EX VIVO EVALUATION OF PIDOTIMOD EFFECT ON IMMUNE RESPONSE

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KEY WORDS
PIDOTIMOD, lymphocytic blastic transformation, capping effect, cytokines.

INTRODUCTION

PIDOTIMOD is the forerunner of a new category of immunomodulant drugs. As a matter of fact, it is a synthetic molecule produced by the Research Centre of POLI INDUSTRIA CHIMICA S.p.A. - Milano, consisting of a single molecule: 3-L-pyroglutamoil-L-thyazolidin-4-carboxilic acid. This drug is able to exert a physiological control of immune response and could be employed particularly in moderate immunodeficit, as occurs in aging (1). As it was not completely clear the optimal dosage of PIDOTIMOD and the duration of its pharmacological effect after the treatment suspension, we performed an ex-vivo study to clarify these questions.
In particular, a double blind trial with 24 healthy adult volunteers was carried out using the following doses: 400 and 800 mg x 2/die per os for 30 days. The effect of the drug on different parameters of immune response throughout the treatment and for a 4 week follow-up period was evaluated.

MATERIALS AND METHODS

The study was carried out on 24 healthy volunteers (mean age 27.12 ± 4.21 years).
Two groups of volunteers, 12 for 800 mg and 12 for 400 mg dosage, were divided randomly in two groups of 6 subjects each who took PIDOTIMOD or placebo in 2 daily doses for 30 days. Blood samples were taken for immunologic evaluations at time 0, time 15 and time 30, respectively. Then, when the opening of the code identified the subjects belonging to the two groups, the volunteers treated with the drug were successively assigned randomly to the following lots of study:

1st lot: blood samples taken at 12hr, 48hr, 96hr, 2nd and 4th week after suspension of treatment
2nd lot: blood samples taken at 6hr, 24hr, 72hr, 1st and 3rd week after suspension of treatment

The following parameters were monitored ex vivo on peripheral blood mononucleated cells (PBMC), as previously described (2):
- α and γ interferon (IFN) production
- lymphocyte blastic transformation induced by lectins: PHA (Wellcome - 1:100), Con A (Sigma - 12.5 μg/ml), PWM (Flow - 1:100) using 1 x 10^6 PBMC.
- IL-1 and IL-2 production induced by PHA (1:40 and/or 1:80 respectively)
- Capping effect of adherent cells from PBMC, evaluated using ConA fluorescein-labeled (Calbiochem) as rating of chemotaxis preparative phase

RESULTS

Fig.1 shows the effect of the drug on blastic transformation induced by PHA expressed as CPM/ml. An evident increase was achieved particularly with PIDOTIMOD 800 mg at T30. This result agrees with the enhancement of IL-2 production, remarkable if a suboptimal dose of inducer (PHA 1:80 rather than 1:40) is used. As a matter of fact, there was an increase in IL-2 production from < 2 U/ml at T0 to 30 ± 10.6 U/ml at T30. With regard to the percentage of PBMC adherent cells presenting capping effect, only the dose of 800 mg was able to increase significantly the expression of this phenomenon ($P < 0.01$ after 30 days of treatment); the values were 36.5 ± 5.5 % at T0 and 34.3 ± 4.5 % at T30 with placebo and 38.3 ± 7.9 % at T0 and 48.6 ± 6.1 % at T30 with PIDOTIMOD 800 mg.
Moreover, a moderate increase of α and γ interferon was observed after PIDOTIMOD 800 mg administration. In fact, α-IFN values were 4.4 ± 1.0 $Ulog_2$/ml at time 0, and 5.7 ± 0.4 after 30 days of treatment; γ-IFN, respectively, 4.6 ± 0.7 $Ulog_2$/ml at time 0 and 5.5 ± 1.0 at the suspension of treatment. PIDOTIMOD 400 mg was less effective and produced an increasing effect only on γ-IFN values: 4.9 ± 1.6 $Ulog_2$/ml at time 0, and 5.5 ± 0.8 at the suspension of treatment.
Finally, the follow-up study showed that PIDOTIMOD effect ceases 48-72 hours after treatment suspension.

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CONCLUSIONS

Reported data show that PIDOTIMOD possesses a dose and time dependent immunomodulating activity and confirm that 800 mg x 2/die, as previously suggested, (3-4) is the optimal dose. Moreover, the drug effectiveness is related particularly to T-helper lymphocytes and its effect is physiologic and ceases after suspension of the treatment.

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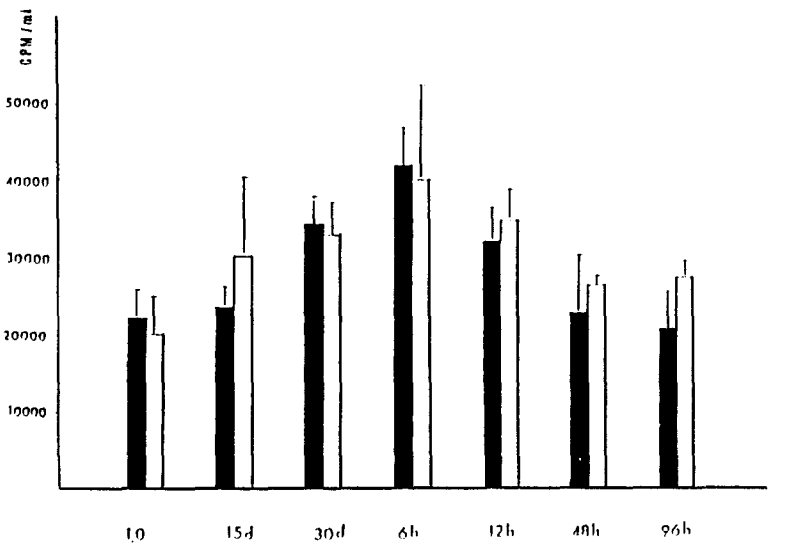

Fig.1 - Blastic transformation induced by PHA on lymphocytes obtained from subjects treated with PIDOTIMOD 800 mg (■) or 400 mg (□) x 2/die