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Antioxidant characteristics of L-histidine

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Proprietary formulations of the amino acid L-histidine are under development as pharmaceutical agents because of the molecule's antioxidant and anti-inflammatory properties. L-histidine has been well characterized in terms of probable dietary requirements, plasma and tissue concentrations, pharmacokinetics, metabolism and excretion, and medical conditions related to physiologic handling. Previous experience with histidine dosing in the literature is extensive, and both clinical and preclinical data suggest that histidine administration is very safe. L-histidine has been shown to scavenge both the hydroxyl radical and singlet oxygen ($^1O_2$) in many studies. These interactions may involve free histidine, small histidine-containing peptides such as carnosine, and histidine residues in proteins. Histidine appears to interfere with redox reactions involving iron and perhaps other metal ions and to interact directly with $^1O_2$; the ability of histidine to scavenge $^1O_2$, a toxic oxygen species of increasing concern, has been well established in the laboratory. Many recent studies have demonstrated the therapeutic efficacy of "pharmacologic" doses of L-histidine in animal models of inflammatory conditions, particularly gastrointestinal conditions and cardiac ischemia-reperfusion injury, and have specifically linked the anti-inflammatory capabilities of histidine to its ability to scavenge toxic oxygen species. The maintenance of histidine pools, therefore, may contribute to the body's physiologic antioxidant capacity. Taken together, the data suggest that histidine supplementation could provide a safe, efficacious method to increase antioxidant protection. (J. Nutr. Biochem. 9:308–315, 1998) © *Elsevier Science Inc. 1998*

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Introduction

L-histidine, like most of the amino acids, has been extensively studied from a nutritional and metabolic point of view. Nutritional data, pharmacokinetic data, metabolism and excretion data, medical conditions related to histidine concentration, previous experience with nondietary histidine dosing in humans, and safety data are summarized briefly below.

Nutritional requirements and dietary intake levels

Human dietary requirements for "essential" amino acids such as L-histidine (as well as the issue of whether these amino acids are always "essential" in adults) are controversial.[1] The World Health Organization suggested daily requirement for L-histidine for human adults was 12 mg/kg, or 840 mg in a 70 kg adult. For reference, the amount of L-histidine in 100 g protein from beef (16 g nitrogen) is approximately 3.7 g.[2]

Normal plasma level and pharmacokinetics

Plasma L-histidine in normal, fasted adults ranges from approximately 4 to 20 mg/L, or 26 to 130 μM.[3–7] This plasma concentration is at the lower end of the scale for amino acids on a molar basis. Methionine, at 16 to 30 μmol/L, is the least abundant amino acid in plasma, whereas glutamine, at 390 to 650 μmol/L, is the most abundant. As with most amino acids, intracellular concentrations of L-histidine are greater than those of extracellular fluids; one study found a plasma L-histidine of 80 ± 12 μmol/L in normal male subjects, while the L-histidine in

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homogenized muscle tissue from these subjects was 370 ± 14.5 μmol/L.[7] Changes in the relative proportions of the other plasma amino acids following oral and intravenous (i.v.) loads of L-histidine suggest that cellular uptake of histidine utilizes transport pathways for both basic and neutral amino acids.[8,9]

Pharmacokinetics

The pharmacokinetics of L-histidine administered orally or intravenously in humans are well characterized in the literature. As would be expected for an amino acid, L-histidine is rapidly and extensively absorbed, with the bioavailability of oral doses reaching 80% or higher. Peak plasma concentrations are observed roughly 1 hour after both oral and i.v. doses, and clearance is similar following both routes of administration.[3,4,9] Sitton and colleagues[3] administered 100 mg/kg L-histidine (mean dose 6.4 ± 0.19 g) to 13 normal adults (mean age 39 ± 4 years) and calculated a mean peak plasma concentration (C_{max}) of 158 ± 5 mg/L (8.8-fold above baseline), a time to peak plasma concentration (T_{max}) of 0.72 ± 0.04 hours, and a serum half-life ($T_{1/2}$) of 1.69 ± 0.19 hours. In a companion study, oral doses of 50 mg/kg were administered to six normal female subjects, resulting in a C_{max} of 73.1 ± 6.6 mg/L (4.7-fold above baseline) and a $T_{1/2}$ of 1.14 ± 0.13 hours.[3] Other studies of oral doses of L-histidine administered to normal subjects have shown similar absorption and clearance and have demonstrated that oral doses as low as 3.7 g can produce increases in plasma histidine (1.35-fold above baseline) at least 4 hours after dosing.[5,6,8] An analysis of data from the literature suggests that the relationship of L-histidine dose (oral or intravenous) and peak plasma concentration is linear up to at least 200 mg/kg (M. Wade, unpublished data).

Metabolism and excretion

The pathways involved in human histidine metabolism are well characterized, and several of the required enzymes and breakdown products have been described.[5,10] Metabolic pathways other than incorporation into proteins or biologically important dipeptides involve oxidation and transamination. Histidine is degraded by histidase (histidine ammonia-lyase), an enzyme found primarily in the liver and skin, to urocanate. In the liver, urocanate is metabolized to glutamate in a series of reactions initiated by urocanase. *Trans*-urocanate accumulates in skin, which lacks urocanase, and may be isomerized to *cis*-urocanate in the presence of ultraviolet radiation; the energy absorbed by isomerization may be protective, and *cis*-urocanate has been linked to ultraviolet radiation-induced suppression of contact hypersensitivity.[11,12]

After histidine loading doses, urinary excretion of unchanged L-histidine, urocanate, imidazolepropionic acid, imidazoleacetic acid, and imidazolelactic acid increased.[5] A very small percentage of intravenously administered histidine (estimated at 0.008–0.02% in humans in one study) also may be enzymatically decarboxylated to histamine,[13] although it is unclear whether orally administered histidine increases urinary histamine excretion in humans.[13,14] Behavioral and central nervous system (CNS) effects attributed to increased brain histamine following large histidine doses have been reported in rodents. However, physiologic effects attributable to histamine have not been produced in humans even with very large oral and i.v. doses of histidine.

One intermediate in normal human L-histidine metabolism, formiminoglutamic acid (FIGLU), requires folate for its breakdown. Therefore, in folate-deficient patients, FIGLU accumulates in the urine after L-histidine loading. This observation led to the use of 10- to 15-g oral loading doses of L-histidine in pregnant women and other patients in the 1960s as a diagnostic tool for folate deficiency.[15-17]

Histidine deficiency and hypohistidinemia

To our knowledge, there has been no reported medical syndrome relating to a dietary deficiency of histidine. Hypohistidinemia has been repeatedly reported in patients with rheumatoid arthritis (RA) and anemia associated with uremia. This finding led to several small clinical studies in which patients with anemia and RA were given daily doses of 1.5 to 8 g L-histidine per day for periods of up to 210 days.[18–21] A within-patient, placebo-controlled study of doses of 1 to 8 g/day for an average of 162 days in 33 RA patients suggested improvements in grip strength, walking time, and erythrocyte sedimentation rate (ESR) in most patients.[20] A 30-week, double-blind, placebo-controlled trial of 4.5 g/day L-histidine was then conducted in 60 RA patients. The double-blind trial failed to confirm the findings, although a retrospective subgroup analysis that suggested improvement in a subgroup of patients with the worst disease at the start of the study led the authors to propose future trials in these patients.[21] Nineteen patients received open-label histidine following the end of the study for a mean period of 10 months.

Histidine clearance and metabolism appears to be normal in RA patients, despite the low plasma concentration.[3] Whether hypohistidinemia contributes causally or is merely symptomatic in RA is controversial.[22]

Histidinemia

An inherited deficiency of histidase activity results in an accumulation of histidine in blood, urine, and cerebrospinal fluid. Clinical abnormalities including speech problems and mental retardation have been associated with hereditary histidinemia in some but not all children in whom the biochemical defect has been identified. Low-histidine diets have been developed and apparently used successfully in some affected children.

A screening study of 400,000 Massachusetts newborns by Levy and colleagues found an incidence of 1 in 20,000 for histidinemia (plasma histidine roughly 8- to 12-fold above normal), but none of the 20 affected children showed mental retardation.[23] The authors concluded that hereditary histidinemia may be benign and that dietary therapy may be unnecessary.[23]

Previous human exposure to histidine

A review of studies in the literature over the last 40 years shows that oral or i.v. doses of 1 g/day or more of L-histidine have been administered to approximately 700 subjects, accounting for roughly 30,000 patient-days of exposure. The maximum daily oral dose reported was 64 g.[24] The maximum i.v. dose reported was 30 g in 30 minutes.[25] As described above, L-histidine has been studied as a diagnostic agent for folate deficiency and as a potential therapeutic agent in patients with RA and anemia. Additional studies have examined the effect of histidine on the sleep/waking cycle in normal adults and narcoleptics (because of studies showing modulation of brain histamine in rodents after large histidine doses). Histidine has also been studied as a potential anorectic agent in scleroderma patients and normal subjects (because of the ability of large doses of histidine to increase zinc excretion, at least during the first week to 10 days of dosing, in some subjects.[24,26–28] L-histidine (20–48 g/day for 5–16 days) had no effect on sleep patterns,[29] and doses of 4 g/day or less in adults had no long-term effect on zinc excretion.[28] However, very large doses of L-histidine (48 or 64 g/day for 2–4 days) resulted in minor neurologic symptoms such as taste and smell distortion in scleroderma patients that resolved immediately upon zinc supplementation.[24,30] Histidine's interactions with metal ions and their potential physiologic relevance, particularly with regard to histidine's antioxidant effects, will be discussed in more detail below.

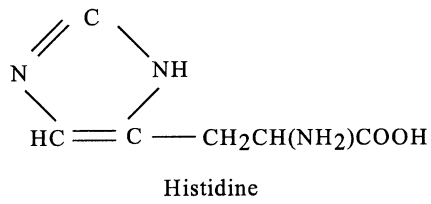
Safety

Other than the mild symptoms observed in a few subjects receiving acute oral doses of more than 40 g/per day, there is little mention in the literature of safety concerns related to acute or chronic dosing with L-histidine. Acute i.v. administration of 30 g in 30 minutes did not produce adverse experiences in one report.[25] Chronic oral dosing in the range of 5 to 10 g/day in RA patients did not suggest any safety concerns.[20,21] The nonclinical studies of histidine in the literature include a 104-week carcinogenicity study in the rat, which showed no carcinogenic potential at doses up to 2.5% of the diet. In metabolic studies, doses of approximately 2000 mg/kg/day for up to 98 days in rat[31] and 330 days in mouse,[32] and approximately 3000 mg/kg for up to 320 days in infant monkeys[33] have been performed. Other than lipidemia in the monkeys, findings in these studies were consistent with metabolic responses to the large amino acid load rather than true toxicities.

Histidine as a physiologic antioxidant

Mechanism of action

Histidine long has been recognized as a scavenger of the hydroxyl radical[34] and of singlet oxygen (delta form of $^1O_2$), a biologically important nonradical toxic oxygen species that is highly reactive because of an excited electron promoted to a higher energy orbital.[35] L-histidine appears to interact chemically with these toxic oxygen species through at least two distinct mechanisms: (1) by interfering with the redox reactions involving metal ions that produce the hydroxyl radical and (2) by direct interactions of the histidine imidazole ring (*Figure 1*) with singlet oxygen.



Histidine

Figure 1 Structure of L-histidine.

Hydroxyl radical. *In vitro* "spin trap" studies using Fenton-type chemistry to generate the hydroxyl radical, [i.e., the reaction of Fe^{2+} complexes of adenosine diphosphate (ADP) or adenosine triphosphate (ATP) and hydrogen peroxide to generate the hydroxyl radical] showed that, when added to the iron-containing reactions prior to the addition of hydrogen peroxide, histidine was among the most effective scavengers among 29 biological compounds tested. These compounds included the other biologically occurring amino acids and several of their derivatives, as well as the proteins albumin, collagen, and histone.[34]

Histidine's ability to bind to and, at high doses, to modulate the absorption of zinc, copper, and iron has been well documented. A potential role for metal binding in histidine's physiologic antioxidant effects was supported in studies by Erickson and Hultin[36,37] involving L-histidine's effects on lipid peroxidation in a fish muscle sarcoplasmic reticulum suspension experimental system. In these studies, L-histidine and certain histidine analogs either stimulated or inhibited the formation of lipid peroxidation products, depending on the order of addition of other free radical-generating reaction components. (These findings may also help to explain several cell-culture studies in the literature indicating that high concentrations of histidine can enhance cellular DNA damage caused by the production of free radicals.) Histidine was found to strongly inhibit the formation of lipid peroxidation products when it was exposed to Fe^{3+} before the iron was added to other reaction components. Erickson and Hultin suggested that, under inhibitory conditions, histidine's ability to inhibit lipid peroxidation may stem from its ability to coordinate with iron, thus preventing reduction of Fe^{3+} to Fe^{2+}.[36,37]

Singlet oxygen. The deleterious effects of singlet oxygen in biological systems have received increasing recognition recently. For example, it has been demonstrated that much of the tissue damage produced by the superoxide anion via the classic Haber-Weiss reaction (reaction of superoxide with hydrogen peroxide to yield molecular oxygen and a hydroxyl radical) may involve singlet oxygen.[38] Evidence is accumulating that singlet oxygen is involved in pathologic processes ranging from ischemia reperfusion injury to ultraviolet-light-induced skin aging and health effects of water chlorination.[39–46] It now has been recognized that part of the protective effect of the lipid-soluble antioxidants beta-carotene, lycopene, and tocopherols, as well as the water-soluble antioxidant ascorbate, is attributable to their

Table 1 Protection by L-histidine against fluid accumulation and tissue damage induced by *S. Typhimurium*

Control	L-histidine	*P*-value
Fluid accumulation (uL/cm of intestinal loop, ± S.E.)		
143 ± 10 (n = 28)	76 ± 14 (n = 22)	0.0002
Morphologic damage (light microscopy)		
Extensive damage, including desquamation of epithelium, loss of villi, infiltration of PMNs	Fewer desquamated cells, villi nearly normal, fewer infiltrated PMNs	—
Ultrastructural damage (electron microscopy)		
Many heavily vacuolated enterocytes, destruction of microvilli on many enterocytes, some mitochondria with swollen cristae	Nearly normal ultrastructure	—

PMN, polymorphonuclear leukocytes.

ability to scavenge singlet oxygen as well as free radical species.[47–50]

Histidine is generally recognized as the most active of the amino acids at scavenging singlet oxygen, with rate constants for reaction with singlet oxygen roughly two- to three-fold higher than those for tryptophan and five-fold higher than those for methionine.[51] Comparisons are difficult because of experimental differences, but in general the chemical rate constant for L-histidine's reaction with singlet oxygen has been calculated in the range of 4×10^7 to 1×10^8 $M^{-1}s^{-1}$.[51,52] For comparison, lipid soluble carotenoids such as beta-carotene are physical quenchers of singlet oxygen, which efficiently accept energy from singlet oxygen with reaction rate constants exceeding 1×10^{10} $M^{-1}s^{-1}$. The water-soluble scavengers histidine and ascorbate are not physical quenchers but rather interact chemically with singlet oxygen: histidine via an endoperoxide and ascorbate via unstable hydroperoxides.[52] The rate constant for the interaction of ascorbate and singlet oxygen may be roughly three-fold higher than the histidine-singlet oxygen rate constant.[52]

Histidine-related compounds

The imidazole ring of L-histidine has been shown to be responsible for the antioxidant activity of several biologically important dipeptides, including carnosine (β-alanyl-L-histidine), anserine (β-alanyl-3-methyl-L-histidine), and homocarnosine (λ-aminobutyryl-L-histidine).[44] Carnosine is found in muscle and brain, and is known to be synthesized by an enzyme in various parts of the brain. Anserine also is found in brain, and homocarnosine is found in cerebrospinal fluid and brain. All three of these compounds play a protective role against oxidative stress in these tissues.[44] Carnosine, which has received the most study, is a more effective singlet oxygen scavenger than L-histidine, although all three compounds have been shown to protect against oxidative DNA damage and against experimentally induced oxidation of liposomes *in vitro*.[44]

Histidine as an anti-inflammatory agent

Histidine's effectiveness as a scavenger of toxic oxygen species, particularly singlet oxygen, has led to many investigations of its efficacy as an antioxidant cytoprotective agent in nonclinical models of inflammation mediated by these species. This work shows that histidine clearly has an anti-inflammatory effect at high local concentrations that is due at least in part to its singlet oxygen and hydroxyl radical scavenging characteristics. Recent work using three models of gastrointestinal conditions is described first, followed by summaries of data from the literature and some additional data from unpublished studies of the potential cytoprotective effects of L-histidine in the CNS, as well as the cardiovascular and respiratory systems. Additional work in a few other animal models and in cell-culture systems is not summarized here. Taken together, these studies suggest a broad protective effect and, in some cases, demonstrate a direct effect of L-histidine on tissue damage known to be mediated by toxic oxygen species.

Gastrointestinal system

Infectious diarrhea. In a mouse model of infectious diarrhea, Peterson and colleagues tested the effect of L-histidine on accumulation of luminal fluid, acute inflammation, and structural damage to the small intestinal mucosa evoked by *Salmonella typhimurium. S. typhimurium* (2×10^8 colony-forming units) was added to sutured loops of mouse intestine, which were analyzed 6 hours later for the pathologies described above. In some experiments, 175 mM L-histidine was added to the bacteria before intraluminal injection and additional doses of L-histidine (100 μL of 175 mM solution) were administered every 2 hours until the animals were sacrificed for examination. Results are shown in *Table 1*.

To examine histidine's ability to reduce the concentration of reactive oxygen species in the intestinal lumen during infection, Peterson and colleagues assessed the capacity of L-histidine to reduce the concentration of these species in U937 human promonocytic cells exposed to lipopolysaccharide (LPS). L-histidine was shown to effectively reduce LPS-mediated oxidation of a dye that fluoresced in response to oxidative stress. In this experimental system, L-histidine was similar in antioxidant capacity to N-acetylcysteine, a cysteine precursor compound known to

[a]Peterson, J.W., Boldogh, I., Popov, V.L., Saini, S.S., and Chopra, A.K. Anti-inflammatory and antisecretory potential of histidine in *Salmonella*-challenged mouse small intestine. Manuscript submitted for publication.

Table 2 Protection by L-histidine against acetic-acid-induced colonic tissue damage and myeloperoxidase activity

Assay	Vehicle	200 mg/kg	300 mg/kg
Histology	14	10*	8*
Macroscopic	6	1*	2*
Myeloperoxidase	5.1	3.5	2.7*

*$P \leq 0.05$ compared with vehicle control; n = 10 animals per group.

have potent antioxidant and anti-inflammatory characteristics in many tissues.[a]

Ulcerative colitis. Keshavarzian and colleagues recently examined the efficacy of L-histidine in a rat model of inflammatory bowel disease in which ulcerative colitis was induced by intra-rectal administration of 4% acetic acid. L-histidine or vehicle was administered intra-rectally 6 hours after the induction of colitis and continued three times per day for 5 days. The rats were sacrificed and colons were assessed by a blinded pathologist for severity of colitis (hyperemia, thickening, and ulceration) and histologically. Myeloperoxidase activity in the colonic mucosa also was measured. Scores in control animals untreated with acetic acid and treated with either L-histidine (200 mg/kg) or vehicle control had similar low scores for the disease parameters. Results are shown in Table 2 for acetic-acid treated animals that received either vehicle or one of two doses of L-histidine (S. Chadhoury, S. Yong, and A. Keshavarian, personal communication).

NSAIDs-induced gastric tissue erosion. The oral administration of indomethacin in rodents produces gastritis similar to that observed in some patients who must take nonsteroidal anti-inflammatory drugs (NSAIDs) on a chronic basis. In the mouse, indomethacin induces mucosal lesions that may be scored by length and number as a numerical index for damage and for the protective effect produced by potential therapeutic agents. Miller has tested oral doses of L-histidine in rats with gastritis produced by 20 mg/kg of indomethacin. Both 10 mg/kg L-histidine and 100 mg/kg L-histidine produced significant, dose-dependent protection, as shown in *Table 3* (M.J.S. Miller, personal communication).

Central nervous system

At least three animal studies have demonstrated a protective effect of L-histidine against pathologic processes in the CNS. At least part of histidine's protective effect in two of these studies may be due to its ability to cross the blood brain barrier.[53] In rats, 250 mg/kg histidine given intraperitoneally was shown to protect against blood brain barrier permeability (BBBP) induced by bacterial infection in a rat model of bacterial meningitis. In this study, 12 rats injected intracisternally with *Haemophilus influenzae* showed a BBBP, measured by the amount of ^{125}I-albumin crossing the blood brain barrier, of 4.75 ± 2.70%. Rats treated with histidine concomitant with bacterial infection ($n = 5$) had a mean BBBP of 1.42 ± 0.45%, and rats treated with histidine 17 hours after the bacteria challenge ($n = 7$) had a mean BBBP of 1.25 ± 0.79%. Histidine alone did not increase the BBBP. Therefore, L-histidine was effective in this model when given either simultaneously with bacterial infection or 17 hours after infection.[54]

L-histidine also was shown to be protective in two studies in animal models of stroke. In recently published work, Kawamoto and colleagues[55] treated male rats with i.v. L-histidine (50 or 100 mg/kg) for 30 minutes before producing transient forebrain ischemia by four-vessel occlusion. Extracellular concentrations of glutamate were measured by cerebral microdialysis, and morphologic changes in the hippocampus were evaluated for delayed neuronal death. Both doses of L-histidine inhibited the elevation of extracellular glutamate produced by transient ischemia ($P < 0.01$) compared with control groups. In addition, the average neuronal density of the L-histidine treated groups was consistently higher 7 days after 10-minute cerebral vessel occlusion ($P < 0.01$) compared to control groups. The authors attributed L-histidine's protective effect to scavenging of singlet oxygen and hydroxyl radical.[55]

L-histidine has also been shown to reduce vasoconstriction produced by induction of subarachnoid hemorrhage in the rabbit. Animals receiving a 50-mg/kg dose of L-histidine 30 minutes before induction of subarachnoid hemorrhage showed a 31% reduction in vasospasm, while a 100 mg/kg dose produced a 52% reduction relative to controls.[56]

Table 3 Protection by L-histidine against indomethacin-induced gastritis

Group	N	Damage Index ± SEM	*P*-value (vs. Indo + vehicle)
Indo	5	37.4 ± 3.6	>0.05
Indo + Vehicle	5	36.4 ± 4.1	–
Indo + 10 mg/kg L-histidine	6	19.3 ± 3.9	<0.05
Indo + 100 mg/kg L-histidine	5	8.2 ± 3.5	<0.001

Indo, indomethacin.

Cardiovascular system

Three nonclinical studies in the literature have shown that histidine directly protects cardiac and vascular tissues from functional and structural damage caused by singlet oxygen. In these studies, rose bengal was irradiated to produce singlet oxygen. In canine heart sarcolemmal vesicles *in vitro*, the activities of both the Ca^{2+}- and Na^{+}-K^{+}-ATPases were protected from singlet oxygen-induced damage by L-histidine. In the case of the Na^{+}-K^{+}-ATPase, the protection was histidine concentration-dependent between 25 and 100 mM, whereas superoxide dysmutase (SOD), catalase, or mannitol had no protective effect. The study of the Ca^{2+}-ATPase directly showed protection of the enzyme by 10 mM histidine and 1 mM ascorbate, whereas SOD and catalase were not protective.[57,58] In a separate study, singlet oxygen inhibited the acetylcholine-mediated relaxation of rabbit iliac arteries after preconstriction by phenlyephrine, and 1 mM histidine restored artery relaxation to 95% of control values.[59]

Additional cardiovascular studies have shown the following results.

- Increased contractility and coronary blood flow, shortened duration of arrhythmias, and reduced ultrastructural damage to myocytes following experimental ischemia by 10 to 50 mM histidine added to reperfusion buffer in the rat.[60]
- Improved left ventricular functional recovery, reduced lactase dehydrogenase in coronary effluent, preservation of high energy phosphates, and protection against lipid peroxidation and morphologic damage in rat heart by 3 mM histidine given 5 minutes before 40 minutes ischemia and added to reperfusion buffer.[61]
- Improved cardiac function and coronary flow, reduction of lactate dehydrogenase release and malondialdehyde formation, and increased release of adenosine and 5'-nucleotidase activity in rat heart by 25 mM histidine in reperfusion solution after ischemia.[62]
- Reduction in infarct size and arrhythmias in rats treated with 3 mM histidine in reperfusion buffer after ischemia.[60]

Respiratory system

An unpublished study by Weintrob and colleagues has shown that rats injected intraperitoneally with endotoxin to produce acute respiratory distress were completely protected from endotoxin-induced lung permeability by a 150 mg/kg i.v. dose of histidine. In this study, rats were injected intraperitoneally either with injection vehicle only ($n = 6$) or with 20 mg/kg endotoxin ($n = 7$). Two measures of acute lung injury, wet lung/dry lung weight ratio and leakage of albumin into the lungs, were used to assess the extent of damage. By both assays, the endotoxin injection led to significant lung damage when compared with that observed in the vehicle-injected control rats. However, i.v. injection of histidine along with the intraperitoneally injected endotoxin completely abolished the increases in wet lung/dry lung ratio and lung leak index in the histidine-treated rats ($n = 4$). With histidine treatment, both these measures of lung damage remained at the levels observed in the rats administered vehicle only.[54]

Discussion

Several lines of evidence suggest that L-histidine may plays a significant role in physiologic antioxidant defenses. L-histidine has long been known as a fairly efficient scavenger of both the hydroxy radical and the nonradical toxic oxygen species singlet oxygen, which is emerging as an important contributing factor in many deleterious oxidative processes. Histidine-containing dipeptides appear to play antioxidant roles in muscle, brain, and other tissues; exposed histidine residues in proteins play an important role in the antioxidant capacity of the protein component of whole plasma.[43,44] In many recent nonclinical studies, L-histidine has been shown to have significant potential as an anti-inflammatory therapeutic agent in a range of diseases, including infectious diarrhea, ulcerative colitis, gastric erosion induced by NSAIDs, cerebral and cardiac ischemia, bacterial meningitis, and respiratory distress syndrome.

L-histidine, like ascorbate, is a hydrophilic scavenger of both hydroxyl radical and singlet oxygen, and it has become clear that both hydrophilic and lipophilic antioxidants are required for antioxidant cytoprotection *in vivo*. Many of the most potent physiologically important antioxidant compounds such carotenes and tocopherols are lipophilic molecules. Furthermore, data indicate that L-histidine is transported into cells by both neutral and basic amino acid transport pathways and that the L-histidine concentration is much higher in cells than in plasma. Therefore, L-histidine may help to provide antioxidant protection within cells even after it has been cleared from plasma.

Histidine's metabolism and pharmacokinetics are very well characterized, and there is considerable human experience with high acute doses and moderate chronic dosing regimens. Although classic toxicology studies with histidine have not been done, the literature contains a wealth of animal data on high chronic doses, including a 2-year carcinogenicity study in rats. No significant safety concerns have emerged from any of these studies. Therefore, supplementation of the diet with doses of L-histidine roughly equivalent to, or possibly somewhat in excess of, the normal adult dietary intake (*e.g.*, 0.5–1 g day) could be a safe method of enhancing antioxidant protection. To our knowledge, the possible antioxidant benefits of histidine supplementation have not been tested clinically.

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