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METHODS OF INHIBITING NITRIC OXIDE SYNTHASE USING CORRIN DERIVATIVES

This application claims the priority of U.S. Ser. No. 60/849,258, the entirety of which is hereby incorporated by reference into this application.

The invention was also made in part with funding under Grant Number CA90548 awarded by the National Institutes of Health. The United States Government has certain rights in the invention.

Throughout this application various publications are referenced. The disclosures of these publications in their entirety are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications can be made while remaining within the spirit and scope of the invention.

TECHNICAL FIELD

This application generally relates to methods for inhibiting nitric oxide synthase (NOS) using corrin derivatives e.g., corrin derivatives that bind NOS but does not bind nitric oxide (NO). These methods can be used to treat diseases or medical conditions associated with elevated nitric oxide synthase levels. In one embodiment, the corrin derivative is a cobinamide such as dicyanocobinamide.

BACKGROUND OF THE INVENTION

Nitric oxide (NO) synthase (NOS) convert L-arginine to L-citrulline and NO in humans (1,2). NOS1 ("neural" NOS) and NOS3 ("endothelial" NOS) generally produce low levels of NO and are constitutively active, while inducible NOS (NOS2) is induced by cytokines and microbial factors. NO plays very important roles in normal physiology and in various pathologic processes (3-5). NO serves as a signal transducing molecule, an effector molecule for the stasis and killing of microbes (e.g., certain viruses, fungi, bacteria, and protozoa), and tumor cells. NO can also block apoptosis by S-nitrosylating caspases (6), and in resting, normal B lymphocytes, the active site cysteine of caspase 3 is nitrosylated (and inhibited by this nitrosylation), and it undergoes denitrosylation upon fas activation and apoptosis (7). NO controls smooth muscle contraction and thus influences vessel, bowel, bronchial, uterine, ureteral, and ductal contraction and tension (3).

There are three major isoforms of NOS (NOS1, NOS2, and NOS3) encoded by three separate genes (1). While these isoforms are noted in various cell types and tissues, NOS1 is found mainly in neural and muscle tissues; NOS2 in monocytes/macrophages, hepatocytes, and chondrocytes; and NOS3 in endothelial cells. A variety of agents have been demonstrated to inhibit the enzymatic function of NOS (1,8).

Most NOS inhibitors bind to the oxygenase domain of NOS with the guanidinium group of the inhibitor binding to NOS glutamate (8). Investigators described the importance of arginine in macrophage-mediated cytotoxicity, and demonstrated that arginine analogues such as N^G-mono-methylarginine (NMMA) could inhibit cytotoxicity (a function they later described as being related to NO production) (9,10). Since then, a variety of NOS inhibitors have been described (8).

Arginine analogues that act as classic competitive inhibitors (e.g., L-thiocitrulline) interact with the NOS oxygenase domain active site through hydrogen bonding interactions

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with glutamate by way of the guanidinium structural motif (8). These are generally isoform nonselective. "Slow on-slow off" arginine analogues (e.g., the S-alkyl-L-thiocitrullines) are not altered by NOS and also offer little isoform selectivity.

Mechanism-based inhibitors [suicide inhibitors (e.g., NIO)] offer the most isoform selectivity. Vinyl-L-NIO {N⁵-(1-imino-3-butenyl)-L-ornithine} is an amidine analogue of this class that is markedly selective for NOS1. Likewise, L-NIL is very specific for NOS2.

NOS oxidase inhibitors (e.g., diphenyleneiodonium which also inhibits NADPH oxidase) inhibits NO formation, and inhibitors of NOS dimer formation (e.g., various pyrimidine-imidazoles) blocks NO formation by NOS.

NOS2-specific inhibitors have been targeted for use in a variety of conditions, most prominently septic shock and arthritis. NOS1-specific inhibitors have been targeted for use in psychiatric diseases such as depression and anxiety, and for neurodegenerative diseases such as Alzheimer's disease and amyotrophic sclerosis.

Others have taken a similar but different approach to negate the effects of NO. They have used compounds that bind, quench or scavenge NO to diminish NO effects (11). These scavengers include heme-containing compounds and cobalt-containing cobalamins and associated molecules. Certain cobalamins bind NO and thus quench their effects (12-23).

There exists a need to inhibit NOS, for example to treat migraine headaches and ill effects of inflammation. Heretofore, no one has ever reported that such cobalamins, or other corrin derivatives, can inhibit the activity of the NOS enzyme itself.

SUMMARY OF THE INVENTION

The present invention provides methods of inhibiting nitric oxide synthase (NOS) using corrin derivatives e.g., corrin derivatives that bind to and inhibit NOS but do not bind and quench/scavenge NO. It also provides methods of inhibiting NOS in vivo by administering corrin derivatives, and methods of treating diseases and medical conditions using this inhibition of NOS. Exemplary embodiments of the present invention are set forth as follows.

In an exemplary embodiment, the present invention provides a method of inhibiting nitric oxide synthase comprising contacting the nitric oxide synthase with a corrin derivative and thereby inhibiting nitric oxide production. In other particular embodiments, the corrin derivative inhibits nitric oxide synthase without sequestering, or while only weakly sequestering, nitric oxide.

In another exemplary embodiment, the present invention provides a method of inhibiting NOS in vivo, comprising administering a corrin derivative to the subject, thereby inhibiting NOS.

In an exemplary embodiment, the present invention provides a method for treating a subject having an inflammatory or neurological disease or medical condition associated with elevated nitric oxide synthase activity and/or NO overproduction comprising administering a corrin derivative so as to inhibit nitric oxide synthase and thereby treating the subject having the disease or medical condition. Examples of such diseases or medical conditions include, but are not limited to: infertility (e.g., gamete-sperm or ovum-defect), inflammation, conjunctivitis, cystitis, inflammatory bowel disease, nephritis, glomerulonephritis, hepatitis, arteritis, vasculitis, cerebritis, vaginitis, dermatitis, sinusitis, proctitis, otitis, pneumonitis, hypotension, arthritis, colitis, nephritis, meningitis, sepsis, septic or cardiogenic shock, myocardial infarction, asthma, or any other inflammatory disease, schizophre-