Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer’s Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Ligustilide

Evidence Summary
Protects against inflammatory and oxidative stress damage through induction of Klotho and Nrf2, but has poor stability and bioavailability. Novel formulations will be needed for it to be clinically viable.

**Neuroprotective Benefit:** Protects against inflammatory and oxidative stress induced cognitive impairment in preclinical studies, but suffers from extremely poor stability and bioavailability in its current form.

**Aging and related health concerns:** Protects against inflammatory damage and vascular dysfunction in preclinical studies, but novel bioavailable formulations will be necessary to offer clinically meaningful benefits.

**Safety:** No major adverse events reported in animals, but pure forms of ligustilide have not been tested in humans.
<table>
<thead>
<tr>
<th>Availability:</th>
<th>Dose:</th>
<th>Chemical formula:</th>
</tr>
</thead>
<tbody>
<tr>
<td>In traditional Chinese medicine (Dong quai)</td>
<td>Wide range used, no clinically beneficial dose established</td>
<td>C_{12}H_{14}O_{2}</td>
</tr>
<tr>
<td>Half-life:</td>
<td>BBB:</td>
<td>Source: Pubchem</td>
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<tr>
<td>3-5 hours (in rats)</td>
<td>Penetrant</td>
<td></td>
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</table>

**Clinical trials:** Dong quai has been tested in 2 RCTS for treatment of menopausal symptoms, but no trials have been conducted with pure ligustilide.

**Observational studies:** No human studies for pure ligustilide.

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**What is it?** Ligustilide (Z-ligustilide) is a phthalide that is contained in Angelica subspecies. It is the primary active ingredient in the root of Angelica sinesis, also known as Dong quai, which is an herb used in traditional Chinese medicine. It is commonly used for cardiovascular health, relief from menstrual pain and menopausal symptoms, such as hot flashes. Ligustilide has anti-inflammatory and antioxidant activity through the induction of Klotho and the Nrf2 antioxidant system.

**Neuroprotective Benefit:** Protects against inflammatory and oxidative stress induced cognitive impairment in preclinical studies, but suffers from extremely poor stability and bioavailability in its current form.

**Types of evidence:**

- Several laboratory studies

*Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function?* None

*Human research to suggest benefits to patients with dementia:* None
Mechanisms of action for neuroprotection identified from laboratory and clinical research:

While there have been no human studies examining the efficacy of ligustilide for dementia prevention, rodent studies have provided evidence of its neuroprotective capabilities. Furthermore, its projected mechanism of action involves induction of two of the body’s most potent defenses against oxidative stress and inflammatory damage: Klotho and the Nrf2 antioxidant system. Due to its poor stability and bioavailability profile [1; 2], novel formulations of ligustilide would need to be developed in order for it to be a viable option for testing in clinical trials.

Alzheimer’s disease: Potential benefit (rodents)

In the APP/PS1 transgenic Alzheimer’s disease (AD) mouse model, ligustilide was able to reduce the progression of cognitive impairment and AD related pathology through induction of Klotho and the reduction of oxidative stress.

Ligustilide treatment (40 mg/kg daily for 15 days) attenuated neuronal loss and Aβ-associated pathology (accumulations of Aβ, APP, p-Tau) in rats following intracerebral injection of Aβ25-35 [3]. The treated rats also had better performance on a spatial memory task (Morris water maze) than untreated animals. Nine month-old AD mice treated with ligustilide (40 mg/kg daily intragastric gavage) for 8 weeks showed improvements in spatial memory on the Morris water maze test, including shortened escape latency, and increased time in platform quadrant [4]. This was accompanied by decreased levels of senile plaques, more structurally intact mitochondria, enhanced mitochondrial respiratory capacity, decreased oxidative stress (ROS, MDA), and less synaptic loss in the hippocampus. Similar cognitive and neuronal protecting benefits were seen with ligustilide treatment (30 mg/kg daily for 14 weeks) in 12-month-old AD mice. In these animals, the neuroprotection was attributed to an increase in Klotho [5]. Ligustilide may act as an enhancer of the protease ADAM10, which promotes the alpha-cleavage and processing of APP, and cleavage of transmembrane Klotho to increase the level of soluble-Klotho. Ligustilide also promotes the expression and secretion of Klotho in the choroid plexus.

Cognitive aging: Potential benefit (rodents)

The Klotho induction and antioxidant activities of ligustilide may help protect against aging-associated cognitive decline.

In 10-month-old senescence-accelerated (SAMP8) mice, ligustilide treatment (40 mg/kg daily for 8 weeks) reduced Aβ1-42 accumulation, p-Tau levels, and neuronal loss, and improved performance on short term (Y maze) and long-term (passive avoidance) memory tasks compared to vehicle [3]. The reduction of AD pathology was correlated with the upregulation of Klotho expression in the choroid plexus.
plexus. Klotho led to the induction of the transcriptional FOXO oxidative stress resistance system by regulating the phosphorylation of FoxO1 and Akt. The antioxidant induction capacity of ligustilide was also able to attenuate accelerated-aging/cognitive impairment induced by reactive oxygen species (ROS) generating d-galactose [6].

**Stroke: Potential benefit (rodents)**

Ligustilide provides neuroprotection in ischemic rodent stroke models (cerebral artery occlusion) by **reduction of inflammation and oxidative stress**. However, ligustilide was more effective at reducing ischemia-related pathology than preventing neurological impairments. The beneficial effects appear to be mediated by the **induction of CNS Klotho and the Nrf2 antioxidant system**.

Ligustilide treatment has been shown to prevent neuronal loss (similar to sham controls)[7; 8; 9; 10; 11; 12], attenuate astroglial and microglial activation [12; 13; 14], greatly reduce induction of pro-inflammatory mediators (NF-kB, TNF-α, IL-6, IL-1β, TLR4) [8; 12; 14], increase anti-inflammatory mediators (IL-10) [12], and reduce levels of oxidative stress (decreased MDA, MPO, and increased SOD) [12; 13]. Treatment also reduced the permeability of the blood brain barrier (BBB) by regulating expression of tight-junction adhesion proteins (occludin, MMP-2, MMP-9, ICAM-1), leading to less brain edema [9; 10; 12; 15]. Ligustilide (32 or 40 mg/kg) administered 2 hours after ischemic injury, reduced infarct volumes to approximately 40% of the infarct size in untreated animals [11; 12], and had similar efficacy as the antioxidant edavarone [11].

Ligustilide promotes activation (nuclear translocation) of Nrf2 and induction of its antioxidant pathway, including expression of the target gene HO-1 [11]. It also reduces oxidative stress damage through activation of Klotho, via modulation of the FOXO transcription factors (by inhibiting phosphorylation of FoxO1 and Akt)[8] and the peroxiredoxin redox system (by reducing secretion of Prx6) [12]. Prx6 promotes the TLR4 signaling pathway and associated microglial activation. In a stroke model (MCAO), ligustilide (40 mg/kg) reduced Prx6 levels to 2.3±1.2 times the level in sham animals, while Prx6 levels increased to 11.7±3.1 times higher than sham in untreated rats following stroke [12].

Despite, the highly neuroprotective effects which reduced neuronal pathology toward levels seen in sham controls, ligustilide treatment was only partially protective in preventing neurological deficits. Neurological deficit scores were reduced up to half relative to untreated animals with MCAO, and ligustilide treated animals had better performance on the Morris water maze, however, they still had significant neurological impairments relative to sham controls [7; 9; 11; 12; 13; 14].
Ligustilide has also been tested for its ability to improve the therapeutic efficacy of adipose-derived stem cells in the MCAO stroke model. Pre-treatment of the stem cells with ligustilide did not enhance their ability to promote recovery [16]. Additionally, high doses of ligustilide (>10 μg/mL) interfered with the proliferation and viability of the stem cells in culture.

APOE4 interactions: Unknown

Aging and related health concerns: Protects against inflammatory damage and vascular dysfunction in preclinical studies, but novel bioavailable formulations will be necessary to offer clinically meaningful benefits.

Types of evidence:
- 2 clinical trials (for ligustilide containing traditional Chinese medicine for menopause)
- Numerous laboratory studies

Inflammation: Potential benefit (rodents)

Ligustilide treatment has been shown to exhibit anti-inflammatory activity in rodent models.

Pretreatment of ligustilide (i.v.) prior to lipopolysaccharide (LPS), attenuated intrathecal LPS-mediated inflammatory pain by reducing the induction of pro-inflammatory mediators (TNF-α, IL-1β, and IL-6) and microglial activation in the spinal cord [17]. Spinal pain induced by injection of Freund’s adjuvant was also alleviated by treatment with ligustilide (60 mg/kg i.v. for 4 days) starting 1 day after injection [18]. The pain relief was mediated by inhibition of TLR4 activation and signaling, likely due to Klotho induction. Pretreatment with Angelica sinensis extract (active ingredients are ligustilide and ferulic acid) was also able to inhibit TNF-α and protect mice against LPS-induced endotoxic shock [19]. Treatment increased survival from 18% to 44%. Ligustilide (40 mg/kg daily for 8 weeks) was also beneficial in reducing cartilage damage and inflammation (TNF-α, IL-6) in a mouse model of osteoarthritis [20].

A nanoemulsion formulation of ligustilide given orally at 20 mg/kg was found to significantly improve the absorption rate (Cmax and AUC), and was superior to untreated ligustilide in reducing inflammation (TNF-α, IL-1β, VEGF, IL-17) in a mouse model of endotoxin-induced eye inflammation (uveitis) [21]. Most notably, this formulation was found to be stable at room temperature for at least 90 days, whereas untreated ligustilide decomposes rapidly (oxidizes) at high temperature [2].
**Vascular dysfunction: Potential benefit (rodents)**

Ligustilide inhibits inflammation and promotes vasodilation through Nrf2 and regulation of voltage-gated calcium channels. In human umbilical vein endothelial cells, ligustilide suppressed monocyte activation, NF-κB activation, and ROS production in response to inflammatory stimuli (TNF-α) [22]. It also promoted the activation (nuclear translocation) of Nrf2 and nitric oxide (NO) production. Ligustilide was able to dose-dependently relax pre-constricted rat arteries by inhibiting receptor-mediated calcium influx and release [23].

**Cancer: Potential benefit (rodents)**

Ligustilide was shown to have anti-tumorigenic properties in cell culture, and possibly utility in helping overcome chemoresistance. In an ovarian cancer cell line, ligustilide promoted oxidative-stress mediated apoptosis [24], and helped to re-sensitize tamoxifen-resistant breast cancer cells by inhibiting autophagy [25]. In a mouse xenograft model, mice treated with Angelica sinesis extract (500 mg/kg s.c.) had reduced glioblastoma tumor size (at day 38) (155.1± 56.4 mm³) compared to controls (849.9 ±150.1 mm³, p<0.05)[26].

**Menopausal symptoms: No benefit**

*Angelica sinesis* (Dong quai) has long been used to provide relief from post-menopausal symptoms because it was believed to have estrogenic effects, however, clinical trials demonstrated that it is no more effective than placebo and does not have estrogen-like effects.

In a Phase 2 non-placebo-controlled RCT of 60 menopausal women treatment with Dang Gui Buxue Tang (composed of Astragalus membranaceus and Angelica sinesis) for 12 weeks improved psychosocial scores on the Menopause Quality of Life scale (P=0.013) relative to baseline, but had no effects on estrogen or luteinizing hormones, or blood lipid content [27]. A separate 24-week placebo-controlled trial of 71 post-menopausal women found that Dong quai (*Angelica sinesis*) treatment was not better than placebo in reducing hot flashes and did not produce any estrogen-like effects on endometrial thickness or vaginal maturation [28].
Safety: No major adverse events reported in animals, but pure forms of ligustilide have not been tested in humans.

Types of evidence:

- 2 clinical trials (for ligustilide containing traditional Chinese medicine for menopause)
- Numerous laboratory studies

Since pure ligustilide has not yet been tested in humans, its safety profile is not known. Dong quai is rated as possibly safe for occasional use by WebMD, but may increase sensitivity to the sun, and there is not enough information to determine whether long-term chronic use is safe. In clinical trials, women taking Dong quai with warfarin were at an increased risk for bleeding [27], and it should not be taken in conjunction with other blood thinners. According to Drugs.com, Dong quai has moderate interaction with 77 drugs, which are primarily anticoagulants.

Sources and dosing:

Ligustilide can be found in a variety of traditional Chinese medicines, Dong quai (*Angelica sinesis*), Dang gui buxue tang, Danzhi xiaoyao san, and Rhizoma chuanxiong. Doses range widely (up to 15g/day), but no clinically beneficial dose has been established. None of these preparations have been rated by reputable sources. Additionally, these herbs contain a variety of other active compounds and due to its poor oral bioavailability, it is unlikely that the consumption of these herbs would provide levels of ligustilide sufficient to provide clinically meaningful benefits. Due to extensive first pass metabolism in the liver, the oral bioavailability in rats was estimated to be 2.6% [1].

**Novel formulations are needed** for ligustilide to be clinically viable. There have been some efforts to improve the stability and oral bioavailability of ligustilide. A nanoemulsion improved the pharmacokinetic profile, and *in vivo* anti-inflammatory activity of ligustilide in rats [21]. A complex of ligustilide with hydroxypropyl-β-cyclodextrin, which increases the solubility of lipophilic drugs, improved oral bioavailability to 35.9% [29].

Research underway:

According to Clinicaltrials.gov, there are currently 6 active or recruiting trials using traditional Chinese preparations that include ligustilide containing *Angelica sinesis*. Notably, one of these is a retrospective study examining the effectiveness of Chinese herbal medicine in Alzheimer’s disease (NCT03221894).
Search terms:

Pubmed, Google: Ligustilide or Angelica sinesis or Dong quai +

Dementia, Alzheimer’s disease, neuroprotection, neurodegeneration, stroke, cognitive, klotho, Nrf2, aging, lifespan, cardiovascular, inflammation, cancer, menopause, clinical trials, safety, meta-analysis, pharmacokinetics, bioavailability

Websites visited for Ligustilide:

- Clinicaltrials.gov (Dong quai)
- Drugs.com (Dong quai)
- WebMD.com (Dong quai)
- PubChem (z-ligustilide)
- DrugBank.ca (Angelica sinesis)
- Patientslikeme.com (Dong quai)

References:


15. Li J, Yu J, Ma H et al. (2017) Intranasal Pretreatment with Z-Ligustilide, the Main Volatile Component of Rhizoma Chuanxiong, Confers Prophylaxis against Cerebral Ischemia via Nrf2 and HSP70 Signaling Pathways. *Journal of Agricultural and Food Chemistry* 65, 1533-1542. [https://doi.org/10.1021/acs.jafc.6b04979](https://doi.org/10.1021/acs.jafc.6b04979)


18. Qian B, Li F, Zhao L-X et al. (2016) Ligustilide Ameliorates Inflammatory Pain and Inhibits TLR4 Upregulation in Spinal Astrocytes Following Complete Freund’s Adjuvant Peripheral Injection. *Cellular and Molecular Neurobiology* 36, 143-149. [https://doi.org/10.1007/s10571-015-0228-0](https://doi.org/10.1007/s10571-015-0228-0)


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