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Ezrin Peptide Therapy: A Potential Treatment for COVID

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Abstract

Ezrin Peptide Therapy was invented in London and developed in Moscow over the previous three decades. Synthetic 14 amino acid peptides that mimic a receptor site in human ezrin, inhibit inflammation and amplify adaptive anti-viral immunity. Human Ezrin Peptide One (HEP1, Gepon) was registered for human use in the Russian Federation in 2001. The successful treatment with ezrin peptides of acute viral respiratory infection with pneumonia complications, led to testing of ezrin peptides as a potential COVID therapy. RepG3, a derivative peptide of HEP1, which has double the IL-6-expression inhibition activity, is being developed as a therapy for COVID and may also help to prevent SARS-CoV-2 re-infection.

Keywords: Ezrin peptide therapy • HEP1 • Gepon • RepG3 • COVID • SARS-CoV-2

Introduction

HIV therapy re-purposed

We recently published a review on how ezrin peptide therapy, originally developed to treat HIV-induced immune-suppression and opportunistic infection, has been found to be an effective therapy in individual human volunteers for mild-to-moderate COVID. The mode of action is a combination of inhibition of inflammation and amplification of adaptive anti-viral immunity [1].

The level of IL-6 expression and inflammation in response to SARS-CoV-2 infection predicts severe COVID [2-4]. SARS-CoV-2 Spike protein induces a TLR8-dependent pro-inflammatory cytokine response from human macrophages followed by high levels of NFκB activation, IL-6 expression IL-6R release, expression of other pro-inflammatory cytokines, leading to damage in human bronchial epithelial cells. It has been recently reported that SARS-CoV-2 Spike also impairs DNA damage repair and inhibits V(D)J recombination which is essential for the adaptive immune response [5]. Ezrin peptides inhibit the expression of inflammatory cytokines, particularly IL-6, while amplifying B cell immunity and serum anti-body titers.

Ezrin peptide therapy was developed after the discovery that the C-terminus of HIV gp120 mimics the hep-receptor of the Alpha domain of human ezrin, a folded alpha-helical zip-like structure comprising of charge-matched amino-acids. Synthetic peptides from five to fourteen amino acids in length that mimic the Hep-receptor in human ezrin are all biologically active in vitro and in vivo. Ezrin Peptide One (HEP1) TEKKRRETVEREKE, brand name Gepon, was registered for human use in Russia from 2001 as a treatment for opportunistic infections in HIV-infected patients. HEP-1 is now off-patent and is produced in the Russian Federation by both Immapharma and Russian Peptide [6,7]. An improved version of HEP1 called RepG3, GEKKRRETVEREGG (a derivative of HEP1), has double the anti-inflammation activity of HEP1 and is patent protected around the world to 2036 [8]. Products based on RepG3 API are being developed by Newal R&D Ltd in London (Figures 1 and 2).

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Ezrin peptide treatment of human COVID volunteers: Early COVID-19 symptoms 7 days after SARS-CoV-2 infection are usually a thick mucus in the throat, sore throat, dry cough, headache, mild fever (+1ºC: 37ºC to 38ºC) and loss of sense of smell. More than twenty human volunteers suffering mild-to-moderate COVID have used 1 mg/ml ezrin peptide (HEP1 or RepG3) aqueous solutions delivered either as an inhaled peptide solution spray (>10 volunteers) or using HEP1 solution injected subcutaneously (>10 volunteers) to treat their COVID symptoms. All volunteers benefited from rapid reduction of fever and other related symptoms of COVID, and became PCR negative for SARS-CoV-2 between 5 and 10 days after start of treatment (so far no treatment failure nor adverse reaction has been reported).

For example, in January 2021 a family in Moscow (grandfather 68 years old, daughter [mother] 35 years old, grandchildren 4 years old and 1.5 years old) were infected with SARS-CoV-2, and developed COVID with its characteristic temperature elevation fever (plus 1-2ºC), loss of sense of smell, cough and headache. Daughter (mother) was confirmed PCR positive for SARS-CoV-2 in two separate tests. The treatment of all four members of the family with 2 mg/2 ml HEP1 inhaled spray 3 × per a day for five days. All recovered within 7 days. Daughter (mother) confirmed PCR negative, SARS-CoV-2 0.6 IgM (control<2) IgG 57 (control<10) ED/ml.

In March 2020, Dr. RP, (Male, 69 years old, previously healthy, 176 cm, 92 kg) was probably infected by SARS-CoV-2 on 01.03.2020 after travelling in a crowded train in Germany. On 08.03.2020, Dr RP developed COVID symptoms: headache, cough, 38.3°C fever, fatigue, and loss of appetite. On 10.03.2020, a PCR test for SARS-CoV-2 was performed and on 13.03.2020 the positive PCR result confirmed SARS-CoV-2 infection. On 14.03.2020 ezrin peptide treatment started: subcutaneous injection of 0.2 mg HEP1 in 1 ml saline once a day. By 16.03.2020 there was significant improvement of

Figure 1. 10 ml Origin spray bottle for peptide solution spray delivery.

symptoms. On 19.03.2020 there were no symptoms of COVID and the patient was healthy. On 20.03.2020 the PCR test was negative for SARS-CoV-2.

In 2020 and 2021, volunteers with mild-to-moderate COVID self-administered an aqueous solution of RepG3 GEKKRRETVEREGG (2 mg in 2 ml water), delivered as a spray aerosol to the throat and inhaled, and repeated 3 × per day over 5 days. For example, in April 2020, two medical doctors were infected with SARS-CoV-2 (confirmed by PCR) during their clinical practice in Moscow and developed COVID symptoms. Fever continued for 10 days. Both doctors went on to develop low blood-oxygen levels and pneumonia. Various antiviral and immune modulating drugs failed to be effective in reversing the disease progression. Both doctors started developing signs of intoxication.

RepG3 treatment was started using an oral throat spray of RepG3 2 mg/2 ml, 3 × per day. Only one hour after the first treatment, the body temperature of one doctor decreased from 39ºC to 37.3ºC, and in 12 hours her fever had disappeared, and body temperature returned to normal 36.6ºC. The other doctor experienced a similar rapid recovery. The daughter of the doctors also developed COVID symptoms, which were promptly terminated by RepG3 therapy.

The general effect of RepG3 in human volunteers with mild-to-moderate COVID was rapid reduction in fever, headache and sore throat, in some cases temporary swelling of lymph-nodes for no more than a few hours, elimination of cough and liquefaction of thick mucus. The reduction of symptoms to zero over the treatment period, and conversion from SARS-CoV-2 PCR-positive to PCR-negative, was usually achieved within seven days. An unvaccinated volunteer, who self-administered RepG3 and successfully treated COVID in March 2020, has remained PCR negative for SARS-CoV-2 variants for over twenty months.

Despite these very encouraging reports from volunteers, only randomized double-blind placebo-controlled clinical trials on RepG3 treatment of COVID and prevention of SARS-CoV-2 re-infection, can formally determine the efficacy of RepG3 therapy.

Earlier clinical trials on other medical applications for Ezrin peptides: Between 1998 and 2004, 21 clinical trials at different hospitals in Moscow, demonstrated the anti-viral, anti-bacterial and anti-fungal efficacy of HEP1 (Gepon) in STD infections, gut infections and respiratory infections. In addition, HEP1 was effective in the treatment and prevention of both Hepatitis B and HCV. Further, HEP1 (Gepon) was found to be effective treatment for inflammation and ulceration of the stomach, duodenum and lower gut (Tables 1-4).

Human ezrin peptides such as HEP1 and RepG3 are active on mucus membrane surfaces found in the airways, gut and reproductive organs. Ezrin peptides activate tissue macrophages to fight infection and amplify adaptive immune defense, while actively reducing inflammation and promoting ulcer

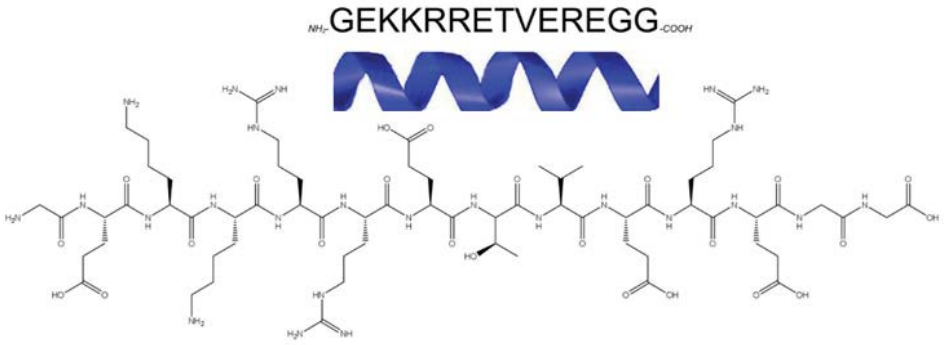

Figure 2. RepG3, single letter amino acid notation, alpha helical structure (blue), and polypeptide chemical structure.

Table 1. Summary results of six clinical trials in Russia on HEP1 treatment of sexually transmitted disease.

HEP1 treatment of sexually transmitted disease					
Sl. No.	**Disease**	**Patients**	**HEP1**	**Duration**	**Result**
1	HIV opportunistic infections	20	10 mg oral, 30 doses	30 days	Reduction in fungal, bacterial and viral opportunistic infection
2	Chronic recurrent candida	70	2 mg vaginal, 3 doses	7 days	Rapid decline in inflammation and clearance in 90% group
3	Chronic recurrent candida	50	2 mg urethral vaginal, topic 3 doses	7 days	Rapid decline in inflammation and clearance in 84% group
4	Chronic Trichomonas+Candida	16	2 mg oral 3 doses	7 days	Rapid decline in inflammation and clearance in 56% group
5	Chronic recurrent Herpes II	35	2 mg oral+ topical 4 doses	7 days	50% reduction in duration and 2.5Xreduction in relapse rate
6	Chronic HPV vaginal warts	27	2 mg oral+ topical 10 doses	20 days	Wart disappearance 4x faster with HEP1 vs controls

Table 2. Summary results of seven clinical trials in Russia on HEP1 treatment (and prevention) of inflammatory gut disease and a diversity of chronic ulcerative diseases.

HEP1 treatment of Ulcers					
Sl. No.	**Disease**	**Patients**	**HEP1**	**Duration**	**Result**
1	Stomach and Duodenal Ulcers PPI AB Treatment failures	37	2 mg catheter 3 doses	7 days	95% patients healed in 10-14 days
2	Stomach and Duodenal Ulcers Elderly Treatment failures	15	2 mg oral 3 doses	7 days	87% patients healed in 10-14 days
3	Irritable bowel syndrome	16	2 mg rectal 5 doses	10 days	reduction in disease index in all patients
4	Ulcerative Colitis	36	1 mg rectal 7 doses	7 days	reduction in disease index in 70% of patients
5	Mucosal radiation damage of rectum and bladder	35	2 mg rectal/bladder 30 doses	15 days	lesions healed 15-20 days, healing 2x faster than controls
6	Mucosal radiation ulcers of soft tissues and epithelium	37	2 mg topical 10 doses	10 days	5 log reduction in bacterial load healing 2x faster than controls
7	Chronic epithelial leg ulcers varicose or diabetic	54	2 mg topical 5 doses	10 days	granulation day 3, scar day 8 healing 3x faster than controls

Table 3. Summary results of two clinical trials in Russia on HEP1 treatment of intestinal infections, and two clinical trials in Russia on HEP1 treatment of viral respiratory infection.

HEP1 treatment of intestinal infections					
Sl. No.	**Disease**	**Patients**	**HEP1**	**Duration**	**Result**
1	Enterocolitis due to Shigellae Salmonellae Klebsiellae	50 children	1 mg oral 10 doses	7 days	Clearance of pathogens and Restoration of normal flora
2	Acute gastroeneteritis due to rotavirus	40 children	1 mg oral 10 doses	5 days	Clearance of rotavirus and restoration of normal flora
HEP1 treatment of Respiratory Infections					
Sl. No.	**Disease**	**Patients**	**HEP1**	**Duration**	**Result**
3	Acute respiratory diseases+laringo-tracheo brochitis immune failure	100 children	0.4 mg nasal up to 90 doses	up to 30 days	Rapid decline in inflammation 2-3x faster recovery than controls 75% cured
4	chronic recurrent pharingitis tonsilitis and allergic rhinitis	58 children	2 mg aerosol 5 doses	5 days	Rapid decline in inflammation, clearance of mucosal infection, prevention of recurrence+6 months

Table 4. Summary results of six clinical trials in Russia on HEP1 treatment (and prevention) of hepatitis disease.

HEP1 treatment (and prevention) of Hepatitis					
Sl. No.	**Disease**	**Patients**	**HEP1**	**Duration**	**Result**
1	Acute or chronic Hepatitis A, Hepatitis B or HCV	77 children	1 mg oral up to 60	up to 30 days	Reduction in viral load, ALT AST and clinical improvement
2	Chronic HCV (moderate)	21	2 mg oral 90 doses and a INF	90 days	Reduction in viral load, ALT AST and clinical improvement
3	Chronic HCV with fibrosis	40	2 mg oral and a INF	daily	Reduction in a INF side-effects long term study
4	Chronic HCV HIV double infection	19	2 mg oral 40 doses	30 days	Reduction in viral load, ALT AST and clinical improvement, one third cleared HCV
5	Chronic HCV HIV double infection	20	2 mg oral 40 doses	30 days	Reduction in viral load, ALT AST and clinical improvement
6	HBS vaccine + HEP 1 adjuvant preventing Hepatitis infection of immuno-deficient children	105	2 mg oral 5 doses	7 days	Higher titers of HBS antibodies and half the infection rate vs HBS vaccine only

healing. In addition, HEP1 (Gepon) activates fibroblasts of the tissue matrix so that granulation tissue forms at the location of wounds or ulcers, and epithelial cells are stimulated to regenerate.

The sequence of electrostatic charges on human ezrin peptides allow specific binding to a "receptor" conformation of human ezrin present on the exterior surface of cell membranes, which on binding to peptide, stimulates the transition to the open-active sub membrane-conformation of ezrin protein, resulting in enhanced receptor clustering and organization of membrane associated cell-signaling complexes.

In epithelial cell culture, ezrin peptides trigger signals down the ras>raf>MEK>ERK and PI3K>PKB pathways in less than 5 minutes, supporting the hypothesis they act rapidly on a specific cell-surface receptor located on monocytes, neutrophils, lymphocytes, epithelial cells and fibroblasts. The ezrin peptide receptor is thought to be a "receptor" transition-conformation between the soluble cytoplasmic inactive form of ezrin, and the active sub-membrane form of ezrin, which stimulates the clustering of cell-signaling protein complexes. The observed systemic effects of ezrin peptides correlate to changes in the expression of different cytokines and interferons in different cell types, on contact with ezrin peptides.

Peptide synthesis and drug-delivery: Fourteen amino acid RepG3 peptide is easy to manufacture by automated solid-phase peptide synthesis: three different peptide manufacturers have produced batches of RepG3 greater-than 98 per cent pure peptide, with acetate as the counter-ion. Newal R&D is investigating different product forms for RepG3 API: peptide-solution sprays and peptide-sugar pills. Generally, the contact between peptide solution and an infected mucus membrane is the target of drug delivery. In Russia, lyophilized peptide is supplied in sealed sterile 2 ml or 5 ml injection-vials. Newal R&D is investigating various peptide solution delivery spray formats comprising of a 5 ml or 10 ml glass bottle with a spray delivery top. Ezrin peptides are stable for years as sterile lyophilized solids. Ezrin peptides are stable in 1 mg/ml aqueous solutions for at least 30 days at 4°C without preservatives or stabilisers.

An alternative product is a saliva-dissolved sucrose-peptide pill that can effectively deliver peptide solution to the throat during an acute viral respiratory infection. In volunteers, sugar-peptide pills have been demonstrated to eliminate cough. During the development work on pill-production, an LFA hand operated pill press was used to make experimental 100 mg sugar pills (Dry-mixed 1 mg RepG3 API, 99 mg Sucrose, 5 microlitres vegetable oil binder) [9].

Conclusion

The development of RepG3 peptide therapy for COVID and related acute & chronic viral respiratory infections is continuing in London and Moscow. Generally, the success in developing ezrin peptides as therapeutic molecules supports the strategy of using synthetic charged peptide mimics of receptor sites in human proteins, for a new range of pharmaceuticals that have specific interactions with their protein targets.

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