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Chronic administration of verapamil, ketoconazole and carbamazepine: impact on immunological parameters

L. Bonhomme-Faivre [a,b,*], F. Forestier [b], D. Auchère [b], M. Soursac [b], S. Orbach-Arbouys [a], R. Farinotti [b]

[a] *Laborotoire de Pharmacologie, Service Pharmacie, Hôpital Paul-Brousse, 14, Avenue Paul Vaillant Couturier, 94800-Villejuif, France*

[b] *Laboratoire de Pharmacie Clinique, Faculté de Pharmacie, Upres, EA 2706, rue Jean-Baptiste-Clément, 92000 Chatenay-Malabry, France*

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Abstract

Inhibitors of P-glycoprotein (P-gp) (verapamil) or cytochrome P-450 (ketoconazole) may reduce IL2 production and T lymphocyte proliferation in vitro. We have examined the effects of chronic oral administration of these drugs and of the cytochrome P450 inductor, carbamazepine, on the hematological and immunological parameters of mice. We found no changes after giving the mice 0.12 mg verapamil, 0.85 mg ketoconazole, or 0.514 mg carbamazepine per mouse for 4 weeks (5 days/week). But giving the drugs for an additional 7 weeks at 0.6 mg (verapamil), 4.25 mg (ketoconazole) or 2.57 mg/mouse (carbamazepine), resulted in significant decreases in monocytes in the verapamil treated group (−51%) and in CD4+ cells in the carbamazepine group (−35%). Chronic oral administration of these drugs reduced the lymphocyte counts of mice by 10–18% and their NK counts by 10–16%. These changes could be due to changes in P-gp function in the transport of IL2, with decreases caused by verapamil and ketoconazole.

Keywords: Verapamil; Carbamazepine; Ketoconazole; Monocytes; Lymphocytes; CD4; NK

1. Introduction

The product of the human mdr 1 gene, P-glycoprotein (P-gp), is involved in the transport of IL2 across the cell membrane. P-gp inhibitors like verapamil or tamoxifen and the anti P-gp UIC2 monoclonal antibody decrease IL-2 secretion by human peripheral T lymphocytes in cell culture without modifying the synthesis of IL2 mRNA (Drach et al., 1996). However, cytochrome P450 inhibitors (such as econazole, miconazole or clotrimazole) reduce proliferation and IL2 synthesis by cell lines such as Jurkat T cells by blocking calcium-dependent CD3 activation (Aussel et al., 1994). Cytochrome P450 may regulate the influx of calcium that is triggered by CD3 TCR activa-

* Corresponding author. Tel.: +33-1-45-59-31-10; fax: +33-1-45-59-37-28.

E-mail address: laurence.bonhomme-faivre@pbr.ap-hop-paris.fr (L. Bonhomme-Faivre).

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tion. We have therefore examined the hematological and immunological consequences of giving mice drugs that alter P-gp and cytochrome P 450 activity. We tested verapamil (P-gp inhibitor), ketoconazole, (cytochrome P450 and P-gp inhibitor) and carbamazepine (cytochrome P450 inducer).

2. Materials and methods

2.1. Protocol

All studies were done on male 8 week-old Swiss mice NMRI (IFFA Credo 69592 L'Arbresles France) (30 g). They were acclimatized for 1 week and then assigned to one of four groups (12 mice per group), three treated and one control. Drugs were given 5 days per week for 4 weeks. All doses were equivalent to those given to humans. The same mice were then given the same drug at a higher dose (five times) for 5 days per week for an additional 7 weeks.

The mice were weighed on day 0 and on the days of blood sampling. Blood was taken by retro-orbital puncture (200 μl), at the same time in the morning, at the end of the 4th and 11th weeks. Hematological and immunological parameters were analysed with a Beckman MD2 type counter, and included erythrocyte counts (RBC), total leukocytes (WBC), lymphocytes, monocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) hematocrit (HCT). and platelets (PLT). CD4 and NK lymphocytes were phenotyped and measured with a Beckman Nepix XL type counter.

Control mice were given 0.1 ml 0.9% NaCl orally on the same days as the treated animals.

2.2. Drugs

The P-gp inhibitor, verapamil, (Isoptine®, Knoll France) was given at 0.15 mg/mouse during the first part of the protocol, and 0.60 mg/mouse in the second part. The cytochrome P450 and P-gp inhibitor, ketoconazole, (Nizoral®, Janssen-Cilag) was given at 0.85 mg/mouse in the first part and at 4.25 mg/mouse in the second part. The cytochrome P450 inducer, (Tegretol®, Novartis) was given at 0.51 mg/mouse during the first part and at 2.57 mg/mouse during the second.

2.3. Statistical analysis

Differences between treated and control mice at a given day were analysed with the Mann–Whitney U-test.

3. Results

There were no significant differences in the body weights of treated and control mice throughout the study (Table 1).

There was no statistically significant difference in the hematological and immunological parameters of the groups (Table 2 and Table 3).

The monocyte count was significantly decreased (51%) at the end of the 11-week treatment in the verapamil group, from 2907 ± 2421 (control) to 1430 ± 1101, and the CD4 count was significantly decreased (35%) in the carbamazepine group, from 5369 ± 2143 (control) to 3450 ± 1270. None of the other changes was significant (Table 4). Althought there was a decrease in the NK counts it was not significant (-10% for the carbamazepine to -16% for verapamil). Similarly leukocyte counts dropped but not significantly

Table 1
Body weights (g) of mice during the study (mean values ± S.D.)

Group	Control	Verapamil	Ketoconazole	Carbamazepine
Day 0	34.4 ± 1.2	35.5 ± 1.6	34.2 ± 1.5	34.4 ± 1.7
Day 30	38.7 ± 0.9	39.2 ± 2.8	37.9 ± 1.2	37.3 ± 2.6
Day 60	42.3 ± 1.6	44.2 ± 2.8	42 ± 2	42.1 ± 2.8

Table 2
Hematological data of Swiss mice at the end of the 4-week study (means ± S.D.)

	RBC (10^{12}/l)	HG (g/dl)	HCT (%)	MCV (μm^3)	MCH (pg)	MCHC (g/dl)	PTL ($\times 10^9$/l)
Control	7.94 ± 0.39	14.1 ± 0.9	43.5 ± 2.4	54.8 ± 1.2	18 ± 0.7	32 ± 1.4	886 ± 70
Verapamil	7.87 ± 0.54	13.9 ± 0.7	40.7 ± 12	55.8 ± 2.8	18 ± 0.6	32 ± 1.28	898 ± 83
Ketoconazole	8.03 ± 0.43	14.1 ± 0.7	44.7 ± 2.4	55.7 ± 2.2	18 ± 0.4	31 ± 1.1	889 ± 53
Carbamazepine	7.9 ± 0.4	13.8 ± 0.9	43.2 ± 6.2	56.1 ± 3.6	17 ± 0.4	31 ± 1.9	819 ± 161

RBC: erythrocytes; HG: hemoglobin; HCT: hematocrit; PTL: platelets; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; weeks 1–4, 5 days/week: verapamil 0.6 mg/mouse; ketoconazole 0.85 mg/mouse; carbamazepine 0.514 mg/mouse.Data are means ± S.D. (Mann–Whitney test).

Table 3
Hematological values at the end of the 4-week treatment (counts/mm^3)

	Control	Verapamil	Ketoconazole	Carbamazepine
Leukocytes ($\times 10^6$/l)	9491 ± 2985	8925 ± 2348	8864 ± 2848	9217 ± 3241
Monocytes ($\times 10^6$/l)	1709 ± 857	1665 ± 884	1438 ± 796	1856 ± 1273
Lymphocytes ($\times 10^6$/l)	7543 ± 2318	7028 ± 1660	7234 ± 2180	7070 ± 2335
CD4	5802 ± 1663	5566 ± 1254	5229 ± 1889	5577 ± 1993
NK	212 ± 64	187 ± 49	205 ± 90	172 ± 79

Table 4
Hematological values at the end of the 11-week treatment (counts/mm^3)

	Control	Verapamil	Ketoconazole	Carbamazepine
Leukocytes ($\times 10^6$/l)	13 010 ± 4291	10 589 ± 2948 (−18.6%)	11 318 ± 5030 (−13%)	11 778 ± 6231 (−9.4%)
Monocytes ($\times 10^6$/l)	2907 ± 2421	1430 ± 1101* (−50.8%)	2073 ± 1278 (−28.6%)	2907 ± 2422 (0%)
Lymphocytes ($\times 10^6$/l)	9538 ± 3284	8521 ± 2702 (−10.6%)	8206 ± 2881 (−14%)	7832 ± 3214 (−17.8%)
CD4	5369 ± 2143	4897 ± 2886 (−8.7%)	5010 ± 1620 (−7%)	3450 ± 1270* (−35%)
NK	4305 ± 1466	3600 ± 1096 (−16.3%)	3718 ± 1300 (−13.6%)	3851 ± 1886 (−10.5%)

From week 1 to week 4 (5 days/week): verapamil 0.6 mg/mouse; ketoconazole 0.85 mg/mouse; carbamazepine 0.514 mg/mouse. From week 5 to week 11: (5 days/week): verapamil 0.6 mg/mouse; ketoconazole 4.25 mg/mouse; carbamazepine 2.57 mg/mouse.
* $P < 0.05$ between treated and control groups (%).

(from −9.4% for carbamazepine to −18.6% for verapamil).

The lymphocyte counts decreased by 11–18%, depending on the treatment.

There was no statistically significant difference in the hematological data on the groups at the end of the 11 week-treatment, except for the MCH of the ketoconazole treated group (Table 5).

4. Discussion

In vitro studies have shown that inhibitors of P-gp and cytochrome P450 can suppress the immune system, since they decrease the production of mediators such as IL2. We gave mice drugs known to affect P-gp and cytochrome P450 to see whether they had any effect on the immune cell populations of mice in vivo. We tested verapamil,

a selective P-gp inhibitor, ketoconazole, which inhibits both P-gp and cytochrome P450, and carbamazepine, a stimulator of cytochrome P450. We found non-significant decreases in most of the groups at the end of the 11th week of treatment. However the decreases in the monocyte count of mice given verapamil, and in the CD4-cell count of mice given carbamazepine were significant.

Since most of the published studies were done in vitro, it is not easy to explain our results. Verapamil inhibits the cytotoxic activity of human peripheral blood monocytes towards tumor cells (Majeski and Cameron, 1988) and of neutrophil polynuclear cells and human peritoneal macrophages. This effect is probably independent of any inhibition of calcium chanels inhibition (Weir et al., 1992). Verapamil also inhibits the release of IL2 and the proliferation of cytotoxic T cells in a dose-dependent manner, thus leading to immunosuppression (Levy et al., 1991; Zanker et al., 1994).

Lastly, verapamil prevents recipient CD8 and CD4 lymphocyte passing through the allogenic graft endothelium in organ-grafted patients (Yamaguchi et al., 1998) and inhibits the release of IL4, IFNα, IL10 and TNF from stimulated blood cells.

Oral carbamazepine, at 5–15 mg/kg body weight, significantly diminishes the cellular and humoral immune responses of mice (Andrade-Mena et al., 1994) and may produce agranulocytosis, lupus and modified immune functions. In man, it suppresses total lymphocyte counts (Silverman and Chapron, 1995).

We find that mice given ketoconazole for 11 weeks have a 29% lower monocyte count, 14% lower lymphocyte and NK counts, and a 7% lower CD4 cell count than untreated mice. Itraconazole, fluconazole, ketoconazole and miconazole all inhibit human T lymphocyte proliferation in vitro via a mechanism which does not involve the secretion of cytokines (Pawelec et al., 1991).

Ketoconazole and cyclosporine together increases the immunosuppressive effects of cyclosporine in sheep (O'Donoghue et al., 1996). Similarly, cyclosporine plus ketoconazole was more immunosuppressive than cyclosporine alone in humans, despite the fact that the blood cyclosporine concentrations were comparable (Watanabe et al., 1997). Ketoconazole does not inhibit the synthesis of immunoregulatory cytokines (IL3, IL4, IL9, GM-CSF TNFα, IFNα) or that of the two chains of the IL2 receptor on human blood cells (Friccius et al., 1992).

The drugs tested do not have strictly selective activities, which makes the results difficult to interpret. Ketoconazole and verapamil are both cytochrome P450 and P-gp inhibitors that mainly affect monocytes and lymphocytes, and have less effect on CD4 than carbamazepine.

Inhibitors of P-gp and cytochrome P450 probably alter the function of P-gp in the transport of IL2, causing a decrease. Our results could be due to the decreased production of total and CD4 lymphocytes and monocytes bearing the IL2 receptor after chronic administration, particularly of verapamil and ketoconazole. These results should be taken into account in the chronic treatment of patients with these drugs and suggests that the immune parameters of such patients should be monitored.

Table 5
Hematological data at the end of the 11 weeks of treatment (means ± S.D.)

	RBC (10^{12}/l)	HG (g/dl)	HCT (%)	MCV (μm^3)	MCH (pg)	MCHC (g/dl)	PTL ($\times 10^9$/l)
Control	7.31 ± 0.91	12.8 ± 1.2	41.2 ± 5.2	56 ± 2	17.64 ± 0.75	31.1 ± 1.5	683.5 ± 327.2
Verapamil	8.06 ± 0.66	13.8 ± 0.9	46.5 ± 4.8	57 ± 2	17.12 ± 0.53	29.8 ± 1.7	827.8 ± 88.9
Ketoconazole	7.11 ± 0.77	12.4 ± 1.4	40.9 ± 5.2	57 ± 3	16.74 ± 0.54*	30.2 ± 1.8	746.8 ± 154.2
Carbamazepine	6.97 ± 2.06	12.3 ± 3.3	40.7 ± 11.8	58 ± 4	17.27 ± 1.02	30.1 ± 2.0	655 ± 202

* $P < 0.05$ compared to control.

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