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Neurologic Damage and Neurocognitive Dysfunction in Urea Cycle Disorders

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Although the survival of patients who have urea cycle disorders has improved with the use of modalities such as alternative pathway therapy and hemodialysis, neurologic outcome is suboptimal. Patients often manifest with a variety of neurologic abnormalities, including cerebral edema, seizures, cognitive impairment, and psychiatric illness. Current hypotheses of the pathogenesis underlying brain dysfunction in these patients have focused on several lines of investigation, including the role of glutamine in causing cerebral edema, mitochondrial dysfunction leading to energy failure and the production of free radicals, and altered neurotransmitter metabolism. Advances in understanding the pathogenetic mechanisms underlying brain impairment in urea cycle disorders may lead to the development of therapies designed to interfere with the molecular cascade that ultimately leads to cerebral edema and other brain pathological findings.
Semin Pediatr Neurol 15:132–139

The urea cycle consists of 6 enzymes (*N*-acetyl-glutamate synthetase, carbamyl phosphate synthetase [CPS], ornithine transcarbamylase [OTC], argininosuccinate synthetase [AS], argininosuccinate lyase [AL], and arginase) that are responsible for eliminating nitrogenous waste. Partial or complete inactivity of any of these enzymes secondary to an inherited urea cycle disorder (UCD) can predispose patients to episodic, life-threatening hyperammonemia. UCD patients often manifest neurologic problems, especially cognitive impairment, if they survive the acute hyperammonemic episode. Acute hyperammonemia is associated with anorexia, vomiting, and altered mental status. Lethargy, progressing to coma, and possibly death if therapy is not instituted quickly may ensue. Seizures, ataxia, asterixis, slurred speech, tremors, weakness, muscle tone abnormalities, and hypothermia are other common manifestations. Patients who have a partial enzyme deficiency typically manifest outside the neonatal period. Clinical features may be subtle in such late-onset cases, leading to delays in diagnosis. Psychiatric symptoms in late-onset UCDs, including hyperactive behavior, mood disturbances, self-injurious behavior, and psychosis, may occur. A tendency to avoid dietary protein is common.[1] Although disease manifestations tend to be milder in patients with late-onset disease, devastating sequelae, including significant neurologic damage and death, may occur if the diagnosis is not suspected, therefore delaying appropriate therapy.

Acute hyperammonemia affects the brain white matter selectively and causes astrocyte swelling and global cerebral edema. Changes involving the deep insular and perirolandic sulci may be reversible.[1] However, in cases of severe hyperammonemia, permanent changes occur. Neuropathological findings in patients who have neonatal-onset proximal UCDs consist of gross cerebral atrophy, ventriculomegaly, delayed myelination, and the appearance of Alzheimer type II astrocytes, ulegyria, and spongiform degeneration of the cortex, gray-white matter junction, and deep gray nuclei, including the basal ganglia and thalamus.[2-4] This review concentrates on describing current hypotheses on the pathophysiology of brain damage in UCDs, after first reviewing historical survival data and developmental outcomes, as well as electroencephalography and brain-imaging findings in these conditions.

Survival

Historically, most children born with a severe UCD enzyme deficiency died as neonates, and few survived infancy.[5] However, pioneering work by Brusilow et al[6] resulted in the development of alternative pathway medications (intravenous sodium phenylacetate plus sodium benzoate and intravenous arginine hydrochloride) for treating acute hyperammonemic episodes. Prolonged survival and improved clinical outcome

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in UCD patients were noted after the initial use of alternative pathway therapy.[7] Recently, a retrospective study of 299 UCD patients treated with alternative pathway therapy who had undergone a total of 1,181 episodes of hyperammonemia showed an overall survival rate of 84% during the 25-year period of study. Patients with late-onset disease were more likely to survive an episode of hyperammonemia than neonates (98% *v* 73%, $P < 0.001$). Dialysis was used in conjunction with alternative pathway therapy in 60% of neonatal and 7% of late-onset episodes.[8] In contrast, a study of 217 UCD patients who were not treated with alternative pathway medications showed a survival rate of only 16% in patients with neonatal-onset disease and 72% in those with late-onset disease.[9]

Neurodevelopmental Outcome

Although alternative pathway therapy and other therapies, especially hemodialysis, for UCDs has led to improved patient survival, cognitive impairment remains a common finding, especially in those who have neonatal-onset disease.[10] However, the age at which the first symptom is noted is not necessarily predictive of outcome in individual cases because patients who have neonatal-onset disease may still have a normal long-term outcome.[11] In a study of 26 children who survived neonatal hyperammonemia, the overwhelming majority (79%) had 1 or more developmental disabilities at 12 to 74 months of age. Interestingly, IQ correlated with the depth of coma but not the peak plasma ammonium level over a range of 351 μmol/L to 1800 μmol/L.[12] Other studies have found correlation between the peak plasma ammonium level and cognitive outcome.[10,11,13] When the concentration of plasma ammonium exceeded 350 μmol/L at the time of the first episode of hyperammonemia, patients either died or had severe neurologic deficits in a Japanese study of 108 UCD patients.[10] This study was performed by questionnaire, and no mention was made of any specific therapeutic interventions. The surprisingly low level of peak plasma ammonium associated with catastrophic outcome may be related to the lack of availability of intravenous sodium phenylacetate and sodium benzoate (Ammonul, Ucyclyd Pharma, Inc. Scottsdale, AZ) and arginine HCl or other factors. In a European questionnaire study, no surviving UCD patients with an initial plasma ammonium level >300 μmol/L or a peak plasma ammonium level >480 μmol/L had normal psychomotor development.[11] Neonatal-onset OTC deficiency is particularly devastating with respect to neurologic outcome.[13,14] However, prospective neonatal therapy, after prenatal diagnosis by DNA or biochemical analysis, decreases the risk of neonatal hyperammonemia and may lead to a more favorable neurologic outcome in OTC deficiency and other UCDs.[15]

Even in late-onset UCDs, the incidence of neurodevelopmental impairment remains high. Despite a relatively low number of overall hyperammonemia episodes (mean of 4), 14 children who had late-onset disease showed significant impairment. The majority (56%) had mental retardation had seizures, and 13% were cortically blind.[16] A large retrospective study of 217 UCD patients showed that 43% of the late-onset UCD cohort (n = 96) had moderate to severe neurologic impairment.[9] On the other hand, some patients appear cognitively normal for years and even decades until the first presentation of illness. Altered mental status after the physiologic stress of illness or fasting, surgery, sodium valproate use, or related to pregnancy or the postpartum period has been associated with late-onset forms of UCDs.[1,17]

Heterozygote OTC females have variable clinical features related to random X-inactivation and allelic heterogeneity.[18] An estimated 15% of OTC females (designated "manifesting heterozygotes") display symptoms such as protein intolerance, cyclical vomiting, behavioral and neurologic abnormalities, and even episodic hyperammonemic coma.[19] In a recent study of 19 mildly symptomatic and asymptomatic women heterozygous for OTC deficiency, comprehensive neuropsychological testing showed significant weaknesses in fine-motor dexterity/speed and nonsignificant weaknesses in nonverbal intelligence, visual memory, attention/executive skills, and math, despite overall normal IQs.[20] When the patients were divided into symptomatic and asymptomatic groups, the asymptomatic cohort outperformed those who had symptoms in all tested neuropsychological functioning domains. Overall, these findings were considered to support the presence of a nonverbal learning disability in OTC heterozygote females, consistent with selective vulnerability of white matter and better preservation of gray matter.[20]

Electroencephalography

Electroencephalography (EEG) most commonly shows changes of a nonspecific diffuse encephalopathy, although a variety of patterns of abnormality have been described in UCD patients, including multifocal independent spike- and sharp-wave discharges, repetitive paroxysmal activity, unusually low-voltage fast activity, and findings consistent with complex partial seizures.[21-23] EEG may not correlate with plasma ammonium level but still has the potential to provide useful clinical information. For example, the reversal of an initially flat encephalogram may represent an encouraging prognostic factor that could lead the clinician to pursue an aggressive plan of treatment.[1] EEG may also provide information that is complementary to physical examination, biochemical evaluations, and neuroimaging studies. Such an assessment may be particularly helpful in cases of severe coma in which neurologic examination has limited utility.[1] There is a lag in the normalization of the encephalographic tracing after the return of plasma ammonium levels to normal.[21] In late-onset UCD cases, EEG may show continuous semirhythmic activity with sharp components, leading to diagnosis of complex partial status epilepticus.[23]

Neuroimaging

The type of brain injury detected in UCDs varies, but 4 broad

2 by extensive infarct-like abnormality[25]; type 3 by presumably ischemic lesions in cerebral intravascular boundary zones; and type 4 by reversible symmetric cortical involvement of the cingulate gyri, temporal lobes, and insular cortex with sparing of the perirolandic cortex.[24] These types roughly correspond to disease severity and age on onset as follows: type 1, neonatal period or infancy; types 2 and 3, infancy to childhood; and type 4, adulthood.[24] Neonatal hyperammonemic encephalopathy may also be associated with injury to the lentiform nuclei and deep sulci of the insular and perirolandic regions.[24] In the acute phase, cerebral edema affecting both gray and white matter with T1 shortening of the gray matter may resemble hypoxic-ischemic encephalopathy.[1,26] Chronic changes include persistent focal abnormalities in the cerebral hemispheres, diffuse cortical and subcortical atrophy, and development of subcortical cysts and ulegyria.[13,27,28]

Magnetic resonance spectroscopy (MRS) has shown elevations in brain glutamine in hyperammonemic animal models and in UCD patients, consistent with the hypothesis that accumulation of intracerebral glutamine contributes to encephalopathy in UCD patients (see later).[29-33] A study of 6 OTC patients undergoing proton MRS showed myoinositol depletion and elevated brain glutamine plus glutamate concentrations that increased in proportion to the clinical stage of disease in 5 symptomatic patients. In addition, choline depletion was detected in 2 severely affected patients.[34] One OTC patient showed normalization of all metabolites on MRS evaluation after liver transplantation.[34] These studies suggest that it may be possible to monitor metabolic control of UCD patients using MRS.[1]

The Pathogenesis of Cerebral Dysfunction

The precise pathogenic mechanisms involved in causing cerebral dysfunction in UCDs are unknown. However, research has focused on several key areas, especially the role of glutamine in causing cerebral edema, mitochondrial dysfunction leading to energy failure and the production of free radicals, and altered neurotransmitter metabolism.[1]

Cerebral Edema: The Glutamine Hypothesis

Hyperammonemia is known to cause increased cerebral cortical glutamine content, activation of astrocytic glutamine synthetase, and astrocyte swelling.[35] Ammonia diffuses freely across the blood-brain barrier and is rapidly incorporated into glutamine via glutamine synthetase. Glutamine synthetase, a cytosolic enzyme primarily localized to the astrocyte in the brain, catalyzes the following reaction: NH_3 + L-Glutamate + ATP $\rightarrow$ L-Glutamine + ADP + P_i.

This reaction, therefore, represents a short-term means of buffering excess plasma ammonium. However, glutamine has been considered to be an organic osmolyte that increases intracellular osmolarity. This leads to increased cellular volume accumulation may represent an increase in osmolality of as much as 30 mOsm per kg.[35]

In vivo nuclear magnetic resonance spectroscopy has been a useful technique to evaluate cerebral glutamine and glutamate concentrations, the rate of glutamine synthesis, energy metabolism, and intracellular pH in rats undergoing intravenous ammonium infusion.[29] In the hyperammonemic rat model, the degree of hyperammonemia correlates with glutamine synthesis activity up to a point of enzymatic saturation, after which ammonia accumulates in the brain and encephalopathy worsens.[30,36] Neuronal presynaptic terminal glutaminase activity is also increased during hyperammonemia, as is cycling of glutamate between neurons and glia, lending evidence to a neuronal-glial neurotransmitter cycle.[31,32,37] The transport of glutamine from the glia to the extracellular fluid and the uptake of extracellular glutamine into neurons are essential components of this cycle. This cycle couples glial glutamine production to the synthesis of neuronal glutamate (Fig 1). Current evidence supports a predominant role for the sodium-coupled amino-acid transporter as a mediator of neuronal glutamine uptake from the extracellular fluid in adult rat brain in vivo.[38]

The central importance of brain glutamine metabolism in the pathogenesis of cerebral edema has been shown by the inhibition of glutamate synthetase by L-methionine sulfoximine (MSO) in rat models of hyperammonemia. Pretreatment with MSO prevents the increase in brain glutamine levels and water content despite elevated plasma ammonium levels.[39] Hyperammonemia also increases the production of reactive astroglial cytoskeletal components, including the intermediate filament glial fibrillary acidic protein (GFAP) and the gap junction protein connexin-43. The number of GFAP immunopositive cells in the cerebral cortex was decreased by pretreatment with MSO, although no change in connexin-43 was observed.[40] Therefore, glutamine synthetase inhibition reduces astrocyte edema and ameliorates some of the reactive astroglial cytoskeletal changes but does not seem to be involved with gap junction alterations.[40]

However, several lines of evidence are not compatible with glutamine causing cerebral edema because of its action as an osmolyte, including (1) in portacaval-shunted rats infused with ammonia, the initial rise in brain water preceded glutamate accumulation; (2) in the same animal model, mild hypothermia prevented the development of brain edema but had no effect on lowering elevated brain glutamine concentration; (3) some nuclear magnetic resonance studies showed that hypothermia normalized brain water content but did not prevent brain glutamine accumulation; (4) in rats with acute liver failure, glutamine levels did not correlate with degree of cerebra edema; (5) although various compounds reduced ammonia-induced swelling in brain slices, this effect was not correlated to a comparable decrease in glutamine concentration; and (6) ammonia-induced astrocyte swelling was delayed with respect to the rise of cytoplasmic glutamine levels,

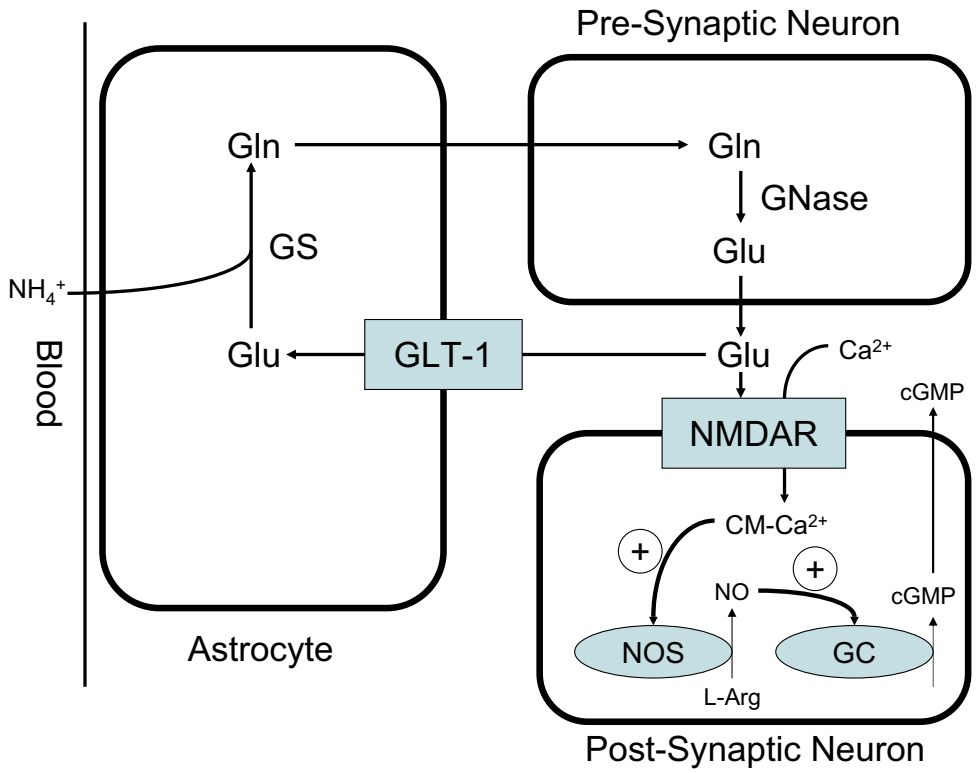

Figure 1 The glutamine-glutamate cycle and NMDA receptor–mediated nitric oxide and cGMP production. The conversion of glutamate to glutamine in astrocytes by glutamine synthetase constitutes the primary mechanism for detoxification of ammonia in the brain (see text). Ammonia removes the NMDA receptor blockade by Mg^{2+} and allows glutamate to activate the receptor. This causes increased calcium flux into the postsynaptic neuron and, in conjunction with calmodulin, stimulation of nitric oxide synthetase. Nitric oxide then activates the soluble form of guanylyl cyclase, which increases cGMP production. Part of the generated cGMP is released into the extracellular matrix.[41,56,59] CM = calmodulin; Gln = glutamine; GLT-1 = glutamate transporter; Glu = glutamate; GS = glutamate synthetase; GNase = glutaminase; L-Arg = L-arginine; NMDAR = *N*-methyl-D-aspartate receptor; NO = nitric oxide; NOS = nitric oxide synthetase; cGMP = cyclic guanosine monophosphate; GC = guanylyl cyclase. (Color version of figure is available online.)

Mitochondrial Energy Failure and Oxidative Stress

Although the "glutamine hypothesis" has been a leading explanation for the development of cerebral edema, recent interest has focused on glutamine-independent mechanisms to explain the pathogenesis of hyperammonemic encephalopathy. One area of active investigation is the role of impaired brain oxidative metabolism in causing cerebral dysfunction associated with hyperammonemia.[41-46] Acute hyperammonemia causes a decrease in brain metabolic rate and decreased high-energy phosphate concentration.[43] Increased production of toxic reactive oxygen species (ROS) by brain mitochondria also occurs, likely secondary to increased formation of these intermediates by the respiratory chain and by xanthine and aldehyde oxidases, as well as decreased activities of free radical-scavenging enzymes, including glutathione peroxidase, superoxide dismutase, and catalase.[44] Ammonium ions also directly inhibit α-ketoglutarate dehydrogenase, which may lead to decreased cerebral energy production.[43] "Peripheral-type" benzodiazepine receptors (PTBRs) are localized to the outer mitochondrial membrane in brain astrocytes and are upregulated control ratio so PTBRs may also play a role in the cellular energy deficit observed during hyperammonemia.[47]

Although glutamine has primarily been considered an osmotically active compound that draws water into the cell when present in high concentration, glutamine also interacts directly with mitochondria. Glutamine enters mitochondria through a histidine-sensitive carrier, a process that is potentiated by ammonia.[42] Phosphate-activated glutaminase is located in the inner mitochondrial membrane and cleaves glutamine into glutamate and ammonia. Because of this localized production of ammonia, intramitochondrial ammonia levels may potentially become very high, leading to the induction of mitochondrial permeability transition (MPT), increased oxidative and nitrosative stress, and astroglial dysfunction.[42,46] The production of ROS and reactive nitrogen species and MPT have been hypothesized to initiate a cascade of events that includes activation of mitogen-activated protein kinases and resultant failure of astrocytes to regulate their intracellular volume.[42] Both treatment with antioxidants and cyclosporine A, an inhibitor of MPT, block

of mitochondrial glutamine transport, completely blocks or significantly attenuates ammonia-induced ROS production, cell swelling, MPT, and loss of adenosine triphosphate.[50] Such findings confirm the importance of glutamine in the production of astrocyte swelling, although its ability to orchestrate an increase in cellular water content may not depend on altered intracellular osmolality. Finally, integral membrane proteins that mediate transmembrane water movement (aquaporins) have also been implicated in ammonia-induced astrocyte swelling. Aquaporin-4 is the most abundant aquaporin in the brain. Cultured astrocytes exposed to high levels of ammonia upregulate aquaporin-4, and this increase precedes the development of astrocyte swelling.[51]

Neurotransmitter Abnormalities

Ammonium ions have a multitude of effects on mammalian neurotransmitters, including systems involving cholinergic, serotonergic, and glutamatergic neurotransmission.[52] Increased brain concentrations of the excitatory amino acid neurotransmitters glutamate and aspartate are present in the sparse-fur (*spf*) mouse, a model of OTC deficiency, which may explain the seizure predisposition in OTC patients.[53] Tryptophan, a precursor of serotonin, and quinolinic acid, an *N*-methyl-D-aspartate (NMDA) receptor agonist known to produce selective striatal cell loss, are also increased in *spf* mice and in rats after portacaval anastomosis.[54,55] In addition, brain pathology in *spf* mice is characterized by a significant loss of medium spiny neurons and increased numbers of reactive oligodendroglia and microglia in the striatum. Ammonia also inhibits high-affinity transport of glutamate in astrocytes, which results in increased extracellular concentration of glutamate.[56] These biochemical and pathological features are suggestive of NMDA-mediated excitotoxic brain injury.[54] The description of an OTC patient who had "stroke-like" episodes is consistent with this theory of neurotransmitter excitotoxicity.[57] Furthermore, in mice exposed to hyperammonemia, either by the induction of acute liver failure or by treatment with ammonium acetate, PTBR upregulation leads to elevated levels of pregnenolone-derived neurosteroids, which have the potential to enhance γ-aminobutyric acid-ergic (GABAergic) neurotransmission.[58] Ammonium ions also depress postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor–mediated currents.[56] α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors mediate fast synaptic transmission and are involved with learning and memory.

Acute hyperammonemia results in the activation of NMDA receptors, which causes increased flux of Na^+ and Ca^{2+} into the postsynaptic neuron. Ca^{2+} then activates calcineurin, which in turn dephosphorylates and activates Na^+-K^+-adenosine triphosphatase, resulting in Na^+ extrusion, consumption of adenosine triphosphate, and increased synthesis of nitric oxide and cGMP (Fig 1).[43,56,59] Excess Ca^{2+} is primarily taken up by the mitochondria, which leads to increased production of ROS and further energy depletion.[43] NMDA receptor blockade prevents brain adenosine triphosphate depletion and increases glutamine synthetase activity, with resulting increase in brain glutamine. These data suggest that ammonia toxicity may be directly related to excessive activation of NMDA receptors, not increased glutamine synthesis per se.[59]

In contrast, chronic hyperammonemia results in a loss of NMDA receptor densities and increased uptake of tryptophan, a precursor of serotonin, into the brain by activation of the L-system carrier.[52,55,56,61] The uptake of tryptophan is further enhanced if plasma levels of branched-chain amino acids are low, as is common in UCD patients who are restricted in dietary protein intake.[61] Serotoninergic symptoms, such as anorexia, altered sleep patterns, and disorders of motor coordination, may be related to the increased brain turnover of serotonin observed in hyperammonemic states.[1,55] The adaptive changes in NMDA receptors that occur in chronic hyperammonemia result in a decrease in excitatory neurotransmission and impaired production of nitric oxide and cyclic guanosine monophosphate (cGMP).[59,62] Decreased cGMP production may inhibit long-term potentiation in the hippocampus. Because long-term potentiation is a long-lasting enhancement of synaptic transmission efficacy, considered to be the basis for some forms of learning and memory, this effect of hyperammonemia may be involved in the abnormal cognitive function observed in patients who have UCDs.[62,63] Abnormal axonal growth, accompanied by decreased creatine and phosphocreatine levels (creatine is essential for axonal elongation), and alteration of brain cytoskeletal elements are also observed in hyperammonemia.[61] Glial fibrillary acidic protein (GFAP) is reduced and microtubule-associated protein-2 and neurofilament protein exhibit decreased phosphorylation, possibly through an effect of ammonia on mitogen-activated kinase function.[61] Although the precise interrelationship between these proposed pathogenetic mechanisms is unclear, it is reasonable to hypothesize that all play at least some role in the mental impairment observed in UCD patients.

Arginase Deficiency

Arginase deficiency has clinical and pathophysiological features that differ from other UCDs and, hence, deserves special mention. Hyperammonemia is rare or, if present, usually mild. The classic neonatal hyperammonemic crisis has been described only rarely in arginase deficiency.[64] Affected patients typically develop signs, including cognitive defects, seizures, spastic paraparesis or paraplegia (more prominent in the lower extremities), brisk tendon reflexes, scissoring, ataxia, and toe walking in later infancy and early childhood.[65] Not surprisingly, a misdiagnosis of cerebral palsy is common. Although elevated plasma arginine levels often point to the diagnosis, the extent of elevation may be mild. Head imaging shows mild cerebral and cerebellar atrophy as well as T2 hyperintensity in the posterior putamen, periventricular

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