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Review Article

Therapeutic potential of selective superoxide dismutase mimetics

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CONTENTS

Summary

Selective superoxide dismutase (SOD) mimetics are potentially useful in pathological conditions in which there is an overproduction of the superoxide anion $O_2^{\cdot -}$. These pathological conditions include inflammation, ischemia/reperfusion, shock, various cardiovascular disorders, amyotrophic lateral sclerosis (ALS) and other neurodegenerative disorders. A major step forward in this field was the development of small-molecule selective SOD mimetics that penetrate cell membranes. These selective SOD mimetics catalytically remove $O_2^{\cdot -}$ without interfering with nitric oxide (NO), peroxynitrite ($ONOO^-$) or other radicals such as hydroxyl radical or hydrogen peroxide (H_2O_2). These selective SOD mimetics (SC-52608, SC-55858, M-40403 and M-40401) have been shown to have benefits in animal models of inflammation, ischemia/reperfusion, shock, thrombosis and diabetes. The next challenge with selective SOD mimetics is to develop therapeutic potential into therapeutic agents.

Introduction

Under normal circumstances, the formation of superoxide anion ($O_2^{\cdot -}$, the one-electron reduction product of oxygen) is kept under tight control by removal with endogenous SOD enzymes. These include the Mn enzymes in mitochondria (SOD2) and the Cu/Zn enzymes present in the cytosol (SOD1) or extracellular surfaces (SOD3). Superoxide dismutase destroys $O_2^{\cdot -}$ by converting it to hydrogen peroxide, or H_2O_2, preventing the production of hydroxyl radical and peroxynitrite, or $ONOO^-$. When the SOD enzymes are saturated, the levels of $O_2^{\cdot -}$ and $ONOO^-$ rise and have pathological effects.

This review begins with a brief discussion of the pathological effects of saturation or lack of SOD. The studies with native enzymes are discussed prior to the main part of this review, which is a discussion of the selective SOD mimetics. Drugs that mimic both SOD and catalase (*e.g.*, EUK-134) (1) are not included in this review.

Pathological effects of the saturation or lack of SOD

Inflammation

In acute and chronic inflammation, the production of $O_2^{\cdot -}$ anions is increased at a rate that overwhelms the capacity of the endogenous SOD enzyme defense system to remove them. The result of such an imbalance is $O_2^{\cdot -}$ mediated damage. Some important proinflammatory roles for $O_2^{\cdot -}$ include endothelial cell damage and increased microvascular permeability, formation of chemotactic factors such as leukotriene B_4 (LTB4), recruitment of neutrophils at sites of inflammation, lipid peroxidation and oxidation, and DNA single-strand damage. Superoxide anion also promotes the formation of $ONOO^-$, a potent cytotoxic and proinflammatory molecule (2).

Ischemia/reperfusion

Following ischemia, $O_2^{\cdot -}$ is produced during the reperfusion phase and it rapidly reacts with free radical nitric oxide, or $NO^{\cdot}$, and forms $ONOO^-$. This reaction has been demonstrated in a variety of tissues, including heart, liver, kidney, intestine and lung, and in neural injury. In

general, prevention of $ONOO^-$ production reduces reperfusion injury (2).

Circulatory shock

Peroxynitrite mimics many of the cardiovascular alterations associated with shock (endothelial dysfunction, vascular hyporeactivity, myocardial failure and cellular energetic failure). Superoxide dismutase has been shown to be protective in various models of endotoxic and hemorrhagic shock and splanchnic artery occlusion/reperfusion (2).

Cardiovascular disease

The nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate (NADH/NADPH) oxidase system accounts for the majority of $O_2\cdot^-$ production in blood vessel walls. Excessive $O_2\cdot^-$ production by the vascular wall will reduce NO bioavailability, and may be responsible for the endothelial dysfunction observed in a number of cardiovascular diseases, including hypercholesterolemia and coronary artery disease (3).

Enhanced NADH/NADPH oxidase-dependent $O_2\cdot^-$ production may also contribute to endothelial dysfunction and vascular hypertrophy in the spontaneously hypertensive rat (SHR) model of hypertension. Angiotensin II may contribute to enhancement of NADH/NADPH oxidase. The resulting excessive $O_2\cdot^-$ increases the oxidative inactivation of NO to produce endothelial dysfunction in the SHR. The $O_2\cdot^-$ may also stimulate the growth of vascular smooth muscle. This excessive $O_2\cdot^-$ production is a result of the hypertension rather than the cause of the hypertension in SHR, as it is not observed in the early stages of hypertension (4).

Enhanced $O_2\cdot^-$ production may also contribute to the endothelial dysfunction observed in heart failure and diabetes, and in heart failure, $O_2\cdot^-$ may impair myocardial function (3).

Amyotrophic lateral sclerosis/neurodegeneration

Mutations in Cu/Zn SOD occur in 2-3% of subjects with amyotrophic lateral sclerosis (ALS), a lethal disease characterized by the relentless death of motor neurons. The other 98% of cases of ALS may have the loss of Zn from wild-type SOD. Zinc-deficient SOD induces apoptosis in motor neurons through a mechanism involving peroxynitrite (5). This suggests a role for SOD mimetics in preventing the death of motor neurons in ALS.

Mitochondrial dysfunction leading to increased $O_2\cdot^-$ may occur in a number of other neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (6).

Studies with native enzymes

Orgotein, or bovine Cu/Zn SOD, shows promising results as a human therapeutic agent in acute and chronic inflammatory conditions including rheumatoid arthritis and osteoarthritis, and as a preventive agent for the side effects associated with chemotherapy and radiation therapy. There are, however, drawbacks to the use of native enzymes as therapeutic agents, including solution instability, immunogenicity of nonhuman enzymes and high susceptibility to proteolytic digestion (7). Another problem with native peptides is that they do not penetrate cells or cross the blood-brain barrier, limiting the dismutation of $O_2\cdot^-$ to the extracellular space.

The production of human recombinant SOD overcame the problem of immunogenicity, but not of cell penetration. Intravenous bolus injections of 1-45 mg/kg recombinant human superoxide were well tolerated, had no detectable effect on cardiac or renal function, and had a half life of about 4 h (8). However, a major clinical trial of intravenous human recombinant SOD prior to angioplasty in patients with acute myocardial infarction showed no additional improvement compared to placebo-treated patients (9).

Recently, an exploratory study was performed of human recombinant SOD in ALS. Intrathecal infusion of recombinant SOD at a dose of 5 mg/day for 3-6 months increased the levels of SOD in the cerebrospinal fluid of 16 subjects with ALS by 40-fold. The treatment was safe, but there was no detectable improvement in the 2 patients with familial ALS and mutations in the gene for Cu/Zn SOD (10).

The first group of compounds developed as SOD mimetics were the manganese-based metalloporphyrin complexes, which also scavenge hydrogen peroxide, $ONOO^-$ and lipid peroxyl radicals (2). Although these agents have been shown to be effective in models of inflammation, they may not be suitable for use in humans as their widespread effects will disrupt physiological processes.

A major step forward in this field was the development of small-molecule selective SOD mimetics, which also have the advantage of penetrating cell membranes. These selective SOD mimetics catalytically remove $O_2\cdot^-$ without interfering with NO, $ONOO^-$ or other radicals such as hydroxyl radical or hydrogen peroxide.

Selective SOD mimetics

A series of manganese-based SOD mimetics have been developed with increasing catalytic activity. These mimetics are low-molecular-weight, manganese-containing, nonpeptide molecules possessing the function and catalytic rates of native SOD enzymes, but having the advantage of being much smaller molecules.

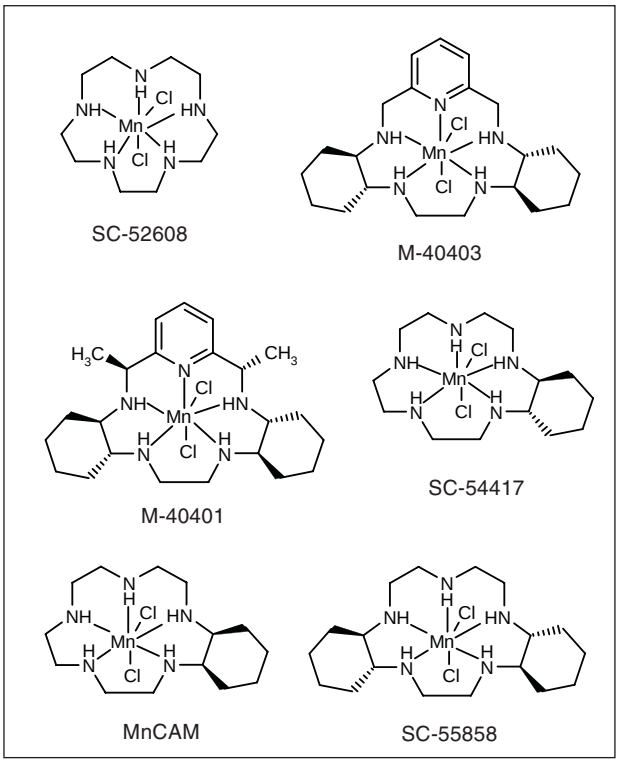

Fig. 1. Chemical structures of selected SOD mimetics.

MnPAM (SC-52608), MnTAM and MnCAM

MnPAM (SC-52608), MnTAM (SC-54417) and MnCAM (Fig. 1) have catalytic rate constants for superoxide dismutation of 4-9 x 10^{+7} M^{-1} s^{-1}. These agents were shown to prevent neutrophil-induced $O_2^{\cdot -}$ mediated human aortic endothelial cell injury *in vitro* (11). Subsequently, these compounds were shown to inhibit neutrophil infiltration and colonic tissue injury induced by intracolonic instillation of dilute aqueous acetic acid in mice *in vivo* (12).

SC-52608, or MnPAM, has protective effects against ischemia/reperfusion damage in the heart and skeletal muscle. Thus, in isolated Langendorff-perfused rabbit hearts subjected to 30 min of global ischemia followed by 45 min of reperfusion, SC-52608 at 20 μM (but not 10 μM) protected against the ischemia/reperfusion damage. SC-52608 decreased the release of creatine kinase and intracellular potassium, and reduced the increase in left ventricular end-diastolic pressure (13). In the anesthetized dog, ischemia/reperfusion damage was caused by 90-min occlusion of the left circumflex coronary artery, followed by 18 h of reperfusion. When SC-52608 was administered 30 and 15 min before ischemia and 15 min and immediately before reperfusion, it reduced the myocardial infarct size (14). In rabbit skeletal muscle, the administration of SC-52608 at the onset of 4 h of ischemia and before reperfusion increased the survival of muscles and functional contractions (15).

SC-52608 was shown to potentiate the effects of endogenous NO and to inhibit platelet-mediated thrombosis in injured and stenotic carotid arteries of anesthetized rabbits. Intravenous administration of 12 mg/kg of SC-52608 also caused a marked decrease in mean arterial blood pressure, which was prevented by N^G-monomethyl-L-arginine, an inhibitor of NO production (16).

SC-55858 and SC-54417

SC-55858 and SC-54417 were developed after SC-52608. SC-55858 is a water-soluble pentaaza macrocyclic Mn(II) complex containing *2R,3R,8R,9R-all-trans*-fused cyclohexano substituents on the carbon atom of the macrocycle (Fig. 1) (17). SC-55858 is an excellent SOD catalyst, with a second-order catalytic rate constant at pH 7.4 of 1.2 x 10^{+8} M^{-1} s^{-1}, rivaling that of the native Mn SOD enzyme (18). SC-54417 is only slightly less potent than SC-55858, with a rate constant of 9.09 x 10^{+7} M^{-1} s^{-1} (19).

Once SC-52608 had been shown to be protective against ischemia/reperfusion injury, further development of SOD mimetics for clinical use required that they be safe for use in animals. In conscious dogs, intravenous infusion of SC-55858 (0.233 and 0.667 μM/kg/min) or SC-55417 (7 and 20 μM/kg/min) increased heart rate and decreased mean arterial pressure and left ventricular systolic and end-diastolic pressures (19). The mechanism underlying these cardiovascular effects is not clear. It is also not clear whether these concentrations correspond to or are higher than protective concentrations.

Numerous cell types release superoxide anions during the inflammatory response, including endothelial cells, epithelial cells, macrophages and neutrophils. The effects of SC-55858 in a rat model of intestinal damage were assessed. The intravenous injection of *Escherichia coli* lipopolysaccharide (LPS) in rats elicits an inflammatory response. This response is characterized by a time-dependent infiltration of neutrophils, lipid peroxidation, microvascular leakage (indicative of microvascular damage) and epithelial cell damage in both the duodenum and jejunum. Administration of SC-55858 (0.03-1 mg/kg) at 3 h after LPS reduced the subsequent increase in microvascular leakage, lipid peroxidation, epithelial cell injury and neutrophil infiltration at 5 h (20). These results suggest that $O_2^{\cdot -}$ plays a role in the pathogenesis of duodenal and intestinal injury during endotoxemia, and that its removal by SC-55858 offers a novel approach to the treatment of septic shock or clinical conditions of gastrointestinal inflammation. Furthermore, the protection of the intestinal epithelium by SC-55858 suggests that it may be useful during chemotherapy and radiation therapy, cancer treatments characterized by gastrointestinal damage (20).

M-40403

M-40403 has a structure similar to SC-55858 (Fig. 1) (21). M-40403, a manganese(II) complex with a

bis(cyclohexylpyridine)-substituted macrocyclic ligand, was designed to be a functional mimic of the SOD enzymes (22). As an SOD catalyst, M-40403 has a second-order catalytic rate constant at pH 7.4 of 1.2 x 10^{+7} M^{-1} s^{-1} (21). The resting oxidation state of M-40403 is the reduced state, or Mn(II) (22). As a consequence, the complex has no reactivity with reducing agents until it is oxidized to Mn(III) by protonated superoxide, whereupon the complex is rapidly reduced back to the Mn(II) state by superoxide anion at diffusion-controlled rates. Since the complex is so difficult to oxidize, many one-electron oxidants (including NO and oxygen) cannot oxidize it. Furthermore, since M-40403 operates via a facile one-electron oxidation pathway, other two-electron nonradical, but nevertheless potent, oxidants are not kinetically competent to oxidize the Mn(II) complex, *e.g.*, $ONOO^-$, H_2O_2, OCl^- (23).

M-40403 is thermodynamically stable, and stable for up to 10 h in whole rat blood at 37 °C (22). After intravenous injection into rats, M-40403 is widely distributed to the heart, lungs, brain, liver and kidney as the intact molecule (20). The complex is excreted intact with no detectable dissociation and is recovered in urine and feces (22).

M-40403 has been shown to be beneficial in several models of inflammation. Intraplantar injection of carrageenan in the paws of rats results in a time-dependent increase in paw volume that is maximal after 3-6 h. Administration of M-40403 (1-10 mg/kg as i.v. bolus) 30 min before carrageenan inhibits the edema. Carrageenan is also associated with infiltration of neutrophils, a marked release of proinflammatory mediators (prostaglandin E_2 [PGE_2], tumor necrosis factor-α [TNF-α], interleukin-1β [IL-1β]) and tissue damage, as evidenced by the release of lactate dehydrogenase. M-40403 inhibits neutrophil infiltration, the release of TNF-α and IL-1β, and the production of lactate dehydrogenase, but has no effect on the release of PGE_2. M-40404, a structural analog of M-40403 which has no SOD activity, had no effect in this model (22).

M-40403 also inhibits the inflammatory response in a rat model of acute pleurisy induced by injection of carrageenan into the pleural cavity. This response is characterized by fluid accumulation in the pleural cavity, which contains a large number of neutrophils. There is also an infiltration of neutrophils in lung tissues and subsequent lipid peroxidation. The production of nitrite/nitrate, PGE_2, TNF-α, IL-1β, IL-6 and IL-10 is increased in this model. M-40403 (5-20 mg/kg) was injected i.p. 15 min before carrageenan. Four hours after carrageenan injection, M-40403 dose-dependently attenuated the fluid accumulation, neutrophil infiltration and production of TNF-α, IL-1β and IL-6, but had no effect on nitrite/nitrate, PGE_2 or IL-10. The pleurisy induced by carrageenan was also associated with upregulation of the adhesion molecules ICAM-1 (intercellular adhesion molecule-1) and P-selectin, as well as nitrotyrosine (a marker of peroxynitrite formation) and poly(ADP-ribose) synthetase (PARS), a nuclear enzyme activated by DNA single-strand damage. M-40403 reduced the staining for ICAM-1 and P-selectin, nitrotyrosine and PARS. A mechanism by which M-40403 attenuates inflammation in this rat model of pleurisy is by reducing $ONOO^-$ formation by removing $O_2{\cdot}^-$ before it reacts with NO. This is important as the proinflammatory and cytotoxic effects of $ONOO^-$ are numerous. Activation of PARS is important in inflammation. Thus, PARS inhibition by M-40403 may also be involved in its antiinflammatory action. A possible mechanism whereby M-40403 inhibits neutrophil infiltration is by inhibiting the expression of adhesion molecules. Finally, $O_2{\cdot}^-$ seems to play an important role in the release of the proinflammatory cytokines TNF-α and IL-1β, as these are inhibited by M-40403 (23).

M-40403 attenuates chronic inflammation, tissue damage and bone damage in arthritis. Collagen-induced arthritis was induced in rats by the intradermal injection of 100 µl of an emulsion of bovine type II collagen in Freund's incomplete adjuvant at the base of the tail, which was repeated 21 days later. These injections induced an erosive arthritis of the hind paws with periarticular erythema and edema in all the hind paws by day 27. There was soft tissue swelling and focal bone resorption, together with osteophyte formation in the tibiotarsal joint. These changes were ameliorated by treatment with M-40403 (2-10 mg/kg/day) starting at the onset of arthritis. M-40403 reduced nitrotyrosine and PARS staining in the inflamed joints (24).

M-40403 may be beneficial for the treatment of inflammatory bowel disease. Inflammatory bowel disease is characterized by oxidative and nitrosative stress, leukocyte infiltration and upregulation of ICAM-1 expression in the colon. In the rat, colitis was induced by intracolonic instillation of trinitrobenzene sulfonic acid, which caused bloody diarrhea and loss of body weight. Colonic damage was characterized by mucosal necrosis, neutrophil infiltration, upregulation of ICAM-1 and P-selectin expression, and high levels of malondialdehyde (an indicator of lipid peroxidation). The colitis increased levels of the cytokines TNF-α and IL-1β. Staining for nitrotyrosine and PARS was also increased in the colitis. M-40403 (5 mg/kg/day i.p.) reduced all of these effects (25).

Shock and ischemia/reperfusion injury were induced in rats by clamping the splanchnic artery. This was associated with neutrophil infiltration into the intestine and lung, profound membrane peroxidation, resulting in high plasma levels of lipid peroxide products such as malondialdehyde, and high plasma levels of TNF-α and IL-1β. Infused for 15 min before reperfusion, M-40403 (0.1-1 mg/kg) inhibited the formation of malondialdehyde, TNF-α and IL-1β, and neutrophil infiltration (measured by myeloperoxidase levels) into the ileum and lung (22).

In a rat model of septic shock, M-40403 has been shown to have some beneficial effects (26). The mortality rate in shock is high. Persistent systemic vasodilatation resulting in multiple organ failure is a frequent cause of death among patients with endotoxic shock. Diverse molecular mechanisms of inflammation and cellular damage have been implicated in shock, including the generation

of superoxide anions and peroxynitrite. Superoxide anion is primarily produced by activated neutrophils and macrophages, and has been associated with the inflammatory response that accompanies tissue damage in septic shock.

The current treatment of shock includes the catecholamines dopamine and noradrenaline to preserve or augment blood flow to vital organs. However, the development of vascular hyporeactivity in shock can limit the effectiveness of these catecholamines. *In vitro*, $O_2^{\cdot -}$ deactivates catecholamines, and this deactivation is inhibited in a concentration-dependent manner by M-40403 (0.1-1 μM). *In vivo,* the ability of noradrenaline to induce a pressor response in anesthetized rats is attenuated in the presence of $O_2^{\cdot -}$. M-40403 (1 μM) protects the vasopressor response to noradrenaline in the presence of $O_2^{\cdot -}$, probably by inhibiting the deactivation of noradrenaline (26).

Administration of *E. coli* LPS (4 mg/kg) to induce shock in rats is associated with the development of hyporeactivity to noradrenaline within 60 min. The hyporesponsiveness to noradrenaline observed 2 h after LPS injection can be reversed by M-40403 (0.25 mg/kg by 15-min i.v. infusion 1 h after LPS). Pressor responses to noradrenaline in rats not treated with LPS are not affected by M-40403. M-40403 also reduced hypotension and mortality in this rat model of shock. Thus, in the absence of M-40403 there was a profound fall in blood pressure and 90% mortality at 9 h. M-40403 prevented the hypotension and reduced the mortality to 10% at 9 h. Even when the administration of M-40403 was postponed to 5 h after LPS, the severe hypotensive phase was reversed and mortality was only 30% at 9 h (26).

The plasma concentrations of noradrenaline, adrenaline and adrenochromes, the product of autooxidation of catecholamines initiated by $O_2^{\cdot -}$, increased after treatment of rats with LPS. Adrenochromes are potential mediators of cytotoxicity and cell damage which have been shown to be cardiotoxic and to cause myocardial necrosis. M-40403 further increased the levels of noradrenaline and adrenaline, while decreasing the levels of the adrenochromes. These results suggest that the deactivation of catecholamines may play a central role in the development of septic shock, and by preserving catecholamines, M-40403 may have potential in the treatment of septic shock (26).

M-40403 does not directly affect diabetes (*e.g.*, blood glucose levels) but does reduce diabetes-induced oxidative stress in the streptozotocin-induced model of diabetes in the rat. In this model, daily injections of 10 mg/kg M-40403 over 3-4 weeks improved the diabetes-induced decrease in endoneurial blood flow. M-40403 also improved diabetes-induced decreases in acetylcholine-mediated vascular relaxation in arterioles that provide circulation to the region of the sciatic nerve, and motor nerve conduction velocity (27).

M-40401

The use of computer-aided design led to the prediction that the 2*S*,21*S*-dimethyl derivative of M-40403, M-40401, should possess superior catalytic SOD activity (Fig. 1). Indeed, M-40401 is a more potent SOD catalyst than M-40403, with a second-order catalytic rate constant at pH 7.4 of $1.6 \times 10^{+9}$ M^{-1} s^{-1} (21). M-40401 has no catalase activity or reactivity with peroxynitrite (28).

M-40401 has been evaluated in a rat model of perfusion injury and shown to have beneficial effects. Occlusion of the splanchnic vasculature followed by reperfusion results in a severe form of circulatory shock, characterized by severe hypotension, hemoconcentration, intestinal injury and a high mortality rate. An important component of this type of shock is endothelial dysfunction, which is attributed to oxygen-derived free radicals released from both the reperfused endothelium and from activated neutrophils. Shock was induced by clamping both the superior mesenteric artery and the celiac trunk for 45 min. This produced an increase in mean arterial pressure, which then decreased until death. Treatment of rats with M-40401 (0.25, 2.5 or 25 μg/kg 15 min prior to reperfusion) attenuated the mean arterial blood pressure increase and migration of neutrophils into the intestine and lung caused by splanchnic artery occlusion shock. M-40401 also attenuated the ileal injury, as well as the increase in tissue levels of myeloperoxidase and malondialdehyde caused by shock in the ileum. Immunohistochemical analysis for nitrotyrosine was positive in ileum from the shocked rats, and this was reduced by M-40401. Reperfused ileal tissue sections from shocked rats showed positive staining for P-selectin and for ICAM-1 in the vascular endothelial cells, and this was reduced by M-40401. M-40401 treatment also improved the survival of the shocked rats from 0% to 100% at 4 h (28). These results demonstrate that M-40401 treatment exerts a protective effect in reperfusion, which may be due in part to inhibition of adhesion molecules and peroxynitrite-related pathways, with subsequent reduction of neutrophil-mediated cellular injury.

Therapeutic use

The next challenge with selective SOD mimetics is to develop therapeutic potential into therapeutic agents. By augmenting the effects of endogenous NO, the SOD mimetic SC-52608 lowered blood pressure. Thus, one of the problems that may be associated with SOD mimetics is overactivity of the NO system. The effects of the SOD mimetics on the cardiovascular, renal and other systems need to be evaluated. The local administration of SOD mimetics may be one way of limiting systemic effects, *e.g.*, injection into inflamed joints. Another approach may be to develop prodrugs that deliver the SOD mimetic locally.

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