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DNA Polymerase as Therapeutic Intervention for Treating Patients with Multiple Sclerosis

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Abstract

In recent years, increasing evidence has pointed to the potential role of fibrinolysis in the pathogenesis of MS. Based on hypotheses describing the aggressive autoimmune responses observed in MS patients, a result of impaired between (t-PA and PA1-1) which are a key molecules in both fibrinolysis and extracellular proteolysis. The present study was done to investigate the therapeutic potential of polymerase enzyme in modulating the changes occurred between levels of Tissue- type plasminogen activator (t-PA) and its inhibitor (PAI-1) in patients with multiple sclerosis. A pilot study was carried out on a total of twenty-one patients (17 females, 4 males; aged 22-46 years) with demyelination suggestive of MS and clinically silent T2 brain lesions on magnetic resonance imaging (MRI). All of the examined patients showed the same clinical symptoms of MS and consented to take the novel therapy by S/C injection of 0.1 cc of DNA polymerase enzyme (termed SS6) twice daily for 24 weeks. At the beginning of the study and at the end of therapy the plasmatic levels of PAI-1, t-PA and anti-MOG IgG antibodies were measured by ELISA and their values were expressed in ng/mg of protein. All patients' showed a significantly association between the decreased levels of PAI-1, anti-MOG IgG titer and the disappearance of annualized relapse rate (ARR), disability progression, and magnetic resonance imaging (MRI) activity. According to these findings, we established that this modality is expected to promote therapeutic remyelination in MS.

Keywords: Tissue plasminogen activator; Multiple sclerosis; Plasminogen activator inhibitor; Anti-MOG IgG antibodies, DNA polymerase enzyme

List of Abbreviations: t-PA: Tissue plasminogen activator; MS: Multiple Sclerosis; PAI-1: Plasminogen activator inhibitor-1

Introduction

In recent years, increasing evidence has pointed out to the potential role of fibrinolysis in the pathogenesis of MS. Specifically; the characteristic inflammation, focal demyelination, and axonal degeneration in MS occur after disruption of the blood-brain barrier (BBB) and entry of serum proteins, including fibrinogen, into the CNS [1]. Extracellular proteolysis represents a potent and irreversible mechanism modulating the extracellular matrix and tissue remodeling, which can affect the breakdown of the BBB [2,3]. Extracellular proteolytic enzymes have been implicated as important factors in demyelinating neuroinflammatory disorders such as MS. Plasminogen activator inhibitor (PAI-1) is a potent inhibitor of fibrinolysis that functions in the regulation of the plasmin-based pericellular proteolytic cascade. PAI-1 also serves to regulate cell migration though binding matrix protein, such as vitronectin and heparin [4-6]. PAI-1 is synthesized in endothelial cells and its release may be stimulated by the onset of inflammation. More reports recorded elevation of the PAI-1 concentrations in patients with multiple sclerosis, encephalitis, viral meningitis, and leukemia [7,8]. Tissue plasminogen activator (t-PA), a neuronal as well as the key fibrinolytic enzyme, is found in high concentration in demyelinated axons in multiple sclerosis lesions together with fibrinogen deposits. t-PA is also present in high concentration in neurons, where upon activation, it has been found to have a role in neuronal development and synaptic remodeling [9,10]. The key molecules in the PA system are tissue-type plasminogen activator (t- PA) and its inhibitor (PAI-1) and due to the formation of t-PA and inhibitor (*e.g.*,PAI) tightly complexes, the fibrinolytic potential in demyelinating MS lesions is greatly diminished because of the formation of the t-PA/PAI-1 complexes is assumed to reduce the ability of t-PA receptors to produce plasmin, which further diminishes the fibrinolytic capacity in MS lesions, possibly resulting in increased axonal fibrin deposition and neurodegeneration [11,12]. In this study we hypothesize that the growing of human brain lesions in multiple sclerosis patients results from excessive fibrin deposition in nerve cells which is being reversibly cleared with action of t-PA [13]. This phenomenon could be considered a defensive mechanism, but with time, this reversible continuous dynamic action

triggers the formation of antibodies against t-PA that interferes with the action of t-PA, thus paving the way for extra expression for PAI-1system.

DNA polymerase is a vital enzyme for the regulation of multiple physiological cellular functions such as DNA repair, gene transcriptions, cell cycle progression, cell death, chromatin function, and genomic stability. It is not surprising that cells in all organisms contain multiple highly specialized DNA polymerases; the majority of it has been recently discovered. One of the main tasks of polymerase is to repair the opposite template lesions by a process known as translation synthesis [14,15].

Aim of the Study

In view for the novel beneficial effects of DNA polymerase, a number of animal studies and human were done to investigate the novel roles of DNA polymerases outside of chromosome replication and repair in processes such as enhancing myelin regenerations in nerve cells, regulate the fluctuations on the concentration levels of PAI-1and t-PA in blood samples, and its positive reflexes in inducing differences in MRI results with stopping the MS diseases activity. In this study we explore the pharmacological action of DNA polymerase enzyme as a potential therapeutic intervention for treatment of MS patients and also to evaluate the potential role DNA polymerase enzyme as anti-inflammatory enzyme.

Materials and Methods

Injection Material

DNA polymerase enzyme (termed SS6) is a sterile, purified preparation of a bacterial protein and is supplied as a liquid containing 5u/IU in vial form contains 6 ml/each, the preparation contains preservatives and is intended for S/C and I/M.

Animal Study

Naïve male and female SJL/J mice 6–8-wk-old (20-23 g) were purchased from (Science Park, Egypt). Transgenic mice were developed in the Transgenic Mice Unit of the Menofiya University.

Anti-inflammatory effect of DNA polymerase: We investigate the Inhibitor action of DNA polymerase for [TNF & Mycobacterium tuberculosis] as inflammatory arthritis inducer [16,17].

A subcutaneous injection of DNA polymerase enzyme (termed SS6) was studied in animal models. About 7 naïve male SJL/J mice 6–8-wk-old (20-23 g) as a test group were injected by multiple intradermal injections of a prepared combination of TNF-alpha, human recombinant for 21 d (Bio vision) and Mycobacterium tuberculosis (Difco) and reconstituted them in H_2O to a concentration of 0.1-1.0 mg/ml to be ready for use. Mice were examined daily for signs of joint inflammation and scored as follows: 0, normal; 1, erythema and mild swelling confined to the ankle joint or mid-foot; 2, erythema and mild swelling extending from the ankle to the mid-foot; 3, erythema and moderate swelling extending from the ankle to the metatarsal joints; 4, erythema and severe swelling extending from the ankle to the digits. The degree of arthritic inflammation was scored as follows: 0, no signs of inflammation; 1, mild synovitis; 2, severe synovitis; 3, severe synovitis with mild cartilage and bone destruction; 4, severe synovitis with severe cartilage and bone destruction. Five Mice from the seven received 0.01 IU of DNA polymerase (termed SS6) two times daily by subcutaneous injection for three weeks while the other two mice didn't receive this injection acts as control group.

Inhibition of EAN activity (experimental autoimmune neuritis): EAN is one autoimmune-mediated inflammation of the peripheral nervous system that leads to disabling disorder of motor, sensory, and autonomic systems [18]. Regarding DNA polymerase as examined therapeutic tool, we investigate its role in recovering the clinical signs of EAN in animal model. About 6 naïve male SJL/J mice 6-8-wk-old (20-21 g) were injected with a 10μg recombinant Human Myelin Protein Zero (Abcam) diluted with bovine albumin solution to 0.05 mg/ml (0.5 gm) as adjuvant, S/C into hind foot pads for 7 Day and into the back on from Day 8 to 22., The mice were clinically examined in blinded fashion to determine disease activity according to a clinical scoring system defined as follows: 0, normal; 1, ruffled fur; 2, floppy tail, weak grip, and slightly reduced motility; 3, mild paraparesis with impaired motility; 4, severe paraparesis with significantly reduced motility; 5, tetraparesis with complete immobilization; 6, death. Four Mice from all six received 0.01 IU of DNA polymerase two times daily starting at the onset of neurological symptoms, (9 d after immunization) and continued for 22 d, while the other two mice didn't receive this injection, acts as control group.

Discovery of DNA and its effects on EAE animal model: 6 naïve female SJL/J mice 6–8-wk-old (20-23 g) were all immunized with myelin oligodendrocyte glycoprotein (MOG) peptide [19]. Each mouse was injected S/C into 4 sites on the back, adjacent to each of the forelimbs and hind limbs (total volume 200 ml) with 200 mg MOG emulsified with 100 ml complete Freund's adjuvant (CFA), one time/d for 15 d. Four female mice (test group) were injected with 0.01 IU of DNA polymerase (termed SS6) SC two times daily starting at the onset of neurological symptoms, (8 d after immunization) and continued for 22 d week in parallel the immunization with MOG, while the other two female mice were not injected with DNA polymerase as a (control group). All the animals were evaluated for neurological score as follows

1. Decreased tail tone
2. Mild hind limb paralysis

3. Moderate hind limb paralysis
4. Severe hind limb paralysis
Maximal neurological effects were observed 10 d after immunization.

Explore the efficacy of DNA polymerase as fibrinolytic agent:

Reagents:

1. Purified human thrombin was obtained from Sigma Chemical Co. as a lyophilized human thrombin and Lyophilized human fibrinogen (St. Louis, MO) as a lyophilized powder was dissolved in 0.05 M Tris-HCl (pH 7.4) centrifuged at 2,000 g for 20 min (40 °C) and the supernatant was frozen in a small aliquots at -75 °C.

Human plasmin (American Diagnostica Inc., Greenwich, CT) and human a2-antiplasmin (Calbiochem-Novabiochem Corp., La Jolla, CA) were reconstituted, centrifuged at 2,000 g for 20 min (40 °C), and stored at -75 °C.

2. Recombinant human t-PA was obtained from (Genentech, Inc., South San Francisco, CA) with various concentrations of t-PA from 0.05 to 0.2 U/ml

3. DNA polymerase was obtained from (Bio lab), various concentrations of enzyme was (5, 10, 15 units) dissolved in 6 ml of PBS.

4. Fluorescence labeling, fibrinogen (10 mg/ml) was incubated with1mg/ml FITC (Molecular Probes, Inc., Eugene) with a continuous stirring for 1 h at 22 °C in 0.1 M sodium bicarbonate (pH 9.0) and stored at -75 °C.

5. Enriched bovine plasma: Purified fibrin gels were formed by suction pipetting a rapidly mixed solution of fibrinogen (3 mg/ml) and thrombin (1 U/ml) up into glass capillary tubes (1.5 mm inner diameter). The buffer for fibrin polymerization was 0.05 M Tris-HCI (pH 7.4) with 0.1 M NaCl to obtain turbid, coarse gels .The gels were allowed to polymerize for over 2 h with plasmin as intrinsic mechanism, In this experiment we investigate our suppose that DNA polymerase has similar effect like t-PA on fibrin gel lysis as a fibrinolytic action, a Various concentrations of t-PA from 0.02 to 0.2 U/ml and DNA polymerase 5,10,15 IU were examined, a control sample of bovine enriched plasma, every concentration was added above the purified fibrin clot plus plasmin as intrinsic factor and was allowed to be incubated for 300 s under well mixed conditions with moderately strong stirring using a small single-blade. The Purified fibrin clots were lysed with the addition of the t-PA enzyme and DNA polymerase, while the addition of bovine enriched plasma the lysis rates were considerably very slow. To explore the velocity of fibrin (solving), we used a fluorescence release assay using FITC-labeled coarse fibrin gels. A volume of 200 ul of polymerizing fibrinogen (3 mg/ml) containing 0.1 mg/ml FITC-fibrinogen was placed in the bottom of a cuvette and allowed to be polymerized for over 90 min to form a 2-mm thick coarse fibrin gel to detect the release of fluorescent degradation products The increase in the intensity of fluorescence emission during plasmin degradation of fibrin due to generation of quenched fragments, the changes in the amount of fluorescence were measured with a luminescence spectrometer at a room temperature.

Patients and Methods

Patient's inclusion criteria

All of these patients have not received previously immunosuppressive therapy and none of them showed any laboratory evidence of diabetes mellitus, liver, renal or metabolic disorders, rheumatoid arthritis or other inflammatory neurological diseases. All patients fulfilled the criteria for clinically definite MS [Inclusion Criteria: Patient suffering from either primary or secondary progressive multiple sclerosis with relapse, Patient with EDSS score of [1.0 to 8.0 and has At least two clinically silent lesions on the T2-weighted MRI scan, with a size of at least 3 millimeter (mm)] and all of them were seropositive for anti-MOG IgG antibodies. We excluded patients who had a history of taking any immunomodulatory or immunosuppressive therapy, Oral or systemic corticosteroids within 10 days prior to study Day and patients with liver diseases, kidney and Cardiac disease, such as angina, congestive heart failure or arrhythmia. The patients were classified into three groups, according the numbers of lesions in white matter and the disease severity using the Multiple Sclerosis Severity Score (MSSS), which corrects the Expanded Disability Status Scale (EDSS) [20,21]. Group A 10 patients (9 female and 1 male) included patients with relapsing-remitting (RR) or primary progressive (PP) score ranging from 1.5 to 3 with one to two lesions, Group B 6 patients (4 female and 2 male), with secondary progressive (SP) course score ranging from 3 to 5 with two to three lesions. Group C 5 patients (4 female and one male) with score ranging from 5 to 7.5 with more than three lesions by using MRI scans.

Injection Material

DNA polymerase enzyme (termed SS6), is a sterile, purified preparation of a bacterial protein and is supplied as a liquid containing 5u/IU in vial form contains 6 ml/each, the preparation contains preservatives and is intended for S/C and I/M [22,23]. Each patient was advised to take 6 vials during the duration's therapy. This was given as subcutaneous injection, 0.1 cc twice daily for 24 weeks. All of the patients consented to this therapy, and two consecutive serum samples were obtained from all of them. The serum samples were analyzed before the beginning of the study and two Weeks after the end of the therapy. ELISA kit for PAI-I (American Diagnostic, Greenwich, CN, USA), t-PA (Techno clone) and anti-MOG-IgG antibodies (Ana Spec) were used, plasma

samples were cold-centrifuged, immediately separated and stored in -70 °C, and the quantities were measured using t-PA, PAI-1and anti-MOG IgG antibodies previously diagnostic kits, (normal values PAI-1 n: 2.2-11.2 ng/ml, t-PA (n: 0.5-4.2 ng/ml), anti-MOG IgG (detection range 0.1-10 ng/ml).

Quantitative Assay for PAI-1 and t-PA Level: First samples that were collected before starting the therapy were assayed to measure the concentrations level of PAI-1 and t-PA by comparing the absorbance of each well with a series of absorbance values obtained from known plasma concentrations of PAI-1, t-PA kits. PAI-1plasma level results: (group A) their PAI-1 levels showed 6 higher folds72±66 ng/ml; (group B) gave 4 higher folds 44±39 ng/ml and (group C) demonstrated 2 higher fold 24±21, While the t-PA levels is 0.3±0.2 ng/ml in group A, 1.6±0.4 ng/ml in group B; and 4.5±1.2 ng/ml in group C (Table 1). This is not a randomized controlled study.

		Group A (10 patients) (8F and 1M)	Group B (6 patients) (4F and 2M)	Group C (5 patients) (5F and 1M)	ANOVA (sig.)
PAI-1 level	Before therapy	72±66ng/ml	44±39ng/ml	24±21ng/ml	0.242 b(NS)
	After therapy	18±1.2ng/ml	13.5±1.5ng/ml	11.5±2.3mg/ml	0.000 (HS)
	p (sig.)	0.018 (S)	0.08 (NS)	0.222 (NS)	
t-PA level	Before therapy	0.3±0.2ng/ml	1.6±0.4ng/ml	4.5±1.2ng/ml	0.000 (HS)
	After therapy	4.5±0.5ng/ml	4.0±0.6ng/ml	4.8±0.7ng/ml	0.09(NS)
	p (sig.)	0.0001 (HS)	0.0001 (HS)	0.642 (NS)	

Table 1: Comparison between plasma level for t- PA and PAI-1 before and after treatment

Quantitative Assay for Anti-MOG-IgG Antibodies Titre: Serum sampling of 21 MS patients were examined for anti-MOG –IgG antibodies before the beginning of the study and two Weeks after the end of the therapy. ELISA kit for anti-MOG –IgG antibodies (Ana Spec)(detection range 0.1-10 ng/ml) was used, plasma samples were cold-centrifuged, immediately separated and stored in -20 °C, and the quantities were measured using anti-MOG –IgG antibodies previously diagnostic kits, The level of anti-MOG IgG antibodies were determined in all examined patients with MS and the obtained results were compared with positive and negative controls before the beginning of the study and two Weeks after the end of the therapy. The anti-MOG antibodies titre: in group A their anti-MOG antibodies levels was (32-88 ng/ml), group B (40-380 ng/ml) and group C (400- 850 ng/ml).

Results

Investigate the Fibrinolytic Action

We found that DNA polymerase and t-PA were nearly effective in dissolving fibrin. This similarity may be due to the autocatalytic conversion of Glu-plasmin into Lys-plasmin which was expected to occur during the experiment. This assay showed that the fibrinolytic potential of DNA polymerase enzyme (termed SS6) gave a different degree in the velocity of gel lysis when we using a higher concentrations of DNA polymerase and faster just if we using a very low concentration than the t-PA at a high concentration, with used a fluorescence labeling fibrinogen to evaluate the fibrin lysis process, by the fibrin fibers degradation, the FITCs on each fragment are dequenched due to the loss of fibril-fibril interaction and the loss of interactions within the fibrin monomer subunit .

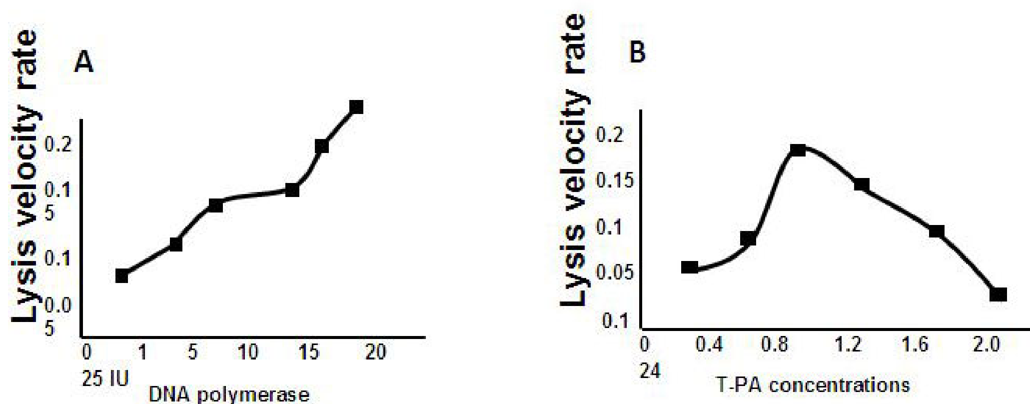

Figure 1: Investigate the velocity rate of fibrin gel lysis for DNA polymerase enzyme and t-PA as fibrinolytic factors when we added them separately above FITC-fibrin gel with 0.1 μM plasmin as intrinsic factor, A Figure described the velocity rate of DNA polymerase enzyme on gel fibrin lysis and B Figure described the velocity rate of t-PA on gel fibrin lysis the figures conclusion showed that DNA polymerase in low concentration gave the same rate of velocity for gel fibrin lysis similarly equal to the high concentration of t-PA showed. These results prove that DNA polymerase can activate a process of fibrinolysis faster than t-PA enzyme and can be used as stimulant for process of fibrinolysis

The release of Fluorescent degradation products with adding 0.05 ml of 10 IU DNA polymerase to FITC-fibrinogen showed an increase in the intensity of fluorescence emission during the process of plasmin degradation of fibrin in compare to the lower degree fluorescence emission when we used 2.0 ng/ml concentration of t-PA, while the control sample showed a minimal emission after long time incubation (Figure 1).

In Studying the Role of DNA Polymerase

In studying the role of DNA polymerase as anti-inflammatory factor we found that Test group mice by clinical examination a completely disappearance of inflammatory arthritis signs and all inflammation were dramatically ameliorated one week after the TNF–tuberculosis mycobacterum combination intradermal injection after receiving a two times daily of 0.01 IU of DNA polymerase, the control groups mice showed a severe synovitis and swelling from the ankle to the mid-food. These results proved that the DNA polymerase has an anti- inflammatory effect and can be used as an inhibitor for arthritis; this is in startling contrast to TNF, which initiates and exacerbates arthritis and inflammation (Figure 2).

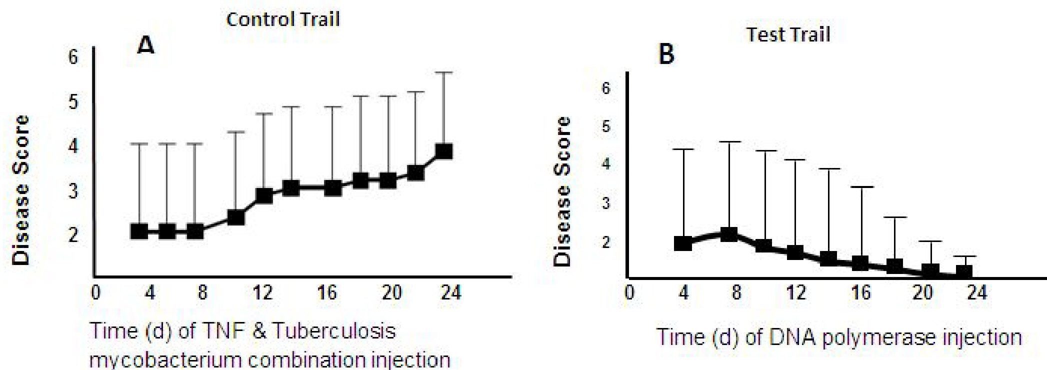

Figure 2: A. Exacerbation of arthritis manifestation for the controlled mice group that received a multiple intradermal injections of a prepared of TNF & Tuberculosis mycobacterium combination injection for 21 d and didn't receive the therapeutic intervention of DNA polymerase, .Statistically the degree of arthritic inflammation was scored as follow: 0, no signs of inflammation; 1, mild ; 2, severe; 3, severe synovitis with mild cartilage and bone destruction; 4, severe synovitis with severe cartilage and bone destruction. This figure showed that the score of inflammation is correlated to the time duration of TNF & Tuberculosis mycobacterium combination injections
B. The test group that received two times of 0.01 IU of DNA polymerase showed that after one week of subcutaneous injections the clinical examination and the signs of inflammatory arthritis was dramatically ameliorated, significant differences between the two groups were observed and proved that the DNA polymerase effect as anti- inflammatory and can be used as an inhibitor of arthritis

Exploring the Role of DNA Polymerase

Exploring the role of DNA polymerase as therapeutic intervention for neurological diseases in experimental autoimmune neuritis (EAN) in mice we found that immunization it with recombinant Human Myelin Protein Zero (Abcam), the outcome data for the novel function of DNA polymerase enzyme in treating mice with (EAN) revealed that the effect DNA polymerase (termed SS6) has a new intervention therapeutic potential for treatment neurological disorders. Test group mice showed a significantly reduction in neurological score when we started the injection of 0.01 IU of DNA polymerase SC two times daily at the onset of neurological symptoms, (7 d after immunization) and complete recover of all neurological signs at the end of 23 d, control group showed no any reduce for EAN activity (Figure 4). Our experiments raise the clinically relevant question of whether these data can be used as a promising concept in MS therapy and other neurological diseases, either prophylactically or therapeutically.

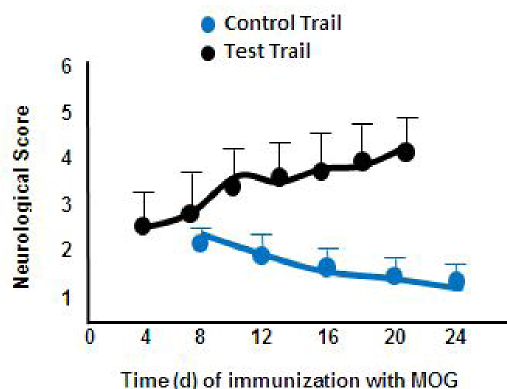

Figure 3: Effect of DNA polymerase on EAE animal model, the black line in the figure showed the control trail mice. This group didn't receive our intervention compound and the effect of immunization for 23 d with myelin oligodendrocyte glycoprotein (MOG) and starting the onset of neurological symptoms from day 7 with progress of the neurological scored 4 degree at 23 d, the test mice group remarked by blue line showed marked reduction in neurological score gradually from day 8 till complete animal recovery at d 23, this results indicated that DNA polymerase has a therapeutic value for treatment of neurological disease.

Discovery of DNA and its Effects on EAE Animal Model

6 naïve female SJL/J mice 6–8-wk-old (20-23 g) were all immunized with myelin oligodendrocyte glycoprotein (MOG) peptide. Each mouse was injected S/C into 4 sites on the back, adjacent to each of the forelimbs and hind limbs (total volume 200 ml) with 200 mg MOG emulsified with 100 ml complete Freund's adjuvant (CFA), one time/d for 15 d. Four female mice (test group) were injected with 0.01 IU of DNA polymerase (termed SS6) SC two times daily starting at the onset of neurological symptoms, (8 d after immunization) and continued for 22 d week in parallel the immunization with MOG, while the other two female mice were not injected with DNA polymerase as a (control group). All the animals were evaluated for neurological score as follows 1-decreased tail tone; 2- mild hind limb paralysis; 3- moderate hind limb paralysis; 4- severe hind limb paralysis. Maximal neurological effects were observed 10 d after immunization.

Examining the Effect of Both DNA Polymerase

In examining the effect of both DNA polymerase as inhibitor for the signs of Experimental Autoimmune Encephalitis (EAE) We demonstrate that DNA polymerase treatment markedly ameliorated the neurological score in the active mouse EAE model the test groups mice showed a marked change in the neurological score when we started the treatment at onset of symptoms, control group showed no effect in reducing the active model of EAE. Pharmacokinetic studies of DNA polymerase enzyme (termed SS6) showed that Laboratory Tests shortly after I/M or S/C injection of DNA polymerase enzyme (termed SS6) will cause slightly decrease in in thrombin time (TT), clotting time and fibrinogen, activated partial thromboplastin time (APTT), platelets counts, prothrombin time (PT), increase in t-PA level, slightly decrease in PAI-1 level and marked increase in CD4+ T-cells, Tregs cells. Studying the Clinical pharmacology of the DNA polymerase enzyme (termed SS6) revealed that DNA polymerase enzyme acts by convert's plasminogen to the proteolytic enzyme plasmin which activate the t-PA enzyme and diminish the t-PA & PAI-1 complex structure, increase in level of t-PA enzyme play the main role in reduce the neurological score. There is no recorded any adverse reactions have been associated with our enzyme specific concentrations with S/C and I/M injection but some patients recorded slightly hypotensive effect. Drug interaction for the DNA polymerase enzyme (termed SS6), with other drugs has not been well studied. The mechanism by which dissociated DNA polymerase enzyme is eliminated is clearance by sites in the liver which usually normalize within 2-4 hours. This clear effect for the DNA polymerase enzyme (termed SS6) in the rat model further supports the efficacy of the peptide and its potential appropriateness for MS patients. Moreover, the relatively fast recovery of animals treated at the acute phase of disease (7 d after (MOG immunization) indicates that DNA polymerase might also be of therapeutic value for MS patients at advanced stage of disease and for those suffering from the progressive form of MS which currently has no available effective therapies.

Results of Voulenteers Studies

Statistics: Study was carried on 21 patients 4 male (19.04%) and 17 female (80.96%) with clinical diagnosis of MS, their age ranged between 22 and 46 years with a mean of 33.57 years old (SD±7.65) (Table 2).

		Range	Mean± SD
Age (years) "n=21"		22-46	33.57± 7.65
		Number	%
Sex	Male	4	19.04
	Female	17	80.9

Table 2: Descriptive data of the studied patients

Statistical analysis was carried out using SPSS 21.0. Results are presented as percentage, mean ±SD. Continuous variables were compared using t test, and categorical variables were analyzed with χ^2 test. Correlation analyses were performed using Pearson. In all statistics, two-sided tests were used and the results were considered statistically significant at P≤0.05 and highly significant P≤0.005.

Effect of DNA Polymerase in Regulates the Fluctuations of PAI-1and t-PA in Blood Samples

The second samples were collected two weeks after last injection, and the collected data revealed that PAI-1level in group A decreased significantly after intervention to become 18±12 ng/ml, group B 13.5±1.5 ng/ml and 11.5±2.3 ng/ml in group C. The t-PA levels increased to 4.5±0.5 ng/ml in group A, 4.0±0.6 ng/ml in group B and 4.8±0.7 ng/ml in group C, this increase was statistically significant in groups A & B while it was non-significant in group C as shown in figure 4,5.

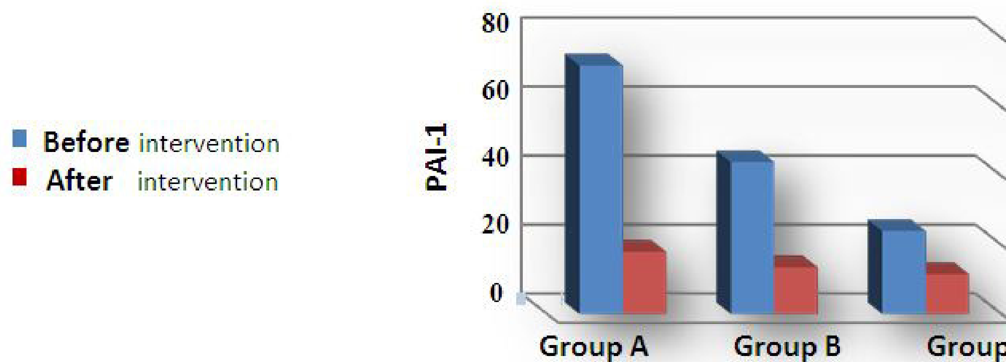

Figure 4: Comparison between plasma levels for PAI-1 before and after treatment

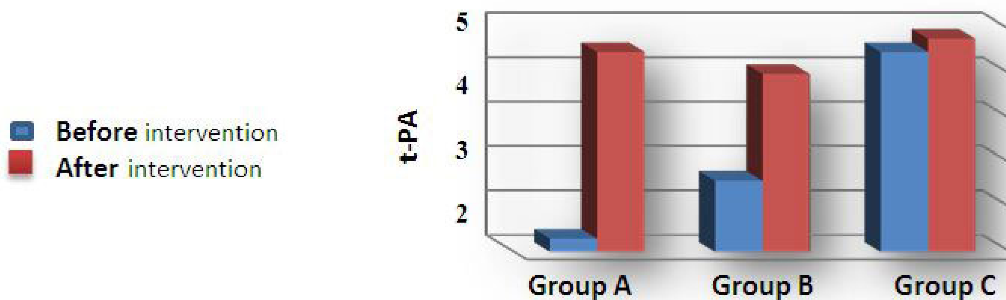

Figure 5: Comparison between plasma levels for t.PA before and after treatment

Effect of DNA Polymerase in Regulates the Fluctuations of Anti MOG Antibodies in Serum Samples

The collected data two weeks after last injection for the titer of anti-MOG antibodies in serum samples of MS patients revealed that the seropositive patients for [group A 7 patients showed a significantly decreased in antibodies titre to 8-12 ng/ml, 2 patients showed 10-14 ng/ml], group B 5 patients showed 15-19 ng/ml, one patient 23 ng/ml While the group C all tested seropositive patient's showed marked decrease in the titre of antibodies 30-67 ng/ml. These results showed significant relation between the decrease in level of antibodies titre and the changes in the brain magnetic resonance imaging (MRI) lesions during the course of the therapy and the anti-MOG antibodies can serve as a diagnostic and maybe prognostic tool in patients with MS patients. The outcome results measures showed for Disability Status Scale (EDSS) Group A Change from 3 to 0 with, reducing in number and volume of lesion and no new or enlarging lesions on T2-weighted MRI image, Group B change from 5 to 1 reducing in number and volume of lesion and no new or enlarging lesions on T2-weighted MRI image and Group C change from 8 to 3 with reducing in number and volume of lesion and no new or enlarging lesions on T2-weighted MRI image.

Discussion

Currently available drugs used for treatment of relapsing remitting MS are corticosteroids like methylprednisolone and other immunomodulators including recombinant interferon-b forms, and cyclophosphamide [24]. Based on hypotheses describing the aggressive autoimmune responses observed in MS patients, a result of impair between (t-PA and PA1-1) which are a key molecules in both fibrinolysis and extracellular proteolysis and more reports recorded the potential role of t-PA and its inhibitors (PAI-1) in the pathogenesis of MS [25,26]. Specifically; the characteristic inflammation, focal demyelination. This study was done to explain that polymerase enzyme has a role in process of coagulation and fibrinolysis, also to investigate the therapeutic potential of polymerase enzyme in modulating the changes occurred between levels of Tissue- type plasminogen activator (t-PA) and its inhibitor (PAI-1). depends on a new mode of action a several mechanisms have been proposed describe that after the injection of the polymerase enzyme a numbers of blood activators factors, like the plasmin, Treg cells, CD4+ T-cells, the plasmin play a vital role in reduce the formation of the t-PA/PAI-1 complexes which assumed to reduce the ability of t-PA receptors to produce plasmin, which further enhances the fibrinolytic capacity in MS lesions, possibly result in an increased removal of the axonal fibrin deposition this regulative manner represents a protective mechanism to remove fibrin deposits, which exacerbate axonal injury and promote regeneration while the increase in Treg cells, CD4+ T-cells may play another complementary role in reducing the cycles of cytokines, this suggests that DNA polymerase has an immunomodulating function [27,28]. In a summary for the all discovered benefits we found that DNA polymerase enzyme (SS6) has a fibrinolysis action and can activate a process of fibrinolysis faster than the previously known effect of the t-PA enzyme and can be used as one of the preferable stimulant for the process of fibrinolysis our results proved that DNA polymerase. One of expected pharmacological action of DNA polymerase is the stimulation of Treg and CD4+T-cells which play role in reduction a number of inflammatory mediators and cytokines which in turn shows a

significantly complete reduction in the neurological score in two of neurological disorders in animal diseases (EAN and EAE) as an animal model of MS and can use this benefit as anti- inflammatory molecule for treatment arthritis. Our experiments raised the clinically relevant question of whether these data can be used as a promising concept in MS therapy and other neurological diseases, either prophylactically or therapeutically treating in lab animals. In explore the therapeutic intervention The potential effect for the DNA polymerase was detected clearly in examined patient suffering from either primary or secondary progressive multiple sclerosis with relapse, were showed a positive relationship between active MS processes and elevated plasma PAI-1levels, as well as a direct relation between the injections of DNA polymerase, decreasing the levels of PAI-1 the regression in numbers of brain lesions and improving their ability levels, this will explain the role of this therapy in treating relapsing remitting MS which will ultimately lead to a new generation of drugs as a regenerative medicine, also the obtained results that describe the decrease in level of anti-MOG –IgG antibodies before the beginning of the study and evaluating their levels Weeks after the end of the therapy and the changes in the brain magnetic resonance imaging (MRI) lesions during the course of the therapy can open a new gate for the functions of DNA polymerase as immunomodulating and can use its effect as diagnostic and maybe prognostic tool in patients with MS patients.

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References

1. East E, Baker D, Pryce G, Lijnen HR, Cuzner ML, et al. (2005) A role for the plasminogen activator system in inflammation and neurodegeneration in the central nervous system during experimental allergic encephalomyelitis. Am J Pathol 167: 545-54.

2. Lemarchant S, Docagne F, Emery E, Vivien D, Ali C, et al. (2012) tPA in the injured central nervous system: different scenarios starring the same actor? Neuropharmacology 62: 749-56.

3. Cuzner ML, Opdenakker G (1999) Plasminogen activators and matrix metalloproteases, mediators of extracellular proteolysis in inflammatory demyelination of the central nervous system. J Neuroimmunol 94: 1-14.

4. Gveri´c D, Herrera B, Petzold A, Lawrence DA, Cuzner (2003) Impaired fibrinolysis in multiple sclerosis: a role for tissue plasminogen activator inhibitors. Brain 126: 1590-8.

5. Sutton R, Keohane ME, Van der Berg SR, Gonias SL (1994) Plasminogen activator inhibitor-I in the cerebrospinal fluid as an index of neurological disease. Blood Coagul Fibrinolysis 5:167-71.

6. Aota S, Nomizu M, Yamada KM (1994) The short amino acid sequence Pro-His-Ser-Arg-Asn in human fibronectin enhances cell-adhesive function. J Biol Chem 269: 24756-61.

7. Gorog DA (2010) Prognostic value of plasma fibrinolysis activation markers in cardiovascular disease. J Am Coll Cardiol 55: 2701-9.

8. Rijken DC, Lijnen HR (2009) New insights into the molecular mechanisms of the fibrinolytic system. J Thromb Haemost 7: 4-13.

9. Lo EH, Broderick JP, Moskowitz MA (2004) tPA and proteolysis in the neurovascular unit. Stroke 35: 354-6.

10. Young AR, Ali C, Duretete A, Vivien D (2007) Neuroprotection and stroke: time for a compromise. J Neurochem 103: 1302-9.

11. Candelario-Jalil E, Yang Y, Rosenberg GA (2009) Diverse roles of matrix metalloproteinases and tissue inhibitors of metalloproteinases in neuroin- flammation and cerebral ischemia. Neuroscience 158: 983-94.

12. Dietzmann K, von Bossanyi P, Krause D, Wittig H, Mawrin C, et al. (2000) Expression of the plasminogen activator system and the inhibitors PAI-1 and PAI-2 in posttraumatic lesions of the CNS and brain injuries following dramatic circulatory arrests: an immunohistochemical study. Pathol Res Prac 196: 15-21.

13. Salah S (2016) A Novel Approach for Treatment Patients with Multiple Sclerosis by Using DNA Polymerase. J Alzheimers Dis Parkinsonism 6: 235.

14. Brutlag,D, Kornberg A (1972) Enzymatic synthesis of deoxyribonucleic acid. 36. A proofreading function for the 3' leads to 5' exonuclease activity of deoxyribonucleic acid polymerases. J Biol Chem 247: 241-8.

15. Muzyczka N, Poland RL, Bessman MJ (1972) Studies on the biochemical basis of mutation. I. A comparison of the deoxyribonucleic acid polymerases of mutator, antimutator, and wild type strains of bacteriophage T4. J Biol Chem 247: 7116-22.

16. Song K, Chen Y, Göke R, Wilmen A, Seidel C, et al. (2000) Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (Trail) Is an Inhibitor of Autoimmune Inflammation and Cell Cycle Progression. J Exp Med 191: 1095-104.

17. Brunn Anna, Mirna Mihelcic, Mariana Carstov, Lisa Feind, Eva C, et al. (2017) Toll-Like Receptor 2, Toll-Like Receptor 4, Myeloid Differentiation Response Gene 88, and Toll–IL-1 Receptor Domain-Containing Adaptor-Inducing Interferon-γ (TRIF) Selectively Regulate Susceptibility of P0106-125-Induced Murine Experimental Autoimmune Neuritis. Am J Pathol 187: 42-54.

18. G Deretzi, S Pelidou, L Zou, C Quiding, E Mix et al. (1999) Suppression of chronic experimental autoimmune neuritis by nasally administered recombinant rat interleukin-6. Immunology. 97: 69-76.

19. Miller SD, Karpus WJ (2007) Experimental Autoimmune Encephalomyelitis in the Mouse. Curr Protoc Immunol Unit-15.1.

20. Lassmann H (2007) Multiple sclerosis: is there neurodegeneration independent from inflammation? J Neurol Sci 259: 3-6.

21. Hebert JR, JR Corboy (2013) The association between multiple sclerosis-related fatigue and balance as a function of central sensory integration. Gait & posture 38: 37-42.

22. Anderson S, Gait MJ, Mayol L, Young IG (1980) A short primer for sequencing DNA cloned in the singlestranded phage vector M13mp2. Nucleic Acids Res 8: 1731-43.

23. Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74: 5463-7.

24. Falaschi A, Spadari S (1978) in DNA Synthesis: Present and Future/ edited by Molineux I, Kohiyama M. 487-515.

25. Emeis JJ, Van Hinsbergh VW, Verheojen JH, Wijngaards G (1983) Inhibition of tissue-type plasminogen activator by conditioned medium from cultured human and porcine vascular endothelial cells. Biochem Biophys Res Commun 110: 392-98.

26. Loskutoff DJ, Van Mourik JA, Erickson LA, Lawrence D (1983) Detection of an unusually stable fibrinolytic inhibitor produced by bovine endothelial cells. Proc Natl Acad Sci USA 80: 2956-60.

27. Hübscher U, Kuenzle CC, Spadari S (1979) Functional roles of DNA polymerases beta and gamma. Proc Natl Acad Sci USA 76: 2316-2320.

28. Weissbach A (1977) Annu Rev Biochem 46: 25-47.

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