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Isoprinosine Induces a Rapid Lympho - Mononuclear Response in Adult Participants

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Abstract

Isoprinosine™ is a drug with immunomodulatory and antiviral properties.
It exerted beneficial clinical effect in patients with HSV, HZV, HPV, HBV, influenza and subacute sclerosing panencephalitis. We established an elevation of PBMC and absolute number of small and large lymphocytes in adult participants prior and after a single-day intake of Isoprinosine™. The study design permits to observe the mononuclear circadian fluctuation and helps to eliminate the natural diurnal variation when evaluating Isoprinosine™ induced enhancement of PBMC. An increased number of lymphocytes in the afternoon were more expressed after Isoprinosine™ intake and rapid IsoprinosineTM response was observed in ¾ of the participants. This is consistent with previous observations for rapid resolution of the common cold at the first day after initiation of Isopriosine™ therapy. This study provides a model to evaluate the potential immunomodulatory effect of other drugs.

Keywords: inosine pranobex, rapid Isoprinosine response, large lymphocytes / monocytes number

Introduction

Inosine pranobex (Isoprinosine™) is a synthetic purine derivative, consisting of inosine and salt of p-acetaminobenzoic acid, with immunomodulatory and antiviral properties. It has been previously reported that this drug potentiates both T-lymphocytes and phagocytic cell function *in vitro* and *in vivo* (1). It also induces the appearance of phenotypic markers of differentiation on immature precursor T-cells, augments helper or suppressor T-cell functions and increases the production of TNF-β (2). Inosine pranobex increases cytokine IL-1 production and enhances IL-2 production, upregulating the expression of the IL-2 receptor *in vitro* (3, 4). There are also data regarding antiviral and antitumour activities *in vivo*, secondary

to the immunomodulating effects (5). Inosine pranobex was studied in several diseases including mucocutaneous Herpes simplex virus (HSV) infections, subacute sclerosing panencephalitis, genital warts, influenza, zoster, and type B viral hepatitis, where it exerted beneficial clinical effects. Recent *in vitro* and *in vivo* studies have shown immunomodulating and antiviral activities of inosine pranobex in children with cellular immunodeficiency as a prophylaxis of recurrent infections, mainly of viral origin (6). Inosine pranobex demonstrated a significant pharmacological activity in subclinical Human papilloma virus (HPV) infection and should be considered an alternative treatment for the condition (7).

The circulating immunocompetent cells are the large lymphocytes, including natural killer cells (NK cells) and the monocytes which move quickly (approx. 8–12 hours) into the tissues in case of infection. Both types of cells can be recognized in blood smears by their morphological features.

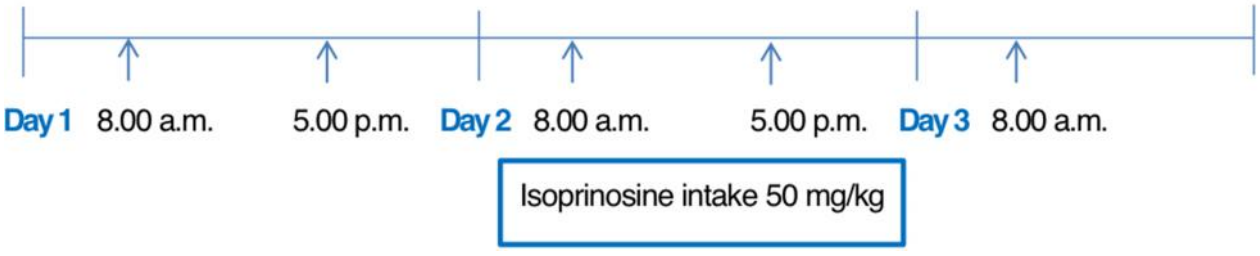
We have empirically observed a rapid clinical improvement even in the first day of inosine pranobex intake in patients with common cold and therefore have performed the following study to determine the variations in the counts and morphology of circulating monocytes and lymphocytes.

Materials and Methods

Twenty-two participants were recruited among the employees of one urban office and one hospital. All of them were healthy adults, without current complaints and not receiving medication known to influence the study parameters. Participants were instructed not to change their routine diet, exercise plan, or lifestyle while participating in the study. The study was approved by the Institutional Ethics Committee, and all participants signed an informed consent prior to recruitment.

Five venipunctures were performed for each participant. Three of them were prior to inosine pranobex intake at 8:00 a.m. and at 5:00 p.m. on Day 1 as well as at 8:00 a.m. on Day 2 while the remaining two venipunctures were after dosing at 5:00 p.m. on Day 2 and at 8:00 a.m. on Day 3 (Fig. 1). Each participant was assigned to receive Isoprinosine™ (Newport Pharmaceuticals Ltd., tabl. 500 mg) 50 mg/kg b.w. 3 or 4 times for 1 single day – starting at Day 2, immediately after the venipuncture at 8:00 a.m. The intake was limited to every 2 hours after the third venipuncture - at 8:00 a.m., 10:00 a.m., 12:00 a.m. and 2:00 p.m. The morning blood samples at 08:00 a.m. were drawn after an overnight fast.

Fig. 1. Study design schedule.



All venipunctures were performed by a single nurse in the same order to assure equal intervals in every recruited participant. The samples were anonymized.

Blood smears were prepared and Gimsa stained by a single person and evaluated by an appropriated specialist.

A full blood count was performed for each sample by automatic blood cell counter. A differential WBC count was also performed in a consistent scanning pattern, using the 100 x objective of digital microscope Olympus BX51. In addition, a calculation of the percent of large lymphocytes and monocytes, counting at 100 mononuclear cells was performed. The absolute number of large lymphocytes and monocytes was calculated (per cent x Ly in 10^9/l) and further evaluated in all subjects (figures 2-4).

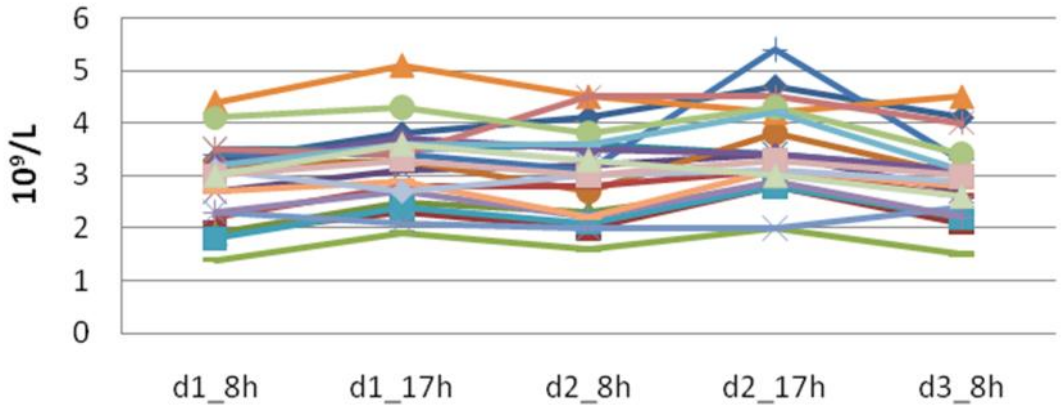
Statistical analysis was made by STATISTICA™ software. Wilcoxon matched paired rank test was used mainly (based on the distribution and sample number).

Results

Twenty-one participants finished the study and were included in the final analysis. One participant withdrew his consent and dropped out from the study.

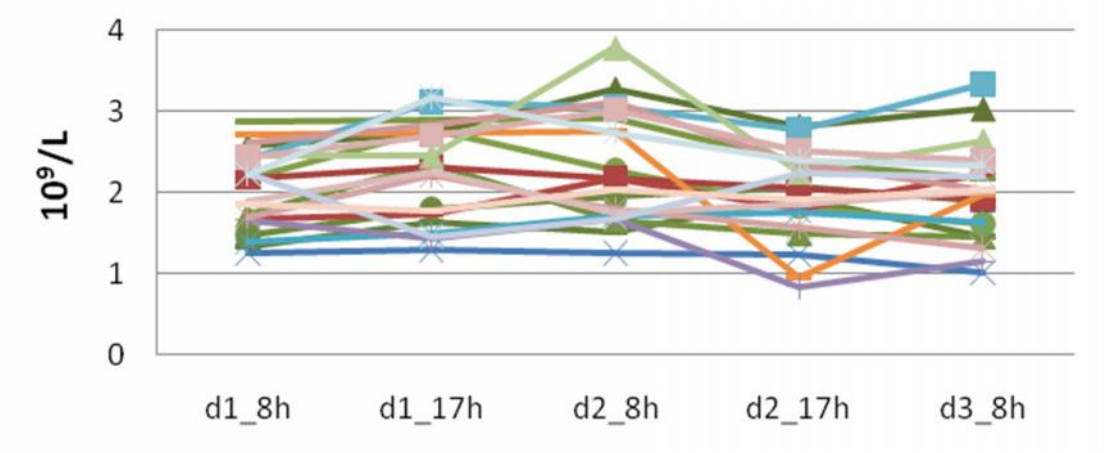
Afternoon values of all automatically measured parameters of peripheral blood mononuclear cells were higher than morning ones (p=0.01) both in pre- and post-treatment measurements at Day-1 and Day-2 of the study, respectively. There was no difference between baseline values of Day 1 and morning readings of Day 2 and Day 3. The increase in 5:00 p.m. at Day 2 was higher than in 5:00 p.m. at Day 1 (p=0.031) – Fig. 2. This effect was lost up until next morning and the last measurement was not different from baseline.

Fig. 2. Mononuclear cells before and after inosine pranobex intake.



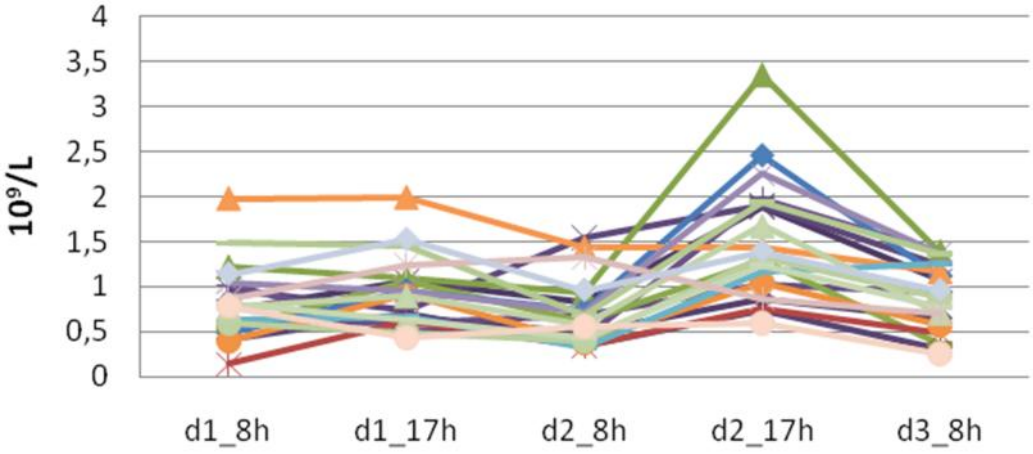
More interesting results were observed when values counted from the blood smears were compared. We observed an increase in the absolute number of small lymphocytes only at Day 1 (p=0.008), which was lost up after the drug intake at Day 2 (p=0.031) – Fig. 3.

Fig. 3. Small lymphocytes before and after inosine pranobex intake.



The number of large lymphocytes and monocytes increased only at Day 2 after Isoprinosine™ intake at 5:00 p.m. ($p<0.001$, Wilcoxon). Moreover, it was higher than Day 1 morning and afternoon values, as well as than Day 2 and Day 3 morning values – Fig. 4.

Fig. 4. Large lymphocytes before and after inosine pranobex intake.



Based on the rise of absolute count of monocytes and large lymphocytes > 20% we signed the participants as responders or non-responders. As a result, 15 (71%) of the participants had an increase of the number of large lymphocytes and monocytes (highest level was achieved after Isoprinosine™ intake at 05:00 p.m. at Day 2) and were assigned as rapid responders. One participant (5%) had an increase in the number of the active cells 24 hours after the Isoprinosine™ intake – late responder. Overall, the total number of responders was 16 (76%). Among the rapid responders about 60% kept the number of presumably activated cells until the next day.

The number of monocytes alone increased in one of the participants. In four others there was a simultaneous increase in both large lymphocytes and monocytes (30%). The rest of the responders were

only with increased number of large lymphocytes, which suggests rapid lymphocytes activation triggered by Isoprinosine™.

Discussion

Fluctuations including circadian rhythm of lymphocytes circulating in the peripheral blood might result from several factors: the distribution of circulating and marginal cell components of tissues and organs, influx from storage sites, cell proliferation, release of *de novo* cells into the circulation, age and cell destruction and removal (8, 9). In the present study we found an increased number of lymphocytes in the afternoon more expressed after Isoprinosine™ intake even in the oldest participant (69 years). In the elderly an alteration of circadian rhythmicity of T helper lymphocytes was recently reported (8). The design of our study permits to observe the mononuclear circadian fluctuation. Moreover it helps eliminate the natural diurnal variation when evaluating Isoprinosine™ induced enhancement of mononuclear cells.

Under physiological conditions T and B cells circulate continuously between the different tissues. Lymphocyte numbers in the blood are used to evaluate the immune status on a daily basis. The lymphocyte subsets in the peripheral blood mirror changes in the lymphocyte populations of the whole body (10). There is an association between the subpopulation of peripheral blood mononuclear cells, morphologically identified as large granular lymphocytes (LGL), and natural killer (NK) activity (11). Both spontaneous and IFN-boosted LGL are the main effector cells exerting NK cytotoxicity (12).

It has been shown that the immunotherapy with IL-2 can increases the number of lymphocytes and prolongs the half-life of CD4 that can exceed 3 years in HIV-infected patients (13). Isoprinosine™ can also enhance IL-2 production, upregulating the expression of the IL-2 receptor *in vitro* (3). Treatment (of human PBMC) with Isoprinosine™ enhanced IL-2 production by PBMC in 7 of 10 normal individuals and may be mediated through the activation of a distinct subset of IL-2 producing cells (4).

Our previous study on healthy volunteers has also shown that Isoprinosine™ treatment leads to an increase in serum levels of IFN-γ, IL-2, IL-10, and TNF-α at 7th to 10th day (14).

In the present study the highest level of mononuclear cell count was observed in the afternoon on the second day, 9 hours after the first Isoprinosine™ intake. Our results show an increase of mononuclear cells in 3/4 of the participants, suggesting a rapid Isoprinosine™ response (RIR). The oldest subject of 69 years had a remarkable RIR. The dose of 4 g/daily was totally enough to assure response even in the subject with the highest body weight (up to 140 kg).

Conclusion

The used model for evaluation of the lymphomonunuclear distribution suggests a circadian fluctuation and a RIR in a high percentage of participants after intake of 6-8 tablets Isoprinosine™ daily. This is consistent with clinical observations for rapid resolution of common cold symptoms. This study provides a model to evaluate the potential immunomodulatory effect of other drugs.

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