and HEK293T) were cultured in RPMI 1640 or Dulbecco modified Eagle medium/F-12 medium (LoVo) supplemented with 10% heat-inactivated fetal bovine serum, 100 μg/ml streptomycin, and 250 ng/ml amphotericin B (all from Invitrogen, Carlsbad, CA). Cells were cultured at 37°C in a fully humidified atmosphere of 5% CO_2 and were passaged approximately every other day.

Reagents

The following statins were used: atorvastatin (Pfizer Pharmaceuticals, Inc, Groton, CT), cerivastatin (Bayer Corp, West Haven, CT), fluvastatin (Novartis Pharma AG, Basel, Switzerland), lovastatin, and simvastatin (both from Merck, Sharp & Dohme Res. Lab., Rahway, NJ). Lovastatin and simvastatin were obtained in the inactive lactone form. They were converted to the active form by dissolving in ethanol, heating for 2 hours at 50°C in 0.1N NaOH and neutralizing with HCl. Distilled water was added to obtain the final stock concentration of 10 mM. Stock solution was aliquoted and stored frozen (–20°C). Mevalonic acid (MA), farnesyl pyrophosphate (FPP), methyl-β-cyclodextrin (MβCD), and water-soluble cholesterol were purchased from Sigma (St Louis, MO). Farnesyltransferase inhibitor L-744,832 was obtained from Merck KGaA (Darmstadt, Germany); 6-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-6-deoxyglucose (6-NBDG) was from Invitrogen and was dissolved in dimethyl sulfoxide to

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