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Tuftsin-phosphorylcholine attenuate experimental autoimmune encephalomyelitis

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ABSTRACT

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) which carries a significant burden of morbidity and mortality. Herein we examine the effects of acute treatment with tuftsin-phosphorylcholine (TPC), a novel immune-modulating helminth derived compound, on a murine model of MS. Experimental autoimmune encephalomyelitis (EAE) mice received acute treatment with TPC showed an improved clinical score and significantly less signs of inflammation and demyelination in CNS tissue compared with vehicle treated EAE mice. Our findings suggest that TPC may provide a beneficial clinical effect in EAE and may therefore have a potential value for ameliorating clinical manifestations and delaying disease progression in MS.

1. Introduction

Multiple Sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) affecting > 2.5 million individuals worldwide. In MS, aberrant pro-inflammatory immune processes target various myelin constituents, leading to multifocal demyelination, neuronal degeneration, oligodendrocyte depletion and astrocytosis. (Compston and Coles, 2008; Lassmann, 2008; Steinman, 2014).

While the precise etiology of MS is still unknown, several environmental factors and genetic features are implicated. The hygiene theory represents one environmental aspect that modulate the risk for developing MS (Fleming and Cook, 2006). It relates to the steady rise in incidence of certain autoimmune diseases (such as MS) in developed countries, parallel to the improvement of sanitary conditions and to the decline in incidence of many infections in these areas. The protective properties of certain infections against the development of autoimmunity are attributed to their stimulation of immunoregulatory pathways (Bach, 2017; Versini et al., 2015). Several intriguing epidemiological studies point to a negative correlation between MS rates and the prevalence of certain parasites, suggesting a possible protective role of these infections against MS (Correale and Farez, 2011a). Some exapmples are the increase in MS in Sardinia following the eradication of malaria in the island (Sotgiu et al., 2008), the steep fall in MS prevalence observed in areas where the prevalence of the helminth Trichuris trichiura exceeded 10% (Correale and Farez, 2011a), and the rise in MS noted in the French West Indies parallel to the reduction of parasitic infections in the area (Cabre et al., 2005). Furthermore, studies have shown an improvement in disease activity score among MS patients naturally exposed to or intentionally treated with helminths (Correale and Farez, 2007, 2011b; Dixit et al., 2017; Fleming et al., 2017, 2011).

The immune-modulating effects of helminths derive from interactions between expressed and secreted helminths products to the host immune cells. One of the secreted products encompasses the phosphorylcholine (PC) moiety (Goodridge et al., 2007; Pineda et al., 2014).

In search of an effective treatment for autoimmune conditions, utilizing a small molecule with minimal side effects, PC was conjugated with tuftsin, a self-immunomodulatory molecule produced in the spleen (Najjar and Nishioka, 1970; Siebert et al., 2017; Siemion and Kluczyk, 1999), to form tuftsin-phosphorylcholine (TPC).

TPC was shown to induce beneficial immunomodulatory effects in murine models of three autoimmune diseases (*i.e.* DSS induced colitis,

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collagen induced arthritis and lupus nephritis) as well as on *ex-vivo* peripheral blood mononuclear cells and temporal artery biopsies of giant cell arteritis patients (Bashi et al., 2016, 2015b; Ben-Ami Shor et al., 2015; Ben-Amram et al., 2017; Blank et al., 2018; Shemer et al., 2018).

These findings, along with the growing body of evidence implying a attributing effect to helminths in MS patients, led us to examine the potential value of acute treatment with TPC in experimental autoimmune encephalomyelitis (EAE), a murine model of MS (Aharoni et al., 2013).

We evaluated the clinical outcome and conducted histological assessments of CNS tissues for the hallmark histopathological imprints of MS, consisting of inflammation, demyelination, neuronal damage and reactive gliosis (Aharoni et al., 2016; Compston and Coles, 2008; Lassmann, 2008).

2. Material and methods

2.1. TPC- TPC was synthetized by Novotide.LTD, Haifa Israel

2.1.1. 2.2 Mice and experimental design

C57BL/6 female mice at the age of 8 to 10 weeks were purchased from Envigo, Jerusalem, Israel, and were used and kept under specific pathogen free (SPF) environment. All experiments were approved and executed according to the protocols of the Ethical Committee of the Israeli Ministry of Health.

EAE was induced by the peptide derived from myelin oligodendrocyte glycoprotein (MOG 35–55), synthesized by Genscript (Piscataway, NJ, USA) as previously described (Aharoni, 2014; Aharoni et al., 2016). Mice were injected subcutaneously with 100 µl emulsion containing 200 µg of the peptide in Freund's adjuvant enriched with 3.3 mg/ml heat-inactivated *Mycobacterium tuberculosis* (Sigma, St. Louis, MO USA). Pertussis toxin (Sigma), 150 ng/mouse, was injected intraperitoneally immediately after the encephalitogenic injection and 48 h later. TPC was subjected twice a week (5 µg/mouse) as an acute treatment starting one day before disease induction. The TPC was injected subcutaneously (sc) $n = 10$ mice per group. PBS was used as control group for EAE/TPC. Additional control groups included PBS treated naïve mice $n = 5$ and naïve mice administered with TPC $n = 5$.

2.1.2. Clinical assessment of disease severity

Mice were examined daily during 25 days from disease induction. EAE was scored as follows: 1 - loss of tail tonicity, 2 - hind limb weakness or partial paralysis, 3 –hind leg complete paralysis with hind body paresis, 4 - hind and foreleg paralysis, 5 - death. Differences in clinical score between groups were analyzed by SPSS software (version 23, IBM, Armonk, NY) applying the non-parametric Mann Whitney *U* test (Baker and Amor, 2012).

2.1.3. Brain and spinal cord fixation

After mice were sacrificed (day 25), four mice were selected from each experimental group. Mice were anesthetized and perfused transcardially with PBS containing 20 U heparin, followed by paraformaldehyde (4% paraformaldehyde in PBS with 0.2% picric acid 7.4 pH). Brains and spinal cords were removed, postfixed (12 h, 4 °C), paraffin embedded and sectioned coronally (brains) or transversely (spinal cords) cut (5 µm) by microtome (Leica,DE).

2.1.4. Histopathological analysis

Sections were deparaffinized with o-xylol at 60 °C followed by rehydration with a series of increasingly diluted ethanol solutions. Parallel sections were stained by Hematoxylin-Eosin (HE) staining and Luxol Fast Blue (LFB) staining to identify areas of infiltration by inflammatory cells and demyelination.

2.1.5. Immunohistochemistry

Activated astrocytes were detected by an avidin-biotin immunoperoxidase method using primary rabbit polyclonal antibody to GFAP (1:500, Abcam) (Bourne, 1S983).

2.1.6. Quantitative analysis

For quantitative analysis the Video-TesT-Morphology version 5.2 software was used. Quantification of demyelination was performed by measuring the percentage of myelin loss in 0.09 mm^2 fields, 4–5 fields (magnification 40 ×, from 15 to 20 sections of segmenta thoracicae and lumborum of each animal, 4–5 mice per group) (Li et al., 2017). Quantification of cellular accumulation was performed by measuring the percentage of region of cellular accumulation in 0.09 mm^2 fields, 4–5 fields (magnification 40 ×, from 12 to 15 SC sections and 15–20 brain sections of each animal, 4–5 mice per group). Quantification of activated astrocytes in SC was performed by counting the GFAP-immunopositive cells in white and grey matter in 0.09 mm^2/fields (magnification 40 ×, from 15 to 20 SC sections and 5–10 brain sections of each animal, 3–4 mice per group). We analyzed summarize quantity of activated astrocytes on the each brain slices both in control and in experimental groups. These regions of the brain were selected are accordingly the pre-existing data on the involvement of various regions of brain in MS. Thus the quantification of activated astrocytes in brain was conducted in basal nuclei, hypothalamus, thalamus (level 59–65 in accordance Allen Brain Atlas (2008). Difference in myelin loss, cellular accumulation and activated astrocytes number between the groups were analyzed by StatSoft, Inc. (2007) STATISTICA version 8.0. (Tulsa, USA) and R-studio (R-Tools Technology, Canada). Descriptive statistical methods included estimation of mean and standard deviation of the mean (SD). Differences between the groups were evaluated by nonparametric Mann-Whitney *U* test for two independent groups. A probability value (p) of < 0.05 was considered significant.

2.2. Microglia cell cultures and treatment

Murine N9 microglia cell line was maintained at 37 °C in a 95% atmospheric air and 5% CO_2 humidified atmosphere in RPMI medium supplemented with 5 mM glucose, 100 U/mL penicillin and 100 µg/mL streptomycin and 5% fetal bovine serum (all from Biological Industries). Microglial cells were plated at a density of plated at a density of 5×10^5 cells per well in 24-well plates (Thermo plates Fisher Scientific Inc., Waltham, MA, USA) for 24 h. Then, the cells were exposed to lipopolysaccharide (LPS) (100 ng/ml) for 24 h followed by addition of different concentrations of TPC, tuftsin or phosphorylcholine (PC) (5, 10, µg/ml). Viability was checked by a trypan-blue staining. Secretion of IL-6, and IL-10 in the culture fluid was followed by ELISA using R&D DuoSet kits according to the manufacturer recommendations. To define the neuropilin-1 on microglia cells through which TPC may affect the M1 phenotype switch, we incubated the M1 phenotype cells with neuropilin-1 (NP1) inhibitor, 50 µM EG00229 N^2-[[3-[(2,1,3-Benzothiadiazol-4-ylsulfonyl)amino]-2-thienyl]carbonyl]-L-arginine (Tocris Bioscience, Ellisville, MO, USA) for 1 h before adding the TPC, tuftsin or phosphorylcholine.

2.2.1. Statistical analyses

Differences in clinical score between groups were analyzed by SPSS software (version 23, IBM, Armonk, NY). The data was first tested for normality using the Shapiro-Wilks test, followed by a parametric comparison using independent samples *t*-test. MS Excel (MS Office 2016) and R-studio (R-Tools Technology, Canada) was used for immunohistochemical statistical analyses. Descriptive statistical methods included estimation of the arithmetic mean and standard deviation of mean (SD). Nonparametric statistical methods were used as well. Differences between the groups were evaluated by Mann-Whitney *U* test. A probability value (p) of < 0.05 was considered significant.

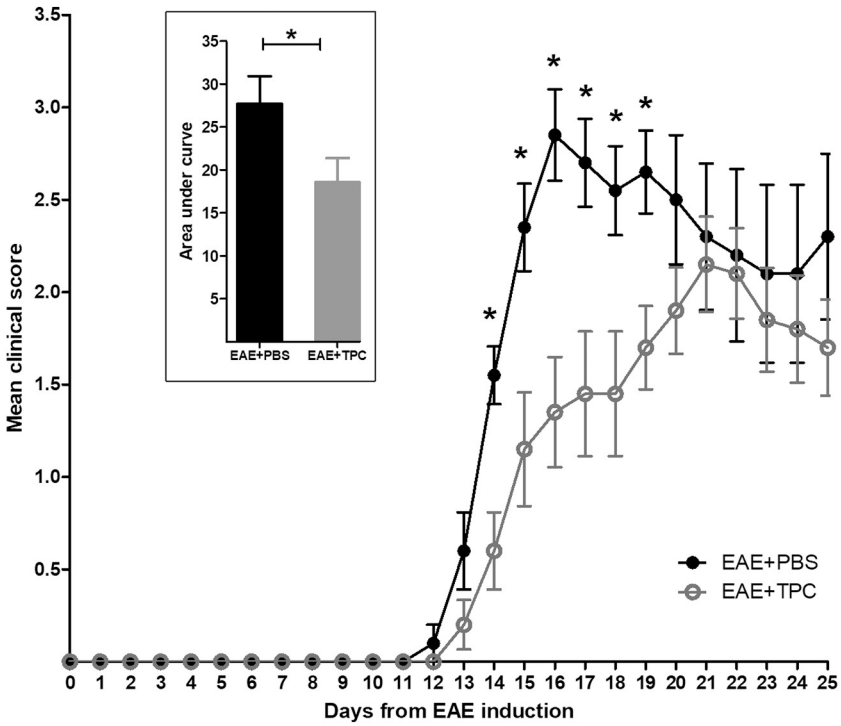

Fig. 1. The effect of TPC on clinical manifestations of EAE. The daily mean clinical scores of EAE mice induced by the MOG 35–55 peptide + SEM. TPC treatment in EAE mice (EAE + TPC), $n = 10$. Control EAE-mice were similarly injected by PBS (EAE + PBS), n = 10. EAE was scored as follows: 1 - loss of tail tonicity, 2 - hind limb weakness or partial paralysis, 3 – hind leg paralysis, 3.5 hind leg complete paralysis with hind body paresis, 4 - hind and foreleg paralysis, 5 - death. * indicates significant differences between the groups.

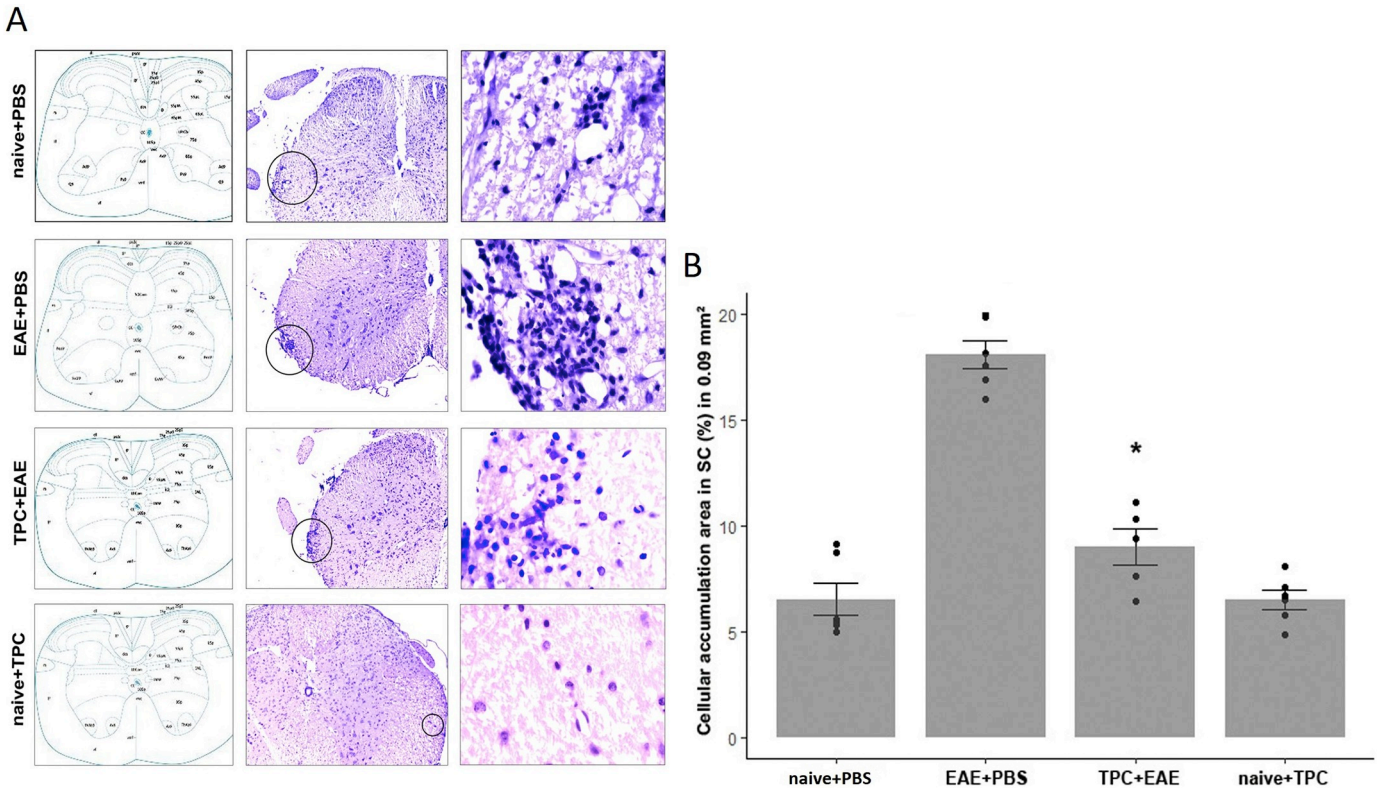

Fig. 2. The effect of TPC on cellular accumulation in spinal cord.
A - Perivascular infiltration in the spinal cord in the PBS treated naïve mice (naïve + PBS), PBS treated EAE mice (EAE + PBS), TPC treated EAE mice (TPC + EAE) and naïve mice administered with TPC (naïve + TPC). Demyelination was detected using LFB staining (10× and 40× magnification, respectively).
B - Quantification of cellular accumulation area in the spinal cord in the PBS treated naïve mice (naïve + PBS), PBS treated EAE mice (EAE + PBS), TPC treated EAE mice (TPC + EAE) and naïve mice administered with TPC (naïve + TPC).

3. Results

3.1. TPC attenuate the clinical score of EAE mice

The effect of TPC on EAE manifestations was investigated in the $MOG_{35–55}$ peptide-induced chronic (non-remitting) model. TPC was given to EAE mice in an acute protocol. Control EAE mice were similarly injected by PBS (EAE + PBS). The daily mean clinical scores (determined according to the manifested motor impairments) of all the animals examined in this study (10 mice per group) are shown in Fig. 1. In the PBS-injected mice clinical manifestations typically appeared 12 days after disease induction, increasing in severity to score of nearly 3 (complete hind body paralysis), mean score of 2.85 ± 0.25 at day 16. Thereafter, motor impairments gradually decreased to a mean score of 2.1 ± 0.48 and 2.3 ± 0.45 by days 24 and 25 after disease induction, respectively.

Mice treated with TPC exhibited delayed and milder clinical manifestations, reaching a maximal mean score of 2.15 ± 0.26 on day 21, and 1.7 ± 0.26 at the end of the experiment - day 25. Mann Whitney U test, sought for differences in clinical manifestations between the groups, indicated a significant reduction of the clinical score in EAE + TPC mice compared to EAE + PBS mice at day 14 ($p = .007$), day 15 ($p = .009$), day 16 ($p = .002$), day 17 ($p = .011$), day 18 ($p = .023$) and day 19 ($p = .015$). A significant difference was evident also for the area-under-curve parameter (AUC, insert in Fig. 1), which indicates the combined clinical scores for the entire experimental period (days 0–25); EAE + PBS: 27.7 ± 3.22, EAE + TPC: 18.55 ± 2.83, $p = .04$.

Thus, TPC delayed disease development and ameliorated the clinical manifestations of EAE.

3.2. TPC attenuates signs of inflammation and demyelination in the spinal cord

Signs of inflammation and demyelination in the spinal cord were determoned by H&E and LFB staining, respectively, on day 25 of the experiment. Perivascular infiltration and demyelination was observed mainly in the lateral white matter adjacent to the dorsal horns (Fig. 2A, 3A). Regions of cellular accumulation were measured in 0.09 mm^2fields, 4–5 fields (from 12 to 15 sections of segmenta thoracicae and lumborum of each animal, 4–5 mice per group) ($P < .0001$) (Fig. 2B). Regions of myelin loss were measured in 0.09 mm^2fields, 4–5 fields (from 15 to 20 sections of segmenta thoracicae and lumborum of each animal, 4–5 mice per group), and were larger in size in the Vehicle treated EAE group compared with the TPC treated EAE group ($P < .0001$) (Fig. 3B).

The quantitative measurements of GFAP-positive astrocytes in the spinal cord sections (day 25) are presented in Fig. 4. The average number of astrocytes in spinal cord was significantly increased in EAE group comparing EAE + TPC group (20.9 + 1.69 *vs* 10.63 + 3.75), $p < .01$. No significant changes were depicted in naive + PBS group (the average number of astrocytes in spinal cord was 9.14 + 1.98, and in naive + TPC group it were 10.51 + 2.51), $p > .05$. Astrocytes were observed predominantly in dorsal horns of the spinal cord.

3.3. TPC attenuate signs of inflammation in the brain

The process of demyelination in the spinal cord correlated with cellular accumulation and astrocytes activation in the brain. Signs of inflammation were observed mainly in the basal nuclei and thalamus (level 60–66 in accordance Allen Brain Atlas). Regions of cellular accumulation in the brain were larger in the Vehicle treated EAE group compared with the TPC treated EAE group ($P < .0001$) (Fig. 5).

As demonstrated in Fig. 6A, TPC treatment in EAE mice led to a decrease in the expression of GFAP in the brain compared to the vehicle treated EAE mice ($P < .0001$). Furthermore, the expression of GFAP in the naïve mice administered with TPC resembled the PBS treated naïve mice ($P > .05$).

The quantitative measurements of GFAP-immunopositive astrocytes in the basal nuclei, preoptic nucleus of the hypothalamus and thalamus structures (level 59–65 in accordance Allen Brain Atlas) are presented in Fig. 6B.

3.4. TPC shift microglia cells in-vitro from M1 to anti-inflammatory M2 phenotype

Naïve N9 mouse microglia cells (M0) were treated with LPS to drive the cells into M1 phenotype secreting elevated proinflammatory cytokines. The M1 cell were exposed to TPC (5,10 μg/ml) for 72 h and cytokines secretion (IL-6, IL-1β, IL10) was measured, data are illustrated in Fig. 7A-C. Mann Whitney *U* test, sought for differences in cytokine concentrations in the culture fluid of the treated microglia cells with TPC, tuftsin and PC.

Data ilustrated in Fig. 7A show that TPC and tuftsin at 5 μg/ml moderetly inhibited IL-1β secretion ($p = .037$, $p = .039$ respectively), wherase TPC at 10 μg/ml prevented significantly IL-1β secretion ($p = .0027$). Tuftsin reduced less signficantly ($p = .0089$), all compared to M1 cells. PC did not have any effect on IL1-β secretion, ($p \geq .05$).

The data indicate a significant reduction in the IL-6 concentrations in the culture fluid upon presence of TPC or tuftsin 5 μg/ml and 10 μg/ml, ($p = .0019$, $p = .0014$ respectively) in comparison to the secretion by M1 microglia cells. No significant difference was obsereved for IL-6 secretion after exposure to 5 μg/ml tuftsin, 5 μg/ml and 10 μg/ml of PC ($p \geq .05$) (Fig. 7B). IL-6 secretion due to exposure to tuftsin showed a border line of significsnce ($p = .047$).

Inversely, TPC accelerated IL-10 production at at 5 μg/ml ($p = .009$) and at 10 μg/ml, ($p = .0001$ respectively) in comparison to M1 cells. Likewise tuftsin alone elevate IL-10 production only at 10 μg/ml ($p = .028$), in comparison to M1(Fig. 7C).

Moreover, as illustrated in Fig. 7C, neuropilin-1 (NP1), a commercial inhibitor, prevented the secretion of IL-10 by microglia cells, mediated by TPC or tuftsin, ($p = .00011$ and $p = .0017$ respectively). PC did not have any effect on IL-10 secretion ($p \geq .05$), all compared to cells in the absence of NP1 inhibitor.

4. Discussion

Multiple sclerosis is a chronic demyelinating autoimmune disease involving significant morbidity and takes a substantial toll on the quality of life of afflicted patients. The disease pathogenesis involves impairment of immune tolerogenic mechanisms leading to over activation of specific populations of T helper cells (Th) 17 and Th1, as well as aberrant activation of certain B cells and components of the innate immune system (Baecher-Allan et al., 2018; Thompson et al., 2018).

While a clear contribution of genetic traits was established for the disease, several environmental elements have been implicated to be involved in the instigation and attenuation of the aberrant immune responses at the root of the MS (Baecher-Allan et al., 2018; Cabre et al., 2005; Cooke, 2009; Fleming and Cook, 2006; Thompson et al., 2018). One note-worthy environmental element explored is the interaction between helminths and the human immune system. Helminths have been vastly demonstrated to possess immune modulating effects which may have served them in their evolutionary quest for symbiotic existence within human hosts (Ben-Ami Shor et al., 2013; Cooke, 2009; Gause et al., 2003; Redpath et al., 2014; Versini et al., 2015). Furthermore, treatment of MS patients with helminths and their products has been shown to produce beneficial effects in several studies (Dixit et al., 2017; Fleming et al., 2017, 2011).

Previously, the immunomodulatory effect of TPC was demonstrated in several murine autoimmune experimental models (*e.g.* lupus nephritis, collagen induced arthritis and DSS induced colitis) (Bashi et al., 2016, 2015b; Ben-Ami Shor et al., 2015; Ben-Amram et al., 2017; Blank

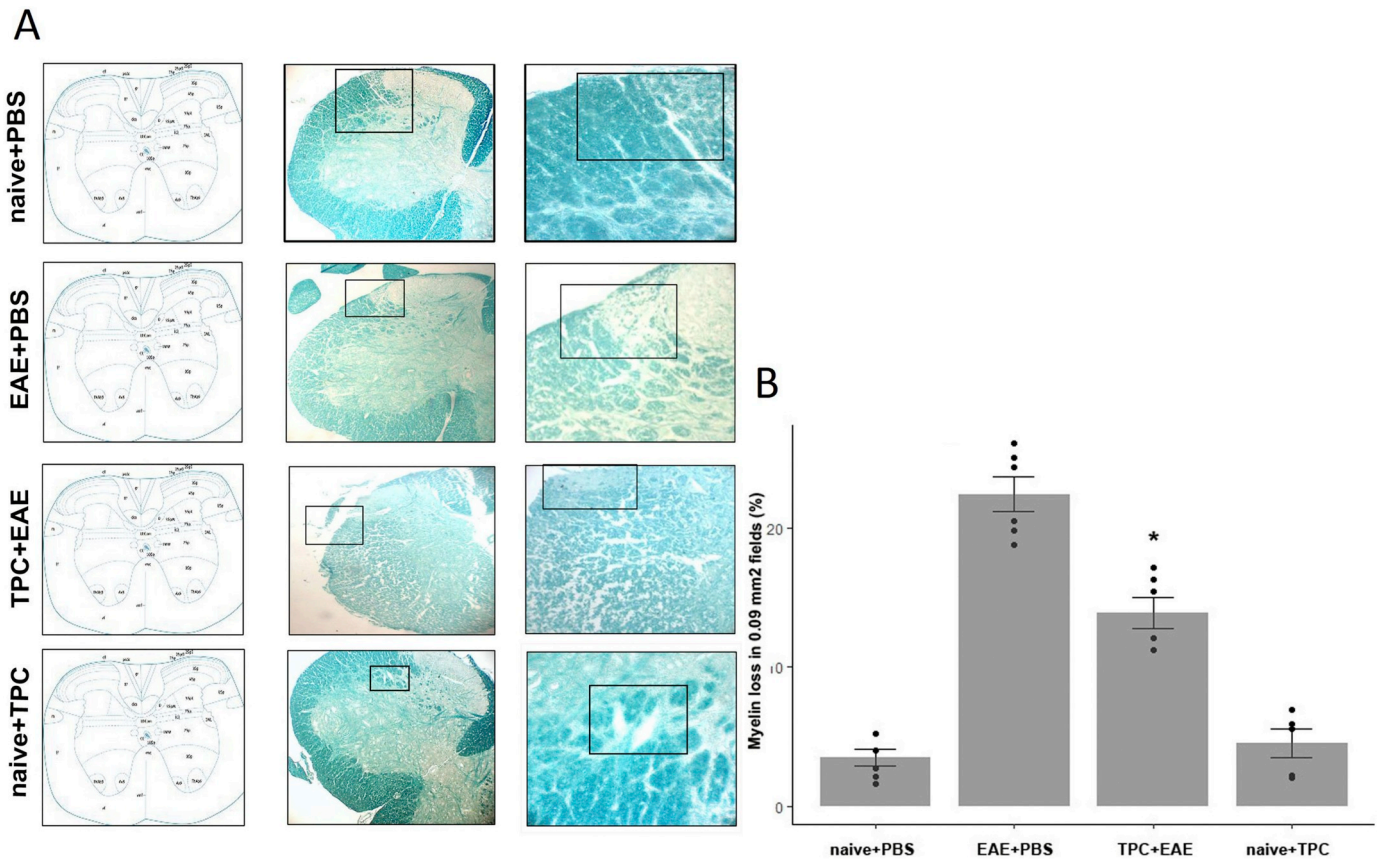

Fig. 3. The effect of TPC on demyelination in the spinal cord

A - Demyelination in the spinal cord in the PBS treated naïve mice (naïve + PBS), PBS treated EAE mice (EAE + PBS), TPC treated EAE mice (TPC + EAE) and naïve mice administered with TPC (naïve + TPC). Demyelination was detected using LFB staining (10× and 40× magnification, respectively). The magnification in Figure shows the white matter areas of the posterior horns of the spinal cord.

B - Quantification of demyelination in the spinal cord in the PBS treated naïve mice (naïve + PBS), PBS treated EAE mice (EAE + PBS), TPC treated EAE mice (TPC + EAE) and naïve mice administered with TPC (naïve + TPC).

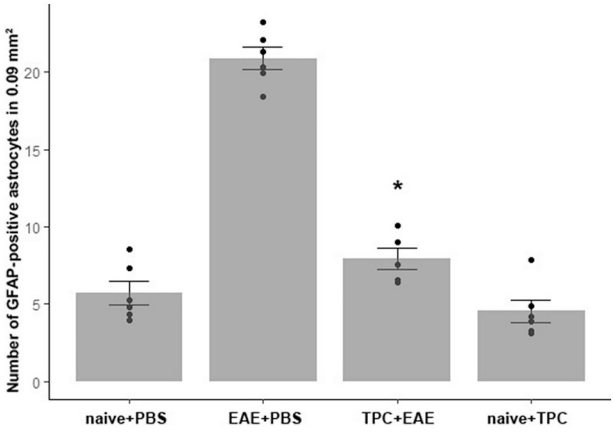

Fig. 4. The effect of TPC on the number of GFAP-positive astrocytes in the spinal cord.

Quantification of average number of GFAP-positive astrocytes in the spinal cord in the PBS treated naïve mice (naïve + PBS), PBS treated EAE mice (EAE + PBS), TPC treated EAE mice (TPC + EAE) and naïve mice administered with TPC (naïve + TPC).

et al., 2018; Shemer et al., 2018).

Herein, we examined the potential therapeutic value of TPC in EAE, a murine model of MS (Aharoni, 2014; Aharoni et al., 2016; Baecher-Allan et al., 2018; Nair et al., 2008).

TPC acute treatment in EAE mice reduced the clinical score and severity of the disease in comparison to the vehicle treated EAE mice.

Moreover, tissue samples from both the spinal cords and the brains of TPC treated EAE mice demonstrated significantly less perivascular infiltrations and cell accumulations as well as lower rates of GFAP-positive astrocytes, compared with parallel samples from the vehicle treated EAE mice.

Additionally, spinal cord tissues of TPC treated EAE mice presented with lower rates of demyelination compared with the PBS treated EAE mice. The reduced demyelination observed following TPC treatment may be the result of its immunomodulatory effect on the pathological inflammation mediating the demyelination process. Lastly, evidence of a neuronal granulovacuolar degeneration and neuronal karyorrhexis were found exclusively among the vehicle treated EAE mice, solely among mice with a clinical score of 4, a reflection of neuronal damage in mice with high disease scores. These findings suggest that acute treatment with TPC reduces the occurrence of inflammation, reactive gliosis, demyelination and neuronal damage, considered as the hallmarks of the pathologic process in the basis of MS (Compston and Coles, 2008; Baecher-Allan et al., 2018). Furthermore, the results suggest that TPC acute treatment may potentially reduce physical disability among MS patients. The beneficial effect of TPC may be attributed to its bifunctional activity. TPC, through neuropilin-1 binding, shifts macrophage cells from inflammatory macrophages M1 phenotype to anti-inflammatory macrophages M2 phenotype secreting IL-10, *via* the tuftsin part of the molecule (Blank et al., 2018). Furthermore, in the current study we show that TPC as well as tuftsin (in a less significant way), are able to shift *in-vitro* microglia cells to anti-inflammatory phenotype, producing elevated IL-10 concentration. This effect may have a role in

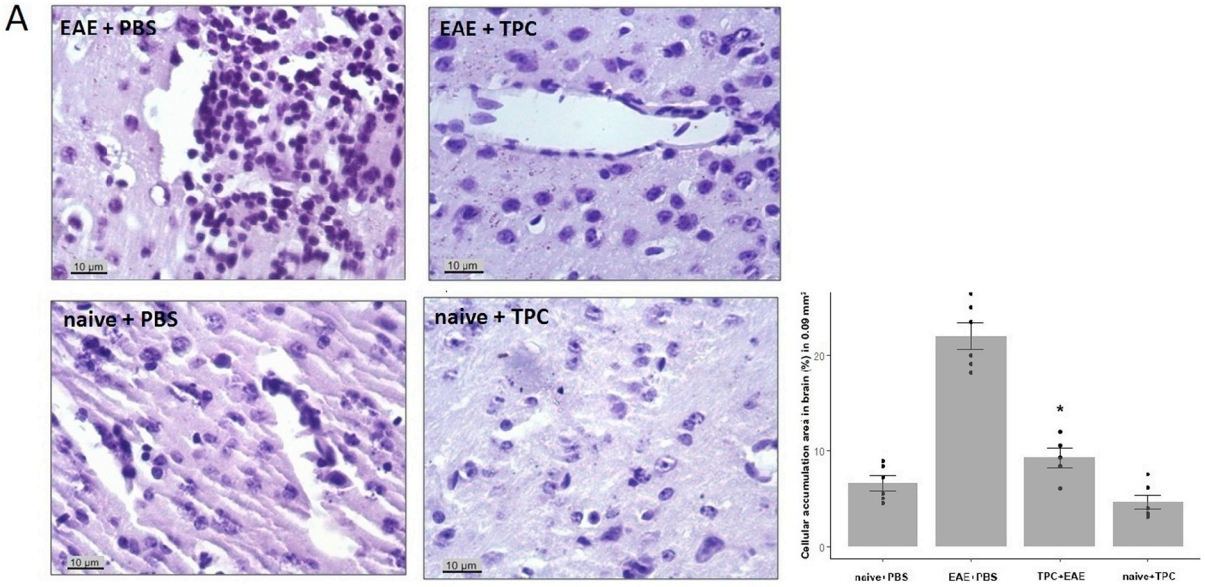

Fig. 5. The effect of TPC on infiltration in the brain.
Quantification of cellular accumulation area in the brain in the PBS treated naïve mice (naïve + PBS), PBS treated EAE mice (EAE + PBS), TPC treated EAE mice (TPC + EAE) and naïve mice administered with TPC (naïve + TPC).

the inhibitory effect on the immune network and the delay in the development of experimental EAE. These data support the reported tuftsin effect on microglia cells in murine model of EAE (Nissen and Tsirka, 2016; Bhasin et al., 2007). The bi-functional activities of TPC was represented also by the reduction of mouse TLR4 expression through NFkB pathway in HEKTM cells, *via* the phosphorylcholine site of TPC molecule point to its anti-inflammatory activity (Blank et al., 2018). Previous studies demonstrated that the TPC inhibited expression of inflammatory cytokines (*e.g.* IL-1β, IFNγ, TNFα, IL-6), enhance IL-10 secretion by splenocytes and cause an expansion of Tregs and Bregs in lupus mice, collagen induced arthritis mice and mice with DSS induced colitis (Bashi et al., 2016, 2015b; Ben-Ami Shor et al., 2015; Ben-Amram et al., 2017; Blank et al., 2018; Shemer et al., 2018). These findings bear resemblance to the reported enhancement of Treg

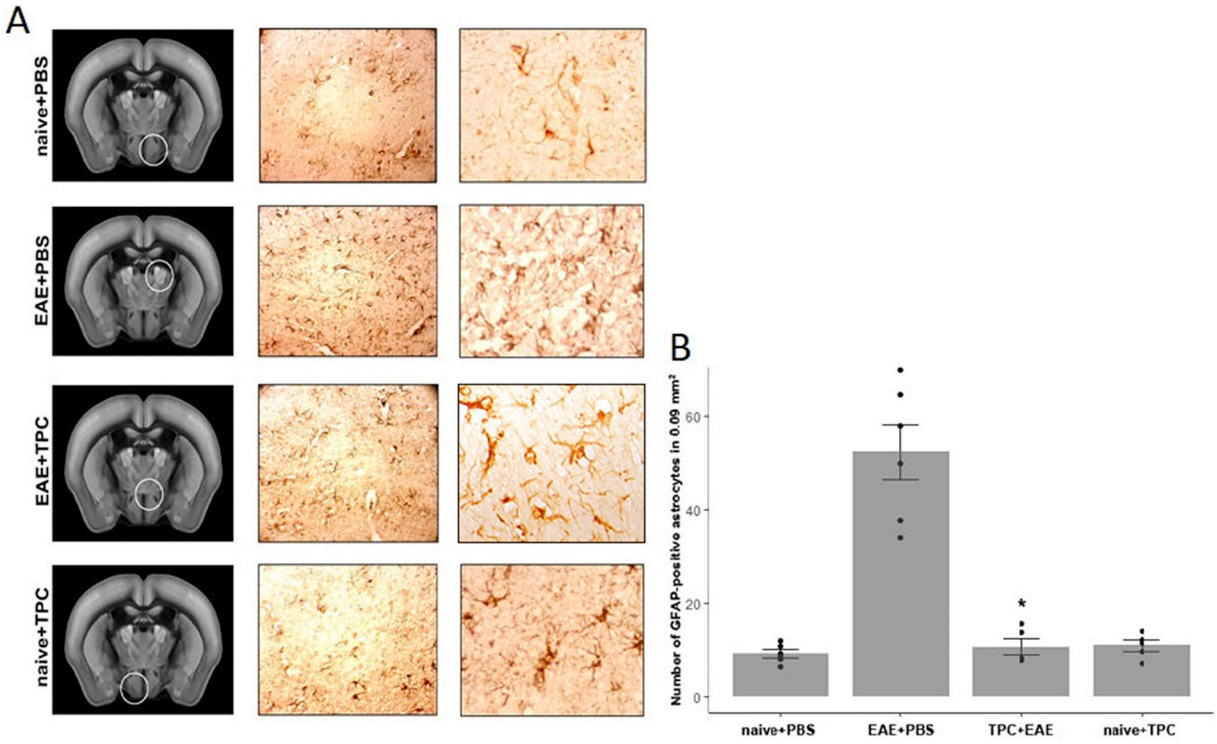

Fig. 6. The effect of TPC on the number of GFAP-positive astrocytes in the brain
A - Immunohistochemical depictions of thalamus brain sections from PBS treated naïve mice (naïve + PBS), PBS treated EAE mice (EAE + PBS), TPC treated EAE mice (TPC + EAE) and naïve mice administered with TPC (naïve + TPC) demonstrating expression of GFAP in astrocytes.
B - Quantification of the number of GFAP-positive astrocytes in the brain in the PBS treated naïve mice (naïve + PBS), PBS treated EAE mice (EAE + PBS), TPC treated EAE mice (TPC + EAE) and naïve mice administered with TPC (naïve + TPC).

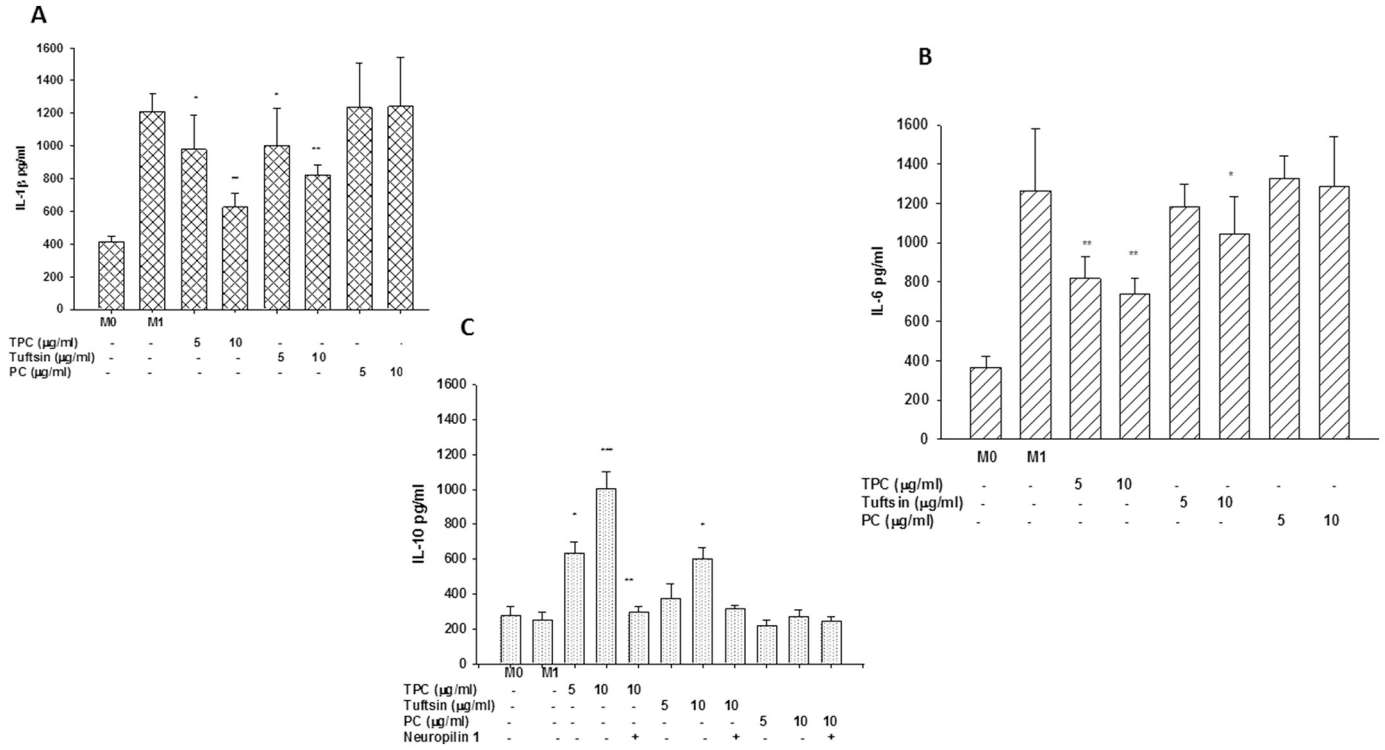

Fig. 7. TPC shift microglia cells towards anti-inflammatory phenotype, *in-vitro*.
IL-1β, IL-6 and IL-10 secretion by M1 microglia cells exposed to TPC, tuftsin and PC *in-vitro*. M0 = naïve cells, M1 = LPS stimulated microglia naïve cells. ManWitney *U* test was used to calculate the significance towards M1 secretion upon exposure of the cells to the treatments. Neuropilin was used to confirm specificity for IL-10 secretion, significance was tested by *t*-test ± neuropilin inhibitor. The data are presented as mean ± SD of 3 separate experiments.
A: IL-1β production by microglia upon LPS induction (M1), TPC, tuftsin or PC. TPC inhibited IL-1β production: * $p < .04$ and ** $p < .003$ respectively.Tuftsin reduced secretion of IL1-β by * $p < 0.04$and ** $p < .02$ respectively. PC had no effect on IL-1β production by microglia cells, $p > .05$.
B: IL-6 production by microglia upon LPS induction (M1), TPC, tuftsin or PC. IL-6 production by microglia cells M1 upon exposure to TPC, tuftsin or PC. TPC reduced IL-6 secretion in a significant manner at 5 µg/ml and 10 µg/ml ** $p < .002$. tuftsin at 5 µg/ml and PC had no effect on IL-6 production, $p > .05$. Tuftsin at 10 µg/ml had a borderline effect on IL-6 secretion by M1 cells.
C: *IL-10 secretion by microglia M1 cells exposed to TPC, tuftsin and PC.* PC accelerated IL-10 production at 5 µg/ml * $p < .01$ and at 10 µg/ml, *** $p < .0007$ in comparison to M1 cells. Tuftsin alone elevate IL-10 production only at 10 µg/ml $p < .03$, in comparison to M1 cells. Neuropilin-1, prevented the secretion of IL-10 by microglia cells, ** $p < .001$ and $p < .02$ respectively. PC did not have any effect on IL-10 secretion $p > .05$, all compared to cells in the absence of NP1 inhibitor.

populations and increased production of IL-10 associated with improved clinical outcomes among helminth-infected MS patients (Correale and Farez, 2011a).

Limited data exist regarding the direct effects of helminth-derived products on microglial cells, however there is evidence for helminth induced suppression of their pro inflammatory phenotype (Bashi et al., 2015a; Chauhan et al., 2015), alluding to the possible modulatory effects of TPC directly on microglia.

5. Conclusion

In the current study, we demonstrated that acute treatment with TPC delays EAE development in EAE mice, indicated by a lower clinical score and reduced histopathological features of neuroinflammation, the hallmark of MS, compared to vehicle treated EAE mice. These findings point to a potential value for TPC as a novel treatment for delaying disease progression and preventing exacerbations of multiple sclerosis.

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Declaration of Competing Interest

YS and MB are shareholders in TPCera Ltd.

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