Nutrition and Metabolic Insights

ORIGINAL RESEARCH

Decreased Serum Hepatocyte Growth Factor (HGF) in Individuals with Anxiety Increases After Zinc Therapy

A.J. Russo

Research Director, Health Research Institute/Pfeiffer Treatment Center, 4575 Weaver Parkway, Warrenville, Illinois 60555, USA. Corresponding author email: ajrusso@hriptc.org

Abstract

Aim: To assess serum Hepatocyte Growth Factor (HGF) levels in individuals with anxiety and to test the hypothesis that there is a relationship between HGF levels and zinc therapy.

Subjects and methods: Serum from 19 individuals with anxiety and 19 controls were tested for serum HGF using ELISAs. HGF serum concentration in individuals with anxiety before zinc and anti-oxidant therapy was compared to levels after therapy. Zinc and copper levels in anxiety patients, pre and post therapy, were also measured and compared.

Results: Individuals with anxiety had significantly lower serum levels of HGF compared to controls ($P = 0.0005$). HGF concentration rose significantly (normalized) after zinc therapy ($P = 0.04$) and zinc levels increased significantly in these same patients ($P = 0.0002$).

Discussion: These results suggest an association between HGF serum levels and individuals with anxiety and demonstrate that zinc therapy is associated with increasing HGF levels.

Keywords: anxiety, hepatocyte growth factor, zinc, zinc therapy

Nutrition and Metabolic Insights 2010:3 43–48

This article is available from http://www.la-press.com.

Introduction

Anxiety is a normal emotional response to a threat or potential threat. However, when this emotion is inappropriate, extreme and persistent, and is not proportionate to the nature of the peril, it is classified as pathological.[1,2] In response to threatening situations, the feeling of anxiety is usually accompanied by emotional stress, which involves behavioral, expressive and physiological features, such as an avoidance of the source of the danger, assuming defensive postures and increasing blood pressure, respectively.[1,3]

Anxiety disorders are the most common class of psychiatric disorders in the US[4] and many other countries.[5–8] Yet, population-based studies have shown that this disease frequently goes untreated.[9,10] Anxiety is implicated in a number of psychiatric disorders, such as depression, panic attacks, phobias, generalized anxiety disorder, obsessive-compulsive disorder and post-traumatic stress disorder[2] and affects approximately 30% of the US population,[4] and one-eighth of the total population worldwide.[11] This imposes a social burden that amounts to billions of dollars each year.[12]

Hepatocyte growth factor (HGF), an 82 kDa, 674 amino acid residue heterodimeric glycoprotein, was originally isolated from rat platelets.[13,14] This growth factor has also been called scatter factor, hepatopoietin A, and mammary growth factor.[15] It is one of a small family of factors lacking significant homology with other known growth factors, but including an HGF-like factor known as macrophage stimulating protein (MSP).[16–19] HGF has mitogenic, morphogenic, and motogenic effects on hepatocytes, as well as endothelial, mesenchymal and hematopoietic cell types,[18,20,21] and demonstrates noticeable species cross-reactivity.[22]

HGF regulates cell growth, cell motility, and morphogenesis by activating a tyrosine kinase signaling cascade after binding to the proto-oncogenic c-Met receptor (translated by the MET gene). HGF is secreted by mesenchymal cells and, although it was first considered to exert biological effects only on specific target cells, it has since been demonstrated to mediate inflammatory responses to tissue injury, and regulate cell growth, cell motility, and morphogenesis in a wide variety of cells. Its ability to stimulate branching morphogenesis, cell migration, survival and proliferation gives it a central role in angiogenesis, tissue regeneration, as well as tumorogenesis.[23–29]

Signaling by HGF has also been found to have anti-inflammatory, antifibrotic, and pro-regenerative activity on various types of tissue. But it seems to be particularly active in the nervous system, where it has been found to have neurotrophic and angiogenetic activity on CNS neurons, promote both the survival of neurons and the regeneration of injured nerves, and function as a target-derived axonal chemoattractant, guiding axons to their target. As a result, it plays significant roles in the development of the CNS.[30]

Studies have shown a potential association between oxidative stress and the etiology of anxiety. As an example, oxidative stress-related anxiety can be reversed in mice upon inhibition of NADPH oxidase or phosphodiesterase-2, enzymes that are indirectly implicated in oxidative stress mechanisms.[31] Surprisingly, diazepam, a well-known anxiolytic, does not fully reverse oxidative stress-related anxiety.[31] These results point to a possible utility for antioxidants in the prevention or reduction of anxiety. Further research will be necessary to show whether anxious subjects need more antioxidants than non-anxious subjects. Recent work[31,32] has shown that some dietary polyphenols have both anxiolytic and antioxidant effects, which may be beneficial to anxious subjects. The potential relationship between oxidative stress and anxiety may generate interest in antioxidants.

There is also much support for the role of GABA in mood disorders, particularly anxiety and depression.[33] HGF has also been found to be associated with GABA regulation, particularly through anxiolytic activity, modulating GABAergic inhibition and seizure susceptibility.[34]

HGF expression has also been found to be associated with oxidative stress markers,[35] and HGF receptor gene expression may be suppressed by oxidative stress.[36]

Zinc is well known as one of the most important trace elements in the body. Dietary zinc deficiency is associated with a variety of physiological defects including anorexia, skin lesion, and growth retardation.[37] Mechanistic studies demonstrated that zinc deficiency affects a large number of hepatic genes involved in multiple cellular functions. In particular, zinc deficiency has been shown to down-regulate hepatic gene expression of metallothionein (MT),

insulin-like growth factor I (IGF-I), insulin-like growth factor binding protein 1 (IGFBP1), cyclin D1, and HGF, which are involved in cell proliferation.[38–40]

Zinc supplementation has also been found to prevent liver cell injury through attenuation of oxidative stress,[41] and there is evidence suggesting that alcohol-induced liver damage initiates hepatocyte proliferation, and zinc supplementation accelerates liver regeneration, through up-regulating cell proliferation-related proteins such as HGF.[42]

Because of the potential association between HGF and GABA regulation, oxidative stress and neurological development and differentiation, and its association with the etiology of neurological diseases, and the potential for zinc to up-regulate HGF synthesis, we tested patients with anxiety for serum concentration of HGF and then compared those levels in individuals pre and post zinc therapy.

Materials and Methods

Subjects

Experimental and controls

Serum from individuals with diagnosed anxiety (n = 19; 13 female; mean age 38.2 years) and controls (n = 19; 16 female; mean age 44.3 years) was obtained from patients presenting at the Health Research Institute/Pfeiffer Treatment Center. Most of these individuals were diagnosed using The Hamilton Rating Scale for Anxiety before presenting for treatment at the Pfeiffer Treatment Center, Warrenville, Il*.

Patient consent was obtained from all patients involved in this study and this study was approved by the IRB of the Health Research Institute/Pfeiffer Treatment Center.

Zinc and anti-oxidant therapy

Individuals in this study who presented to the Pfeiffer Treatment Center with anxiety were tested for anti-oxidant levels. Based on deficiencies, they were then prescribed the appropriate dosage of anti-oxidant. Pre-therapy patients represent those who were tested when they first presented and were not previously taking any anti-oxidants. Post-Therapy patients received anti-oxidant therapy (One dose—Vitamin C (125 mg), E (50 IU), B6 (37.5 mg) as well as Magnesium (11 mg), and Manganese (3.75 mg) if warranted), and zinc supplementation (as zinc picolinate) (25 mg), for a minimum of 8 weeks. Pre and post therapy patients were different groups. The same patients were not followed prospectively.

Serum/plasma

All experimental and control serums were treated in an identical fashion—frozen at −70C immediately after collection and cell/serum separation, then stored at −70C until thawed for use in ELISAs.

ELISA to measure serum HGF (ELISA kit, R&D Systems, Minneapolis, Minn.)

All reagents and specimens were equilibrated to room temperature before the assay was performed. A 1:51 dilution of the patient samples was prepared by mixing 10 µl of the patient's sera with 0.5 ml of Serum Diluent. One hundred microliters of calibrators (20–200 Eu/ml antibodies), positive and Negative control serums, serum diluent alone, and diluted patient samples were added to the appropriate microwells of a microculture plate (each well contained affinity purified polyclonal IgG to HGF). Wells were incubated for 60 minutes (±5 min) at room temperature, then washed 4 × with wash buffer. One hundred microliters of pre-diluter anti-human IgG conjugated with HRP was added to all microwells, incubated for 30 minutes (±5 min) at room temperature, then wash 4 × with wash buffer. One hundred microliters of enzyme substrate was added to each microwell. After approximately 30 minutes at room temperature, the reaction was stopped by adding 50 µl of 1M sulfuric acid, then the wells were read at 405 nm with an ELISA reader (BioRad Laboratories, Inc., Hercules, CA, USA).

Copper and zinc serum concentration

Copper and zinc plasma concentration was performed by LabCorp, Inc. (Naperville, IL 60563) using inductively-coupled plasma-mass spectrometry.

Statistics

Inferential statistics were derived from t-test with 95% confidence intervals.

Results

Serum from 19 individuals diagnosed with clinical anxiety and 19 healthy, age and gender similar controls

*The Pfeiffer Treatment Center is a comprehensive treatment and research center, specializing in the care of neurological disorders, including anxiety.

were tested for serum HGF using ELISAs (described above). Each assay was repeated two or more times, with multiple wells for each serum in each assay.

Serum HGF concentration (pg/ml) of individuals with anxiety (N = 19) was significantly lower than age and gender similar healthy controls (N = 19) ($P < 0.0005$) (Table 1).

HGF serum concentration of individuals with anxiety before they began zinc therapy, was compared to HGF concentration after therapy. There was a significant increase in HGF after therapy ($P = 0.04$) (Table 2), normalizing to the levels of healthy controls.

Zinc levels in the serum of these same groups of individuals increased significantly after zinc therapy ($P = 0.0002$), but Cu levels ($P = 0.36$) and Cu/Zn ($P = 0.11$) did not (Table 3).

Discussion

HGF has been found to be associated with a variety of diseases of the CNS. For instance, immunohistochemistry using anti-HGF antibody has revealed more intense immunolabeling in Alzheimer's disease (AD) than in control brains, and there appears to be a significant correlation between cerebral spinal fluid HGF levels and white matter high-signal foci determined on brain magnetic resonance imaging (MRI) in AD patients.[43] In Amyotrophic Lateral Sclerosis (ALS), overexpression of hepatocyte growth factor (HGF) in the nervous system attenuates motoneuron death and axonal degeneration and prolongs the life span of transgenic mice overexpressing mutated Cu^{2+}/Zn^{2+} superoxide dismutase.[44] Overexpression of HGF after gene transfer prevents neuronal death in a Parkinson's Disease rat model.[45] In addition, decreased levels of HGF have been found in autistic children with GI disease.[46]

One of the limitations of this study is that we did not follow patients prospectively, but instead compared HGF and Cu/Zn levels in groups of patients before they received zinc therapy and after. In future experiments, however, we plan to include this type of study.

Regardless, our data suggests that individuals with anxiety have significantly lower levels of HGF than normal controls, and that after zinc and antioxidant supplementation, both zinc and HGF levels normalize.

We suggest that the low levels of HGF, possibly associated with concurrent oxidative stress, may cause lower GABA, having an anxiogenic effect and that zinc supplementation may help raise HGF levels. To evaluate this possible association, future studies will assess more patients with anxiety and evaluate GABA levels along with HGF concentration, pre and post zinc therapy.

Table 1. Serum HGF (Hepatocyte Growth Factor) in individuals with anxiety is significantly lower than in healthy controls.

	HGF anxiety	HGF controls
Mean	383.8	573.9
SEM	37.4	32.8
N	19	19

The two-tailed *P* value equals 0.0005.

Table 2. HGF (Hepatocyte Growth Factor) serum levels in individuals with anxiety are significantly higher post zinc therapy.

	HGF anxiety pre therapy	HGF anxiety post therapy
Mean	319.7	481.8
SEM	34.5	67.9
N	9	8

The two-tailed *P* value equals 0.0436.

Table 3. Zinc (Zn) levels (μg/dL) in individuals with anxiety are significantly higher after zinc therapy (A), but Copper (Cu) levels (μg/dL) (B) and Cu/Zn do not significantly change after therapy.

A	Zinc before therapy	Zinc after therapy
Mean	71.1	100.5
SEM	3.1	5.4
N	9	8
B	**Cu before therapy**	**Cu after therapy**
Mean	98.9	112.8
SEM	7.4	13.0
N	9	8
C	**Cu/Zn before therapy**	**Cu/Zn after therapy**
Mean	1.4	1.1
SEM	0.2	0.1
N	9	8

The two-tailed *P* value equals 0.0002; The two-tailed *P* value equals 0.3557; The two-tailed *P* value equals 0.1090.

Disclosure

This manuscript has been read and approved by the author. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The author and peer reviewers of this paper report no conflicts of interest. The author confirms that they have permission to reproduce any copyrighted material.

References

1. Weinberger DR. Anxiety at the frontier of molecular medicine. *N Engl J Med*. 2001;344:1247–9.
2. Gross C, Hen R. The developmental origins of anxiety. *Nat Rev Neurosci*. 2004;5:545–52.
3. Leman S, Le Guisquet A, Belzung C. Liens anxiété-mémoire: Études expérimentales. In: Ferreri M, editor. Dans: "Anxiété, anxiolytiques et troubles cognitifs". Paris: Elsevier; 2004. p. 71–9.
4. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry*. 2005;62:593–602.
5. Alonso J, Lépine JP. Overview of key data from the European Study of Epidemiology of Mental Disorders (ESEMeD). *J Clin Psychiatry*. 2007;68:3–9.
6. ESEMed/MHEDEA 2000 investigators, author. Prevalence of mental disorders in Europe: results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. *Acta Psychiat Scand*. 2004;109:21–7.
7. Wittchen HU, Jacobi F. Size and burden of mental disorders in Europe-a critical review and appraisal of 27 studies. *Eur J Neuropsychopharmacol*. 2005;15:357–76.
8. Tuthill A, Slawik H, O'Rahilly S, Finer N. Psychiatric co-morbidities in patients attending specialist obesity services in the UK. *QJM: Monthly Journal of the Association of Physicians*. 2006;99:317–25.
9. de Graaf R, Bijl RV, Smit F, Vollebergh WAM, Spiker J. Risk factors for 12-month comorbidity of mood, anxiety and substance use disorders: Findings from the Netherlands Mental Health Survey and Incidence Study. *Am J Psychiatry*. 2002;159:620–9.
10. Issakidis C, Andrews G. Service utilisation for anxiety in an Australian community sample. *Social Psychiatry and Psychiatric Epidemiology*. 2002; 37:153–63.
11. Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, et al. Trends in alternative medicine use in the United States, 1990–1997: Results of a follow-up national survey. *JAMA*. 1998;280:1569–75.
12. Lépine JP. The epidemiology of anxiety disorders: prevalence and societal costs. *J Clin Psychiatry*. 2002;63:4–8.
13. Nakamura T, et al. Purification and characterization of a growth factor from rat platelets for mature parenchymal hepatocytes in primary cultures. *Proc Natl Acad Sci U S A*. 1986;83:6489.
14. Nakamura T, et al. Partial Purification and Characterization in hepatocyte growth factor from serum of hepatectomized rats. *Biochem Biophys Res Commun*. 1984;122:1450.
15. Sakkab D, et al. Signaling of Hepatocyte Growth Factor/Scatter Factor (HGF) to the Small GTPase Rap1 via the Large Docking Protein Gab1 and the Adapter Protein CRKL. *The Journal of Biological Chemistry*. 2000; 275:10772–8.
16. Michalopoulos G, et al. Control of Hepatocyte Replication by Two Serum Factors. *Cancer Res*. 1984;44:4414.
17. Thaler FJ, Michalopoulos G. Hepatopoietin A: Partial Characterization and Trypsin Activation of a Hepatocyte Growth Factor. *Cancer Res*. 1985;45:2545.
18. Zarnegar R, Michalopoulos GK. The many faces of hepatocyte growth factor: from hepatopoiesis to hematopoiesis. *J Cell Biol*. 1995;129:1177.
19. Weidner KM, et al. Evidence for the identity of human scatter factor and human hepatocyte growth factor. *Proc Natl Acad Sci U S A*. 1991;88: 7001–5.
20. Comoglio PM, Graziani A. in Guidebook to Cytokines and their Receptors, Nicola NA, editor, Oxford University Press; 1994. p. 182.
21. Comoglio PM, Graziani A. in Guidebook to Cytokines and their Receptors, Nicola NA, editor, Oxford University Press; 1994. p. 185.
22. Grant DS, et al. Scatter factor induces blood vessel formation in vivo. *Proc Natl Acad Sci U S A*. 1993;90:1937–41.
23. Nakamura T, et al. Purification and characterization of a growth factor from rat platelets for mature parenchymal hepatocytes in primary cultures. *Proc Natl Acad Sci U S A*. 1986;83:6489.
24. Nakamura T, et al. Partial Purification and Characterization in hepatocyte growth factor from serum of hepatectomized rats. *Biochem Biophys Res Commun*. 1994;122:1450.
25. Sasaki M, et al. Identification of mouse mammary fibroblast-derived mammary growth factor as hepatocyte growth factor. *Biochem Biophys Res Commun*. 1994;199:772.
26. Michalopoulos G, et al. Control of Hepatocyte Replication by Two Serum Factors. *Cancer Res*. 1994;44:4414.
27. Thaler FJ, Michalopoulos G. Hepatopoietin A: Partial Characterization and Trypsin Activation of a Hepatocyte Growth Factor. *Cancer Res*. 1985;45:2545.
28. Zarnegar R, Michalopoulos GK. The many faces of hepatocyte growth factor: from hepatopoiesis to hematopoiesis. *J Cell Biol*. 1995;129:1177.
29. Weidner KM, et al. Evidence for the identity of human scatter factor and human hepatocyte growth factor. *Proc Natl Acad Sci U S A*. 1991;88: 7001–5.
30. Hamanoue M, et al. Neurotrophic effect of hepatocyte growth factor on central nervous system neurons in vitro. *J Neurosci Res*. 1996;43(5):554–64.
31. Masood A, Nadeem A, Mustafa SJ, O'Donnell JM. Reversal of oxidative stress-induced anxiety by inhibition of phosphodiesterase-2 in mice. *J Pharmacol Exp Ther*. 2008;326:369–79.
32. Bouayed J, Rammal H, Dicko A, Younos C, Soulimani R. Chlorogenic acid, a polyphenol from Prunus domestica (Mirabelle), with coupled anxiolytic and antioxidant effects. *J Neurol Sci*. 2007;262:77–84.
33. Kalueff A, Nutt D. Role of gaba in anxiety and depression depression and anxiety. 2007;24:495–517.
34. Bae M, et al. Hepatocyte growth factor (HGF) modulates GABAergic inhibition and seizure susceptibility. *Experimental Neurology*. 2010;221:129–35.
35. Borawski J, et al. Relations between oxidative stress, hepatocyte growth factor, and liver disease in hemodialysis patients. *Renal failure*. 2002;24: 825–37.
36. Zhang X, Youhua L. Suppression of HGF receptor gene expression by oxidative stress is mediated through the interplay between Sp1 and Egr-1. *Am J Physiol Renal Physiol*. 2003;284:F1216–25.
37. McClain CJ, Adams L, Shedlofsky S; Zinc and the gastrointestinal system. Essential and Toxic Trace Elements in Human Health and Disease. Edited by AS Prasad. New York, Alan R. Liss Inc; 1988. p. 55–73.
38. McNall AD, Etherton TD, Fosmire GJ. The impaired growth induced by zinc deficiency in rats is associated with decreased expression of the hepatic insulin-like growth factor I and growth hormone receptor genes. *J Nutr*. 1995;125:874–9.
39. Pfaffl MW, Gerstmayer B, Bosio A, Windisch W. Effect of zinc deficiency on the mRNA expression pattern in liver and jejunum of adult rats: monitoring gene expression using cDNA microarrays combined with real-time RT-PCR. *J Nutr Biochem*. 2003;14:691–702, 2002;283:C623–30.
40. Dieck HT, Do¨ring F, Roth HP, Daniel H. Changes in rat hepatic gene expression in response to zinc deficiency as assessed by DNA arrays. *J Nutr*. 2003;133:1004–10.
41. Kahl KG, Bens S, Ziegler K, et al. Angiogenic factors in patients with current major depressive disorder comorbid with borderline personality disorder. *Psychoneuroendocrinology*. 2009;34(3):353–7.
42. Wakatsuki M, Akiyoshi J, Ichioka S, et al. Administration of antisense DNA for hepatocyte growth factor causes an depressive and anxiogenic response in rats. *Neuropeptides*. 2007;41(6):477–83.
43. Tsuboi Y, et al. Increased hepatocyte growth factor level in cerebrospinal fluid in Alzheimer's disease. *Acta Nneurologica Scandinavica*. 2003;107: 81–6.

44. Sun W, et al. Overexpression of HGF Retards Disease Progression and Prolongs Life Span in a Transgenic Mouse Model of ALS. *The Journal of Neuroscience*. 2002;22(15):6537–48.
45. Koike H, et al. Prevention of onset of Parkinson's disease by in vivo gene transfer of human hepatocyte growth factor in rodent model: a model of gene therapy for Parkinson's disease Gene therapy for Parkinson's disease. *Gene Therapy*. 2006;13:1639–44.
46. Russo AJ, et al. Decreased Serum Hepatocyte Growth Factor (HGF) in Autistic Children with Severe Gastrointestinal Disease Biomarker Insights. 2009;2:181–90.
47. Kahl KG, Bens S, Ziegler K, et al. P*sychoneuroendocrinology*. 2009;34(3): 353–7.
48. Wakatsuki M, Akiyoshi J, Ichioka S, et al. *Neuropeptides*. 2007;41(6): 477–83.