Summary of the Science Behind Oligopeptide (Humanofort)

John S. Hayden, MD/PhD and Soledad M. Manaay, PhD

March 25, 2015

Summary of the Science Behind Xicepta's Proprietary Ingredient: Oligopeptide (Humanofort)

Introduction

Xicepta uses Humanofort (Oligopeptide in product labels) also known as Embryonic Peptides (EP), as its main active ingredient. Humanofort is derived from laboratory-grade chicken (Gallus Gallus) embryos extracted from partially incubated hen eggs. Chicken embryo contains growth factors, i.e., Activin, Follistatin, Nerve Growth Factor (NGF), Tumor Necrosis Factor-alpha (TNF-alpha), Neurotrophin-3(NT-3), Brain-Derived Neurotropic Factor (BDNF), Ciliary Neurotropic Factor (CNTF), Fibroblast Growth Factor family (FGF), Transforming Growth Factors Beta-1(TGF-beta1), Insulin Growth Factors family (IGF). IGF family activity tends to maximize when harvested aseptically from the embryo. IGF-II, and IGF-binding proteins in the chicken embryo have been proven in scientific studies. These growth factors act as bio-signaling molecules (Radecki, et al, 1997; Martinez-Maqueda, et al, 2012; Kousaku, 2011).

Xicepta's CTO, Dr. John S. Hayden, MD and a leading molecular biologist and former federal medical scientist has been formulating supplements containing Amino Acids paired with other natural ingredients. He and his research partner, Soledad M. Manaay, PhD, studied the components of Humanofort and found that Humanofort is rich in Gallus Gallus microRNAs (miRNAS). According to research, miRNAs have multiple roles in many biological processes (Brennecke, etal, 2003; Cuellar, McManus, & McManus (2005).

Study shows that eggs are excellent source of biologically active peptides. Embryo tissues are rich in growth factors that regulate growth and development and prevent proliferation of transformed cell. Avian eggs are commonly the source of embryo-peptides (Martinez-Maqueda et al., 2012; Kousaku, 2011). In South Asian cultures, avian embryos have long been considered beneficial to one's health including as aphrodisiac for men (Magat, 2002). Recent studies on the role of miRNAs in reprogramming and therapeutic strategies may explain the benefits derived from chicken egg embryitic extract.

The Inventor

Humanofort was invented and patented by Dr. Gheorghe Mihaescu, MD, an international expert in Experimental Immunology in Oncology, Steroid Biochemistry, Radio-assay Methodologies, and Geriatric Nutrition. He spent 10 years of research at the Department of Nuclear Medicine, Institute of Physical and Engineering Research in Bucharest, Romania. During this time he endeavored to perfect this unparalleled technology of extracting vaccine-graded embryonic stem cell in bio-active state using cutting-edge technology of processing embryo-peptides --by tangential ultra-filtration and using intestinal absorption enhancers to increase its bioavailability when taken orally. The inventor's ingenuity allows Humanofort to cross the intestinal membrane and act beneficially as growth factors

without producing toxicity or immunological side effects (Mihaescu, 1997). Along with his Associates, Dr. Mihaescu conducted several studies to prove the benefits of his invention.

Clinical Trials

Steroidogenesis Test on Laboratory Animals

Dr. Miheascu proved during tests on laboratory animals that chicken embryo-peptides affects steroidogenesis. His experimented on 40 Wistar rats (20 males of weight 97 ± 4 g and 20 females of 95 ± 5 g), feeding them with a standardized diet. Humanofort powder, 50 mg/kg body/day was administered for 60 days. The results of this study showed that administration of Humanofort induced a high significant increase of 17-ketosteroids in urine. This proved steroidogenesis stimulation at the adrenal cortex level. There were no side effects noted during the study.

Tab.1. Effect of rats ingesting 50 mg/kg body/day of Humanofort for 60 days

Parameter	Control		Humanofort administered	
	Males	Females	Males	Females
17-Ketosteroids (mg/g creatinine)	0.11 ± 0.02	0.16 ± 0.05	0.27 ± 0.07 ($p < 0.01$)	0.54 ± 0.04 ($p < 0.001$)

Steroidogenesis Test on Rugby Players

Using 30 volunteer rugby players between 20 to 30 years old Dr. Mihaescu and associates endeavored to prove Humnofort's stimulation of steroidogenesis on humans. The group was divided into three (3) groups of 10 participants each. The control group took Placebo caps; the second group received four (4) caps/day of Embryonic Peptide (EP) while the third group received 12 caps/day. Blood was collected from all subjects before and after the duration of the 21-day study. The experiment resulted in significant increase of androgen hormones when compared to the initial values. A dose-effect relationship is observable for testosterone, the principal androgen hormone (Mihaescu, et al, 1997). Studies show that low androgen hormones in both men and women are detrimental to wellness and enjoyment in life. The suggested symptoms of androgen deficiency in both men and women include, among others: lethargy, loss of muscle mass and strength, loss of libido, lack of motivation, low emotional state, and lowered mood (Better Health, 2014).

Table 2. The effect of Humanofort administration on rugby players' serum steroids

Hormone	Treatment		
	Placebo	6 caps EP/day	12 caps EP/day
DHEA	121.3 ± 16.5	89.7 ± 10.4	115.2 ± 13.8
DHEA sulfate	81.4 ± 14.2	114.7 ± 11.5	108.3 ± 10.6

Androstenedione	88.5 ± 12.4	137.3 ± 21.5	126.5 ± 16.4
Testosterone	102.4 ± 14.7	124.5 ± 15.6	135.3 ± 17.9

Research on Cholesterol Effects

An experiment was conducted using older adult participants composed of 22 women and 20 men between 50 and 70 years of age with satisfactory clinical health. Participants with some medical problems in chronic state were under medical control.

Study participants took 4 caps/day of Humanofort, two in the morning and two in the evening with no diet restriction. Each subject was his own control. Blood analysis were conducted using UE's standard methods and the results were electronically processed. The participants reported no side effects or allergic reactions during the study (Mihaescu, et.al, 1997). The study's quantitative results are presented in Table 3.

Tab. 3. The influence of Humanofort administration on some biochemical parameters in adult human subjects (Mihaescu, et. al, 1997).

Parameter	Men		Women	
	Initial	After Humanofort treatment	Initial	After Humanofort treatment
Total cholesterol (mg/dl)	248.4 ± 6.3	216.6 ± 9.4 ($p < 0.01$)	274.6 ± 4.3	234.5 ± 9.2 ($p < 0.01$)
LDL-cholesterol (mg/dl)	156.5 ± 5.3	117.6 ± 8.5 ($p < 0.01$)	174.8 ± 4.3	140.6 ± 7.2 ($p < 0.01$)
Cortisol (nmol/l)	486.5 ± 24.2	373.6 ± 28.7 ($p < 0.01$)	475.8 ± 24.3.	418.5 (72%) 496.7 (28%)

The above results show that the initial values of cholesterol and its LDL-fraction were influenced. The administration of Humanofort resulted in a significant decrease (mean of 30 mg/dl) for both male and female participants and produced diverse regulatory result which could be the function of gender and initial values. The cortisol level returned to physiological range in 70% of women and 100% of men. In most cases, the decrease of cortisol reached more than 50 nmol/l and most of regulative modifications of steroid hormones amounted for 70% of the subjects (Mihaescu, et. al, 1997.

Test on Metabolic Syndrome

Another experiment was conducted on a group of 18 men and 22 women between 55-75 years of age with moderate hypertension and early stage type 2 diabetes. The participants were home-bound during the study with no diet restriction and no interruption of their specific medication. The local ethics committee approved the study and informed consent was obtained from the participants. This study also intended to investigate Metabolic Syndrome (MS) previously introduced by Reaven in 1988 (Mihaescu, Olinescu & Oancea, 2005).

Each subject took 4 caps of Humanofort per day (two in the morning and two in the evening)

for 60 days after which blood samples were tested. The analyses were performed according to EU standards (Mihaescu, Olinescu & Oancea, 2005). During the study, no side effects were reported. The results are presented in table 4.

Tab. 4. Quantitative Result of The effects of Humanofort administration on some biochemical parameters of the human subject with symptoms of Metabolic Syndrome (Mihaescu, Olinescu & Oancea, 2005).

Parameter.	Initial	After Humanofort treatment	Diff.(P)
Men			
Total cholesterol (mg/dl)	244.8±12	219.6±+9	<0.05
HDL- cholesterol (mg/dl)	38.4±10	47.3±7	<0.05
LDL - cholesterol (mg/dl)	160.7±14	131.4±5	<0.01
Triglycerides (mg/dl)	278.6±12	246.8±8	<0.05
Insulin (U/ml)	12.5±5	6.5±2	<0.05
Cortisol (nmol/l)	546.8±17	470.3±15	<0.05
IGF-1 (ng/ml)	315.8±27	228.7±15	<0.01
Women			
Total cholesterol (mg/dl)	286.5±13	253.7±16	< 0.05
HDL- cholesterol (mg/dl)	55.8±9	63.2±6	NS
LDL - cholesterol (mg/dl)	201.8±16	176.4±13	<0.05
Triglycerides (mg/dl)	269.5±18	234.3±12	<0.05
Insulin (U/ml)	12.5±6.1	7.2±2.6	NS
Cortisol (nmol/l)	512.7±24	443.5±18	<0.05
IGF-1 (ng/ml)	246.27±27	195.4±18	<0.05

As seen in table 4, the administration of Humanofort resulted in significant changes including the improvement of lipid profile, with decrease of triglycerides, decrease of LDL (bad cholesterol) for both sexes, increased of HDL (good cholesterol) for males and changes of insulin and cortisol as well as of IGF-1 towards physiological range (Mihaescu, Olinescu & Oancea, 2005).

Dr. Mihaesco also conducted a study that examined the aging reversal of participants who have taken Humanofort. In this study, Dr. Mihaesco and associates (1997) found 30 percent high improvement and 70 percent general improvement specifically in participants' sexual performance, sleep quality and mood disorders. Result also showed substantial increase in antioxidant capacity and decrease in oxidative stress. This proved that the embryonic peptides (Humanofort) is an effective signaling devise for cells to rejuvenate (Mihaescu, et al, n.d.; Gruia, Olinescu, Mihaescu, 1997).

Current Study on the Role of miRNAs in Cancer Suppression

MiRNAs are single-stranded non-coding RNA polynucleutides that control gene expression. They have been detected in plants and animal species and also found in viruses. They participate in cellular processes such as proliferation, differentiation, metabolism, and gene cluster silencing (Bartel, 2004; Jansson, Lund, (2012). Majority of miRNAs genes happen to reside in fragile areas of the human body. They can act as tumor suppressors; however, their loss of function could transform a normal cell into a malignant cell. The loss of function could be credited to factors such as deletion, mutation, silencing or alteration of miRNA processes (Rothschild, 2014).

In the last decade, miRNAs has been the subject of research including clinical trials for new diagnostic and therapeutic opportunities. Several studies were able to identify cancer-type diseases in which miRNA target binding sites and mirRNAs processing machinery in tumor cells were altered or globally decreased. Along with this finding is the significance of miRNA family that silences anti-metastatic miRNAs and miRNAs that are over-expressed (Hayes, Peruzzi & Lawler, 2014).

A study by Jansson and Lund (2012) points to miRNAs having key roles in stem cells and stem cell differentiation and in induced pluripotency. Jansson and Lund (2012) specifically mentioned miRNA 302 having shown to produce iPSC in both human and mouse fibroblasts (Anokye-Danso et al in Jansson & Lund, 2012); in addition, researchers emphasize that miRNAs could exert their full effects by via multiple targets (Jansson & Lund, 2012).

A study by Zhang, et al (2007) exulted miRNAs as oncogenes and tumor suppressors. In their study, the authors found that miRNA-15 and miRNA-16 destroys the cells that are not needed in a process called apoptosis by targeting antiapoptotic gene B or cell lymphoma 2, a key inducer of leukemias, lymphomas, and carcinomas (Cimmino et al, 2005 in Zhang et al, 2007. Authors also suggest that the following cancers are related to reduced miRNAs: Brain cancer-- reduced miR-21, miR-181 and increased miR-221; breast cancer – reduced miRNA-125b, miRNA-145, miRNA-21, and miRNA-155; Chronic lymphocytic leukemia – miR-15 and MiR-16; Colorectal neoplasia – reduced miR-143 and miR-145; Hepatocellular carcinoma – increased miR-18 and miR-224 and reduced miR-199, miR-195, miR-200 and mir-125; Lung Ccancer – reduced let-7 and increased miR 17-92 (Zhang et al, 2007).

Rothschild (2014) showed that miRNAs were able to regulate the expression and function of another protein-coding RNA. Rothschild stated that miRNAs serving as therapeutic agents have these criteria: 1) findings that miRNA expression is dysregulated in cancer; 2) the cancer phenotype can be altered by targeting miRNA expression (Rothschild, 2014).

Zhang et al (2007) hypothesized that since miRNAs function as tumor suppressor, it is possible that injection of miRNAs could regulate cancer expression. They also suggest that artificial miRNAs can be developed for the purpose of blocking cancer formation.

Conclusion

Data gathered from orally administered Humanofort trials indicate that Humanofort's embryonic peptides with growth factors signal positive changes in steroidogenesis resulting in 1) Decrease of bad cholesterol or LDL; 2) Positive change in Metabolic Syndrome; 3) Increase of the good cholesterol or HDL on men with Metabolic Syndrome; 4) Normalization of cortisol level; 5) Raise androgen levels in young male (Mihaescu, Olinescu & Oancea, 2005); 6) Aging reversal of persons who have taken Humanofort (Mihaescu, et al, n.d.; Gruia, Olinescu, Mihaescu, 1997) and significant modifications in elderly persons' oxidative stress after administration of Humanofort (Mihaescu, et al, 2006); 7) Studies show that miRNAs could regulate or supress cancer genes (Zhang et al, 2007).

It can be gleaned from these studies that Humanofort as a supplement can be used as a versatile therapeutic agent or active component of molecular compounds. Dr. Mihaescu's technology ensures that the bio-availability of Humanofort is safe and its growth factors can effectively reprogram cells and tissues to change into a pluripotent state.

Reprogramming cells has been the subject of many scientific studies since the early 1900s. In 1962, John B. Gurdon's research proved that cellular differentiation is achieved through transplantation. Dr. Shinya Yamanaka (2012) also proved that the introduction of transcription factors into a differentiated cell can indeed convert the cell into a pluripotent state. Yamanaka's research concluded that matured cells can be reprogrammed to replicate. In 2012, Dr. Yamanaka and Dr. Gurdon both received a Nobel Prize in Physiology for their discovery of mature cells being able to be reprogrammed to a pluripotent state (Yamanaka, 2006).

Researchers Onder and Daley (2011) declared that miRNAs are macro players in somatic cell reprogramming and that miRNA-based reprograms "faster than the standard four-factor reprogramming" method such as the Yamanaka factors. In addition, the authors suggest that miRNA-based reprogramming is more significant and practical way to generate pluripotent stem cell induction (Onder & Daley, 2011). Humanofort containing chicken embryotic extract is rich in Gallus Gallus miRNAs which is proven to benefit users. Humanofort also contains growth factors as mentioned above. The miRNAs and growth factors that Humanofort contain when correlated with studies relating to miRNAs and induced pluripotency and cell reprogramming explain why the products have effectively addressed specific conditions.

References

Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanisms, and functions. Cell. 116: 281-297.

Better Health (2014). Androgen deficiency in men and women. Retrieved November, 1, 2014. http://www.betterhealth.vic.gov.au/bhcv2/bhcarticles.nsf/pages/Androgen_deficiency.

Boulland J., Halasi G., Kasumacic N., Glover J.C. (2010). Xenotransplantation of Human Stem Cells into the Chicken Embryo. JoVE. 41. http://www.jove.com/details.php?id=2071, doi: 10.3791/2071.

Brennecke J, Hipfner DR, Stark A, Russell RB, Cohen SM; Hipfner; Stark; Russell; Cohen (2003). "Bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila". Cell 113 (1): 25–36.

Carlson ME, Conboy IM (2007) Loss of stem cell regenerative capacity within aged niches. Aging Cell 6: 371-382. http://www.stembook.org/node/459.

Carlson, M.E. Suetta, C., Conboy, M. J., Aagaard, P., Mackey, A., Kjaer, M., & Conboy, I. (2009). Molecular aging and rejuvenation of human muscle stem cells. EMBO Mol Med. 1(8-9): 381–391.

Chan, J. (2011). Shinya Yamanaka, Induced Pluripotent Stem Cell (iPS) Pioneer. Asian Scientist. News and News and Information from the Asian Scientific community. http://www.asianscientist.com/features/shinya-yamanka-induced-pluripotent-stem-cell-ips/.

Cuellar TL, McManus MT; McManus (2005). "MicroRNAs and endocrine biology". J. Endocrinol. 187 (3): 327–32.

Fernyhough. M. E., Bucci, L. R., Feliciano, J., Dodson, M. V. (2010). The effect of nutritional supplements on muscle-derived stem cells in vitro. International Journal of Stem Cell. 3(1). 63-67.

Gruia, I. M., Olinescu, R., Mihaescu, G. (1997). The modification of oxidative stress in elderly after orgasel and humanofort treatment. Infomedica, 11: 40-44.

Jansson, M. D. & Lund, A. H. (2012). MicroRNA and cancer. Molecular Oncology. 6: 590-610

Kousaku O., (2011). Anxiolytic-like activity of egg protein-derived peptide. J. Jap. Soc. Food Sci. Tech. 58: 346-349.

Hayes, J., Peruzzi, P.P. & Lawler, S. (2014). MicroRNAs in cancer: biomarkers, functions and therapy. Cell. 20(8). 460-469.

Magat, M. 2002, Balut: fertilized duck eggs and their role in filipino culture, West. Folk.

61.1:63-96.

Martinez-Maqueda et al., (2012) Antihypertensive peptides from food proteins: a review. Food & Function, 3: 350-361.

Mihaescu G., Olinescu R. & Oancea F. (2005). Metabolic Syndrome may be improved following a long-time administration of a nutritive supplement containing embryonic peptides. General Endocrinology/Acta Endrocrinologica (Buc), 1(3). 263-270.

Mihaescu G., Olinescu R. & Oancea F. (2006). Significant modifications of lipid metabolism in aged persons following the administration of a nutritive supplement. Rom. J. Intern. Med.

Mihaescu, G. et. al (1997). "The effects of orally administered Humanofort on steroid hormone levels and on oxidative stress of rugby players" Medicine and Pharmacy University. Bucharest, Romania.

Mihaesco, G., et. Al (1997) Oxidative stress decrease in old age patients treated with drugs containing embryonic peptides. National Institute for Geriatrics and Gerontology "Ana Asian." Bucharest.

Onder, T. T. & Daley, G. Q. (2011). micrRNAs become macro players in somatic cell reprogramming. Genome Medicine. 3:40.

Reaven, G. M. (1988). Role of insulin resistance in human diseases. Diabetes; 37: 1595-1599.

Radecki, S.V., Capdevielle, M.C., Buonomo, F.C. & Scanes, C.G. (1997). Ontogeny of insulin-like growth factors (IGF-I and IGF-II) and IGF-binding proteins in the chicken following hatching. Gen Comp Endocrinol. 107(1) 109-17.

Sigurjonsson, O. E., Perreault, M., Egeland, T. & Glover, J. C. (2004). Adult human hematopoietic stem cells produce neurons efficiently in the regenerating chicken embryo spinal cord. National Academy of Sciences. 102(14) 5227-5232.

Takahashi, K. & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 126, 663–676.

Zhang, B., Pan, X., Cobb, G. P. & Anderson, T. A. (2007). microRNAs as oncogenes and tumor suppressors. Science Direct. 302: 1-12.

