

DOI 10.2478/pjvs-2013-0099

Original article

***In vivo* stimulatory effect of multi-component herbal remedy PADMA 28 on mitogen-induced proliferation of mice splenic lymphocytes and their chemokinetic activity**

**P. Skopiński¹, D.M. Radomska-Leśniewska¹, I. Sokolnicka², B.J. Bałan³,
A.K. Siwicki⁴, E. Skopińska-Różewska^{5,6}**

¹ Department of Histology and Embryology, Center for Biostructure Research, Warsaw Medical University, 02-004, Chałubińskiego 5, Warsaw, Poland

² Transfusion Immunology Laboratory with Blood Bank, Children's Memorial Health Institute, Al. Dzieci Polskich 20, 04-730 Warsaw, Poland

³ Department of Immunology, Biochemistry and Nutrition, Warsaw Medical University, Banacha 1, 02-097, Warsaw, Poland

⁴ Department of Microbiology and Clinical Immunology, Warmian-Mazurian University, Oczapowskiego 13, 10-957 Olsztyn, Poland

⁵ Pathology Department, Center for Biostructure Research, Warsaw Medical University, 02-004 Chałubińskiego 5, Warsaw, Poland

⁶ Department of Microwave Safety, Military Institute of Hygiene and Epidemiology, Szaserów 128, 04-349 Warsaw, Poland

Abstract

PADMA 28, a natural herbal multi-compound remedy originates from traditional Tibetan medicine and possesses a variety of beneficial effects on experimental and clinical models of inflammation and atherosclerosis, as well as angioprotective, antioxidative and wound – healing properties. The aim of the present study was to evaluate the *in vivo* influence of this remedy on the *in vitro* mitogen-induced proliferation of murine splenic lymphocytes and their chemokinetic activity in cell culture. The study was performed on 6-8 weeks old inbred Balb/c mice. PADMA28 was administered to mice *per os* in daily doses 5.8 mg (calculated from the highest dose recommended for human) or 0.085 mg (dose from the range of active doses of other herbal extracts containing polyphenolic substances used previously by us in experiments with mice), for 7 days. Control groups received water.

Results: No substantial differences were observed between groups of mice fed with low and high PADMA doses. In both of them, response of splenic lymphocytes to mitogen PHA ($p < 0.001$) and their *in vitro* chemokinetic activity ($p < 0.001$ for low dose and $p < 0.01$ for high dose) were highly significantly increased as compared to the controls.

Conclusion: The results of our investigations suggest that PADMA 28 can stimulate cell-mediated immunity in mice and might be used for this purpose in the wide spectrum of doses.

Key words: PADMA 28, mice, lymphocytes, PHA

Introduction

Padma 28 a natural multi-compound preparation originates from Traditional Tibetan medicine, one of the biggest medicine system of the world. (Schwabl et al. 2006). Padma is comprised of 20 specific herbs and 2 nonherbal ingredients. As phytopharmacological analysis showed, the main active substances in Padma are bioflavonoids, tannins, terpenes and essential oils (Giełdanowski et al. 1992). Cooperation of a variety of ingredients ensures a wide spectrum of biological activities: antiinflammatory, antioxidant, antisclerotic, antiperoxidal, cytoprotective and anabolic. Owing to this, PADMA 28 exerts a variety of beneficial effects on experimental and clinical models of inflammation, degenerative diseases, chronic infections and atherosclerosis, as well as angioprotective, antioxidative and wound healing properties (Jankowski et al. 2001, Melzer et al. 2006, Aslam et al. 2010, Ginsburg et al. 2011).

PADMA 28 was registered in Switzerland in 1977 and in 1992 in Poland. This remedy has been used in Switzerland for over 30 years in the symptomatic treatment of circulatory disorders including intermittent claudication.

In this work we have investigated the *in vivo* influence of PADMA 28 tablets on the *in vitro* mitogen-induced (PHA) splenocytes proliferation and their locomotive (chemokinetic) ability in cell culture.

Materials and Methods

The study was performed on forty eight, 6-8 weeks old, inbred Balb/c mice, female, weighing about 20 g, delivered from the Polish Academy of Sciences breeding colony. PADMA 28 tablets (batch 28/6311, PADMA AG, Suisse) was administered to mice *per os* in two daily doses: 5.8 mg or 0.085 mg. Higher dose was calculated according to the highest daily dose (6 tablets 484 mg each), recommended for humans (applying the factor 7 for differences between mouse and human in relation of the surface to body mass). Lower dose conforms to the range of active doses of other herbal extracts and their polyphenolic compounds used in our previous experiments in mice (Skopińska-Różewska et al. 2002, 2009, 2011, 2012, Siwicki et al. 2007). Balb/c mice were fed with PADMA by Eppendorff pipette, in 40 µl of water, or 40 µl of water (controls), for 7 days, then sacrificed and their spleens were used in mitogen-induced (PHA) splenocytes proliferation and chemokinesis assays.

Splenocytes isolation

Splenocytes were isolated from spleens under sterile conditions by straining through stainless sieve and cotton gauze and centrifugation on Lymphoprep in order to remove erythrocytes. Isolated splenocytes were resuspended in Parker culture medium.

Mitogen-induced (PHA) splenocytes proliferation assay

Before establishing cultures, splenocytes from 2-3 Balb/C mice were pooled. Spleen cells cultures (in multiple repetitions) were incubated in Costar 96 well microplates (10^5 cells in 0.2 ml RPMI-1640 medium, Biomed Lublin, with 2mM L-glutamine, 10% FCS and antibiotics) with mitogen PHA (Murex, G. B.) at a concentration of 0.5, 1 and 2 µg/ml, in a humidified atmosphere, at 37°C, with 5% CO₂. After 48 h of incubation 10 µl of tritiated thymidine (3HTdR, 0.2 mCi/ml, specific act. 2 Ci/mM) was added. After further 24 hours cells were harvested (Skatron) and incorporation of tritiated thymidine was measured using β-scintillation counter (Rack Beta 1218, LKB Wallac). The arithmetical mean of quadruplicate count was calculated and expressed as counts per minute (CPM).

Spleen cells chemokinesis (spontaneous migration) assay

Chemokinesis assay was performed *in vitro* according to the Sandberg method (Sandberg 1976) in own modification (Skopińska-Różewska et al. 2009). Briefly: splenocytes were resuspended in Parker culture medium with 5% inactivated FCS, at the final concentration of 30×10^6 cells/ml. Afterwards, siliconized capillary tubes were filled with cell suspension, sealed with plasticine, centrifuged (5 min, 450 g) and fixed on the glass plates. Cells levels were marked. After 24 h incubation (37°C, 5% CO₂ humidified atmosphere) the distances of migration were measured in millimeters (mm) at a magnification of 6.5 × and presented as migration units (1 MU = 0.18 mm). Stimulatory indices were calculated by dividing the results obtained for individual splenocytes cultures derived from PADMA 28 fed animals by the mean of the results of accompanying control cultures.

For all experiments animals were handled according to the Polish law on the protection of animals and NIH standards. All experiments were accepted by the local Ethical Committee (No 42/N/18.11.2004).

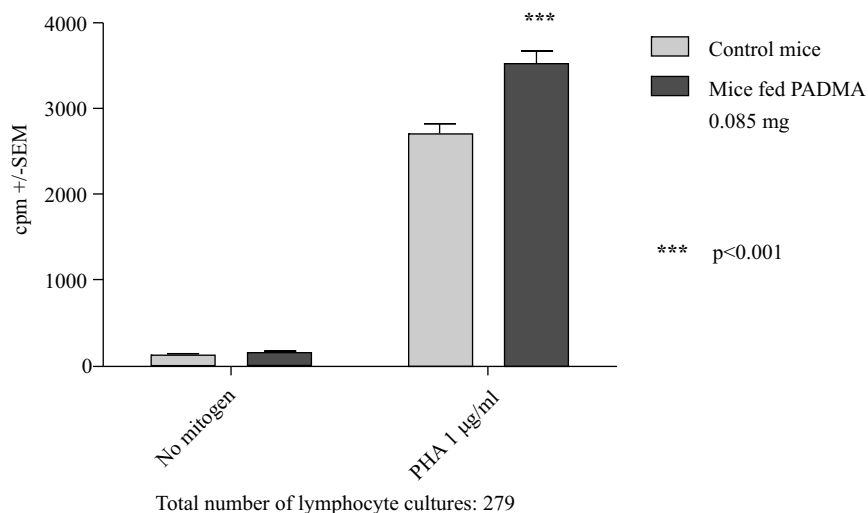


Fig. 1. Stimulatory effect of lower dose of PADMA 28 on the ability of mouse splenic lymphocytes to proliferate in the presence of PHA.

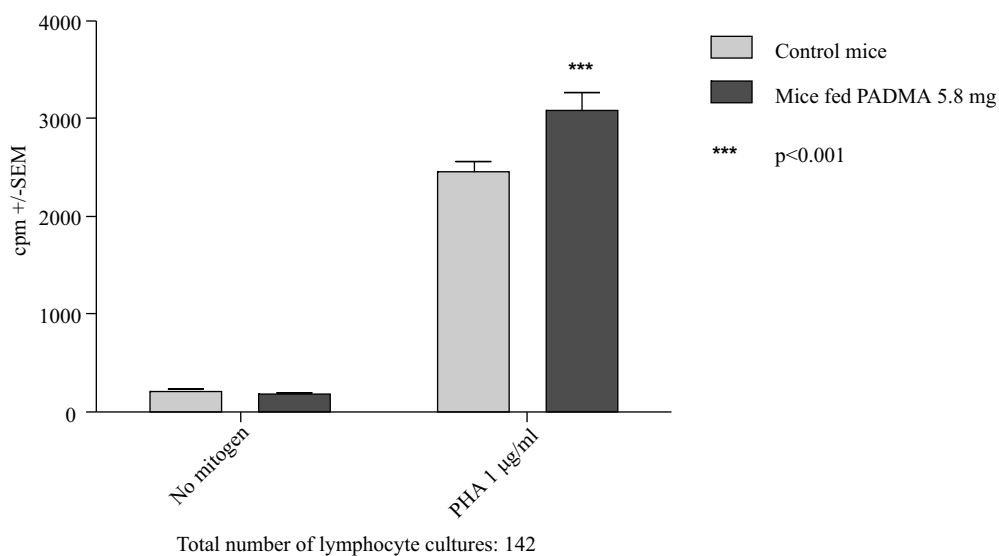


Fig. 2. Stimulatory effect of higher dose of PADMA 28 on PHA-induced proliferation of splenocytes.

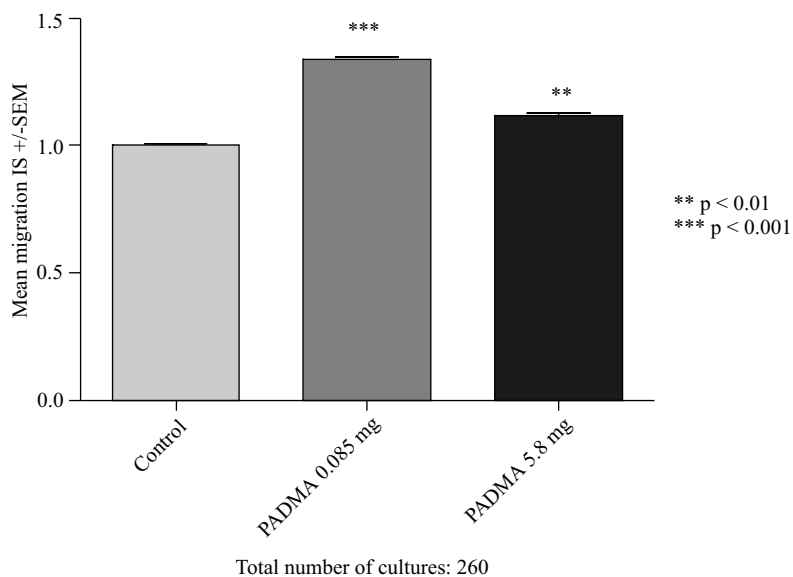


Fig. 3. Stimulatory influence of PADMA 28 on splenocytes chemokinetic activity.

Statistical analysis

Statistical evaluation of the results was performed by two-way ANOVA and the significance of differences between the groups was verified with a Bonferroni Multiple Comparison PostTest (GraphPadPrism software package).

Results

The effect of PADMA 28 on PHA – induced lymphocyte proliferation is presented on Fig. 1 (low dose) and Fig. 2 (high dose). Stimulatory effect on PHA-induced splenocytes proliferation, as compared with control group ($p < 0.001$), was noted in the group of mice fed 0.085 mg per day of PADMA 28 and in the group fed with higher, 5.8 mg daily dose of the remedy.

Stimulatory effect of PADMA administered to mice in lower dose, on *in vitro* splenocytes migration, was more pronounced ($p < 0.001$) than the effect of bigger one ($p < 0.01$) (Fig. 3).

Discussion

Studies on the effect of PADMA 28 on organisms of experimental animals are very scarce. Wójcicki et al (1989) reported the inhibition of ethanol-induced changes in rats by PADMA administration. In rabbits subjected to experimental atherosclerosis Giedanowski et al. (1992) have observed significant reduction of atherosclerotic plaques in the aorta and restoring of some immune functions by PADMA administration. It was also reported, that PADMA 28 improves structure and function of corticosteroid-treated skin, leading to improved wound healing of subsequently induced abrasion wounds in rats (Aslam et al. 2010). The therapeutic effect of PADMA 28 on experimental allergic encephalomyelitis in SJL/J mice was reported by Badmaev et al. (1999). *In vitro* study performed by Moeslinger et al. (2000) have demonstrated inhibition of inducible nitric oxide synthesis by PADMA 28 in mouse macrophage cell line. In the present paper we report for the first time the *in vivo* stimulatory effect of PADMA 28 on such parameters of cell-mediated immunity in mice as PHA- induced proliferation of splenic lymphocytes and their locomotive ability.

Most studies of the effect of PADMA 28 on various organism functions were performed in humans. Study with a big group of children suffering from chronic infective pulmonary diseases was performed by Jankowski in Poland (Jankowski et al. 1992, 1999,

2001, 2003). PADMA was applied to 254 children (2-4 tablets depending on age), while 65 children received placebo. The decreased number of infection (62%), reduction of the disease duration and applying antibiotics number was noted in studied group. These effects were maintained a year after the end of drug application.

The mechanism of immune activity of PADMA involves stimulation the number of rosette forming cells, increase haemolytic activity of complement (Suter and Richter 2000), normalization of granulocytes and macrophages phagocytic function as well as their intracellular metabolic processes.

PADMA was shown to protect DNA from oxidative stress due to content of reducing and metal ion-chelating substances in their composition. Inhibition, in a concentration dependent manner, of inducible nitric oxide synthesis was described by Moeslinger et al (2000). PADMA downregulated iNOS protein and iNOS mRNA level in a macrophage cell line. Ginsburg et al. (2011) presented protective effect of PADMA on viability of PC12 cells pretreated by neurotoxins (eg. amyloid -beta, glutamate) involved in degenerative disease such as Alzheimer's or Parkinson's diseases. Also oxidative capacity of these cells and inflammatory cytokines production were decreased by PADMA indicating that it could be useful in the treatment of inflammatory and degenerative diseases and disorders with the oxidative stress pathomechanism (Barak et al. 2004, Exner et al. 2006).

Jankowski et al (2001) demonstrated that PADMA normalized the disturbed number of CD3, CD4 and CD8 – positive T lymphocytes as well as the CD4/CD8 index of T-cells. In that study mitogen -induced (PHA, Con A) proliferation of lymphocytes was increased in the group of treated children.

Conclusion

Results of our investigations have shown that PADMA 28 can stimulate cell – mediated immunity in mice and might be used for this purpose in wide spectrum of doses.

References

- Aslam MN, Warner RL, Bhagavathula N, Ginsburg I, Varani J (2010) A multi-component herbal preparation (PADMA 28) improves structure/function of corticosteroid-treated skin, leading to improved wound healing of subsequently induced abrasion wounds in rats. *Arch Dermatol Res* 302: 669-677.
- Badmaev V, Kozłowski PB, Schuller-Levis GB, Wiśniewski HM (1999) The therapeutic effect of an herbal formula

- Badmaev 28 (padma 28) on experimental allergic encephalomyelitis (EAE) in SJL/J mice. *Phytother Res* 13: 218-221.
- Barak V, Kalickman I, Halperin T, Birkenfeld S, Ginsburg I (2004) Padma-28, Tibetan herbal preparation is an inhibitor of inflammatory cytokine production. *Eur Cytokine Netw*. 15: 203-209.
- Exner M, Raith M, Holzer G, Gmeiner B, Wagner O, Kapiotis S (2006) Anti-inflammatory mechanisms of the Tibetan herbal preparation PADMA 28 in the vessel wall. *Forsch Komplementarmed* 13(Suppl 1): 13-17.
- Giełdanowski J, Dutkiewicz T, Samochowiec L, Wójcicki J (1992) PADMA 28 modifies immunological functions in experimental atherosclerosis in rabbits. *Arch Immunol Ther Exp (Warsz)* 40: 291-295.
- Ginsburg I, Rozenstein-Tsalkovich L, Koren E, Rosenmann H (2011) The herbal preparation Padma® 28 protects against neurotoxicity in PC12 cells. *Phytother Res* 25: 740-743.
- Jankowski A, Jankowska R, Brzosko WJ (1992) Treatment of children prone to infection with PADMA 28. *Schweiz Zschr für Ganzheits Medizin* 4 (7/8 Suppl 1): 22-23.
- Jankowski A, Lewandowicz-Uszyńska A, Leszczyk-Kapusta I, Borowiec A (1999) Padma 28, oraz inne leki o działaniu immunokorekcyjnym w leczeniu nawracających zakażeń dróg oddechowych u dzieci. The use of immunostimulation in children with recurrent respiratory tract infections *Nowiny Lekarskie* 98: 61-67.
- Jankowski A, Lewandowicz-Uszyńska A, Mleczek J, Augustyniak D, Luchowska M, Jaros B, Kość A (2001) Immunokorekcyjny wpływ roślinnego leku PADMU 28 na wybrane parametry odpowiedzi immunologicznej dzieci chorych na nawracające zakażenia dróg oddechowych. *Terapia* 3: 3-7.
- Jankowski A, Mleczek J, Majkowska-Skrobek G (2003) Immunostymulacja jako postępowanie z wyboru w leczeniu nawracających zakażeń dróg oddechowych u dzieci na podstawie własnych obserwacji. In: *Rola immunomodulatorów pochodzenia naturalnego w zapobieganiu i leczeniu chorób*. Skopińska-Różewska E, Siwicki AK (eds) *Medyk*, Warszawa pp15-26.
- Melzer J, Brignoli R, Saller R (2006) Efficacy and safety of Padma 28 in peripheral arterial occlusive disease. *Forsch Komplementmed* 13: 23-27.
- Moeslinger T, Friedl R, Volf I, Brunner M, Koller E, Spieckermann PG (2000) Inhibition of inducible nitric oxide synthesis by the herbal preparation Padma 28 in macrophage cell line. *Can J Physiol Pharmacol* 78: 861-866.
- Sandberg G (1976) The sealed capillary migration technique and thymocyte migration in vitro. *J Immunol Meth* 12: 365-368.
- Schwabl H, Geistlich S, McHugh E (2006) Tibetan medicine in Europe: historical, practical and regulatory aspects. *Forsch Komplementmed* 13: 1-6.
- Siwicki AK, Skopińska-Różewska E, Hartwich M, Wójcicki R, Bakula T, Furmanowa M, Bałan BJ, Sommer E, Mielcarek S, Buchwald W, Krajewska-Patan A, Mścisz A, Mrozikiewicz PM, Bany J (2007) The influence of *Rhodiola rosea* extracts on non-specific and specific cellular immunity in pigs, rats and mice. *Centr Eur J Immunol* 32: 84-91.
- Skopińska-Różewska E, Furmanowa M, Guzewska J, Sokolnicka I, Sommer E, Bany J (2002) The effect of *Centella asiatica*, *Echinacea purpurea* and *Melaleuca alternifolia* on cellular immunity in mice. *Centr Eur J Immunol* 27: 142-148.
- Skopińska-Różewska E, Bychawska M, Białas-Chromiec B, Sommer E (2009) The *in vivo* effect of *Rhodiola rosea* and *Rhodiola quadrifida* hydro-alcoholic extracts on chemokinetic activity of spleen lymphocytes in mice. *Centr Eur J Immunol* 34: 42-45.
- Skopińska-Różewska E, Wasiutyński A, Sommer E, Skopiński P, Pastewka K, Zdanowski R, Bany J (2011) Modulatory effect of *Echinacea pallida* on cellular immunity and angiogenesis in mice. *Centr Eur J Immunol* 36: 18-23.
- Skopińska-Różewska E, Stankiewicz W, Zdanowski R, Siwicki AK, Furmanowa M, Buchwald W, Wasiutyński A (2012) The *in vivo* effect of *Rhodiola quadrifida* extracts on the antibody production, on the blood leukocytes subpopulations and on the bacterial infection in mice. *Centr Eur J Immunol* 37: 140-144.
- Suter and Richter (2000) Anti and pro-oxidative properties of Padma 28, a Tibetan herbal formulation. *Redox Rep* 5(1): 17-22.
- Wójcicki J, Samochowiec L, Kadłubowska D (1989) Inhibition of ethanol-induced changes in rats by Padma 28. *Acta Physiol Pol* 40: 387-392.