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.

Dihexa

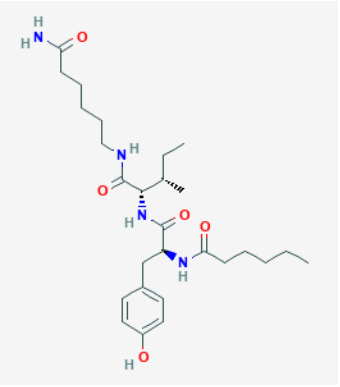
Evidence Summary

Dihexa improves cognition and increases synapses in rat models of cognitive dysfunction, but no studies have evaluated its long-term safety, including potential tumorigenenic and cancer metastatic effects.

Neuroprotective Benefit: In rat models of cognitive dysfunction, dihexa treatment improves cognition and increases spines/synapses. Dihexa did not improve cognitive functions in rats with normal cognition. No studies in humans have been published to date.

Aging and related health concerns: No studies have examined dihexa for age-related diseases. Theoretically, dihexa via activation of HGF and c-Met could promote tumorigenesis and cancer progression.

Safety: No studies in animals or humans have examined the long-term safety of dihexa. Dihexa has a long half-life and there are theoretical concerns of c-Met activation leading to tumorigenesis and cancer progression.

Availability: not available	**Dose**: Not established. In rats, dihexa has been tested orally at 2 mg/kg dose, i.c.v. at up to 1 nmol, i.v. at up to 10 mg/kg dose, and i.p. at up to 20 mg/kg dose.	**Chemical formula:** $C_{27}H_{44}N_4O_5$ **MW**: 504.7  Source: PubChem
Half life: 12 days following i.v. administration and 8.8 days following i.p. administration in rats; unknown for humans	**BBB**: penetrant based on rodent studies	
Clinical trials: none to date	**Observational studies**: none to date	

What is it? Dihexa, also known as N-hexanoic-tyrosine-isoleucine-(6) aminohexanoic amide, is an oligopeptide derived from angiotensin IV (Ang IV). Ang IV is susceptible to metabolic degradation and does not cross the blood-brain barrier (Ho and Nation, 2018). N- and C-terminal modifications of Ang IV have resulted in increased hydrophobicity and metabolic stability, leading to dihexa and other related compounds. Dihexa binds with high affinity to the hepatocyte growth factor (HGF) and promotes mesenchymal-epithelial transition factor (c-Met) signaling and HGF-dependent cellular activity (Benoist et al., 2014). Aside from its essential actions in embryonic development, activation of the c-Met receptor later in life can stimulate neurogenesis and protect against tissue insults in many types of cells including neurons (Wright and Harding, 2015). These activities have been linked to improved cognitive functions and increased dendritic arborization, spinogenesis, and synaptogenesis (McCoy et al., 2013). Of the Ang IV and its analogs, dihexa has been noted to be potentially the most promising for future testing in humans given the hydrophobicity, metabolic stability, blood-brain-barrier penetrance, and preclinical data showing cognitive benefits (Ho and Nation, 2018).

Dihexa was originally developed by Joseph Harding, PhD, and his laboratory at Washington State University. M3 Biotechnology, a Washington State University spin-off company, was formed to further develop dihexa and related compounds (WSU.edu). Further development led to NDX-1017. M3 Biotechnology was renamed Athira Pharma in 2019 and NDX-1017 was renamed ATH-1017 (AlzForum). ATH-1017 is being tested in clinical trials for Alzheimer's disease and Parkinson's disease.

Neuroprotective Benefit: In rat models of cognitive dysfunction, dihexa treatment improves cognition and increases spines/synapses. Dihexa did not improve cognitive functions in rats with normal cognition. No studies in humans have been published to date.

Types of evidence:

- Several laboratory studies
- Several reviews of Ang IV and its analogs

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:
None available.

Human research to suggest benefits to patients with dementia:
None available.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:
In a systematic review of preclinical studies assessing cognitive effects of renin-angiotensin system peptides, 8 of 9 studies found Ang IV and its analogs were effective in improving performance on spatial working memory and passive avoidance tasks (Ho and Nation, 2018). Dihexa has been studied in a few studies in rodents and cell culture. Based on a study in rats using [^{3}H]dihexa and [^{14}C]inulin infusions, dihexa was found to penetrate the blood-brain barrier and accumulate in the brain (McCoy et al., 2013).

In a rat model of cognitive dysfunction induced by scopolamine (via i.c.v.), dihexa treatment given through an i.c.v. cannula (0.1 or 1.0 nmol) significantly improved cognitive performance as measured by the Morris water maze (latency to find the hidden platform), when compared with the scopolamine group (McCoy et al., 2013). The high-dose i.c.v. group (1.0 nmol) was indistinguishable from the scopolamine-untreated control group on all testing days. Further studies showed that intraperitoneal and oral delivery routes of dihexa were also effective. The higher doses of each method of delivery (i.p. at 0.5 mg/kg/day; oral at 2.0 mg/kg/day) produced performances on the water maze that were significantly better compared to scopolamine-treated, dihexa-untreated rats ($p<0.001$), and indistinguishable from scopolamine-untreated (vehicle) controls. In the probe trials (time spent in the target quadrant; used to evaluate the strength and persistence of the spatial memory), dihexa at its highest dose (regardless of the delivery method) significantly increased the time spent in the target quadrant compared with the scopolamine-impaired groups and was not different from scopolamine-untreated controls. There was also a dose-response relationship of dihexa on the probe trial results.

In aged rats (24 months old), orally delivered dihexa (2 mg/kg/day) improved cognitive function as measured by the Morris water maze and this effect was statistically significant on most test days (McCoy et al., 2013). Because some aged rats do not exhibit cognitive decline, the results showed high variability and were not as robust as the data seen with scopolamine.

In rat hippocampal neuronal culture, dihexa administration for 5 days increased the number of spines by nearly 3-fold (41 spines per 50-µm of dendrite with dihexa versus 15 spines with vehicle)(McCoy et al., 2013). An acute 30-minute application of dihexa (10^{-12} M dose) also increased spines on neurons as well as the spine-head width, indicative of larger synapses implicated in memory functions (Hara et al., 2012). The mean spine-head width was 0.80 µm with dihexa treatment and 0.67 µm for vehicle control (McCoy et al., 2013).

These newly formed spines/synapses contained the same synaptic machinery as already-existing spines, as indicated by the presence of VGLUT1, synapsin, and PSD-95. The synapses that were newly formed were also confirmed to be functional (based on a corresponding increase in AMPA-mediated mEPSCs).

A follow-up study to the above revealed that the cognitive and spine/synaptic effects of dihexa were mediated by HGF/c-MET (Benoist et al., 2014). Dihexa bound to HGF with high affinity, inhibited HGF dimerization, and acted synergistically with HGF to promote c-Met signaling, including cellular scattering, decreased cell adhesion, and increased cell motility and proliferation. Dihexa and HGF also acted synergistically to increase hippocampal neuronal spinogenesis.

An HGF antagonist (Hinge) delivered intracerebroventricularly blocked the procognitive effects of orally-delivered dihexa, as measured by the Morris water maze test (Benoist et al., 2014). It is worth highlighting that the HGF antagonist alone, or dihexa in the absence of scopolamine, had no effect on cognitive performance, suggesting that the HGF/c-Met system is not engaged during normal learning under healthy conditions. The HGF/c-Met signaling has been shown to be upregulated in neurodegenerative diseases, injury, and stroke (Kato et al., 2003; Shimamura et al., 2007; Salehi and Rajaei, 2010). It is thought that the HGF/c-Met system is designed to respond to injury and support synaptic plasticity and regeneration.

In a press release, the authors of the 2013 publication noted that dihexa was seven orders of magnitude more powerful than BDNF, based on cell culture assays of new neuronal connections (ScienceDaily). However, effects of BDNF and dihexa were not directly compared in the publication (McCoy et al., 2013).

APOE4 interactions: Unknown.

Aging and related health concerns: No studies have examined dihexa for age-related diseases. Theoretically, dihexa via activation of HGF and c-Met could promote tumorigenesis and cancer progression.

Types of evidence:

- Several laboratory studies
- Reviews on HGF/c-Met

There have not been any studies that have tested dihexa for age-related diseases. There is a theoretical concern for dihexa's mechanism of action. Activation of HGF and c-Met is a key signaling pathway in many cancers (Mulcahy et al., 2020; Meng and Chen, 2021; Fu et al., 2021). In cancer cells, HGF and MET are often overexpressed, and this overexpression correlates with tumorigenesis, metastasis, and poorer prognosis. No studies have tested the long-term safety of dihexa treatment including its potential effects on tumorigenesis and cancer progression.

In preclinical studies, dihexa has been used as one of the growth-factor-free small molecules to differentiate human pluripotent stem cells to hepatocyte-like cells (Siller et al., 2015; Mathapati et al., 2016). Dihexa is used during the hepatocyte maturation phase where hepatoblasts are differentiated to hepatocyte-like cells. In this phase, 100 nM dihexa, and 100 nM dexamethaxone are administered for 10 days.

Safety: No studies in animals or humans have examined the long-term safety of dihexa. Dihexa has a long half-life and there are theoretical concerns of c-Met activation leading to tumorigenesis and cancer progression.

Types of evidence:

- Several laboratory studies

There are no publications documenting the long-term safety of dihexa in humans or animals.

There is a theoretical concern for dihexa binding to HGF and augmenting c-Met signaling as the HGF/c-MET signaling pathway is a key signaling pathway in many cancers (Mulcahy et al., 2020; Meng and Chen, 2021; Fu et al., 2021). No studies have tested the long-term safety of dihexa treatment including its potential effects on tumorigenesis and cancer progression.

In preclinical studies, dihexa solutions were prepared by suspending dry-stock dihexa in dimethyl sulfoxide (DMSO) at 1 mg/ml, and subsequent serial dilutions in 50% DMSO or HPLC-grade water (McCoy et al., 2013). The rats received dihexa dissolved in 75% DMSO intravenously (via a jugular vein catheter) or intraperitoneally. The typical injection volume was 200 µl, yielding an estimated DMSO blood concentration of 0.46%. While DMSO is FDA-approved to treat certain conditions (e.g., painful bladder syndrome), DMSO can cause serious side effects when used in high concentrations (WebMD),

In rats, dihexa exhibited a long half-life ($T_{1/2}$) of 12.68 days following i.v. administration and 8.83 days following i.p. administration (McCoy et al., 2013). Dihexa had a serum half-life of 335.5 minutes in a study in rat serum.

Drug interactions: Drug interactions have not been studied for dihexa. Some sources recommend against using dihexa at the same time as other nootropic or psychoactive substances (Ergogenic Health).

Sources and dosing: Dihexa was originally developed by Joseph Harding, PhD, and his laboratory at Washington State University. M3 Biotechnology, a Washington State University spin-off company, was formed to further develop dihexa and related compounds including NDX-1017 (also known as ATH-1017)(WSU.edu; AlzForum).

In preclinical studies, dihexa was synthesized in Dr. Harding's laboratory using 9-fluorenylmethoxycarbonyl-based solid-phase methods (McCoy et al., 2013). Stock dihexa was kept in powder form and stored at −20°C. Dihexa solutions were prepared by suspending dry-stock dihexa in DMSO at 1 mg/ml, and subsequent serial dilutions in 50% DMSO or HPLC-grade water. The rats received dihexa dissolved in 75% DMSO intravenously (via a jugular vein catheter) or intraperitoneally. The typical injection volume was 200 µl, yielding an estimated DMSO blood concentration of 0.46%.

In rats, dihexa has been tested at up to 2 mg/kg; orally, at up to 1 nmol dose, i.c.v.; at up to 10 mg/kg, i.v.; and at up to 20 mg/kg, i.p. (McCoy et al., 2013; Benoist et al., 2014).

Dihexa is not available commercially in the US. However, there are private clinics that may administer dihexa therapy.

Research underway: No ongoing clinical trials are testing the efficacy of dihexa based on ClinicalTrials.gov. There are also no programs specifically testing dihexa that are currently funded by the NIH. ADDF funded Athira Pharma's early work of dihexa (PI, Joseph Harding, PhD), but since then Athira has moved on to develop ATH-1017.

There are several clinical studies testing ATH-1017, a brain-penetrant small molecule that also activates the HGF and MET receptor system; these trials are in mild-to-moderate Alzheimer's disease and Parkinson's disease (ClinicalTrials.gov). Exclusion criteria for these trials include people with a history of or newly diagnosed malignant tumor, epilepsy, myocardial infarction or unstable angina, clinically significant cardiac issues (e.g., arrhythmia, cardiomyopathy), hypertension, hepatic impairment, and others.

Search terms:
Pubmed, Google: dihexa, PNB-0408

Websites visited for dihexa:

- Clinicaltrials.gov (0)
- NIH RePORTER (0)
- Examine.com (0)
- DrugAge (0)
- Geroprotectors (0)
- PubChem
- DrugBank.ca (0)
- Cafepharma (0)
- Pharmapro.com (0)

***Disclaimer**: Cognitive Vitality Reports® do not provide, and should not be used for, medical advice, diagnosis, or treatment. You should consult with your healthcare providers when making decisions regarding your health. Your use of these reports constitutes your agreement to the **Terms & Conditions**.*

If you have suggestions for drugs, drugs-in-development, supplements, nutraceuticals, or food/drink with neuroprotective properties that warrant in-depth reviews by ADDF's Aging and Alzheimer's Prevention Program, please contact INFO@alzdiscovery.org. To view our official ratings, visit Cognitive Vitality's Rating page.