

PRELIMINARY COMMUNICATION

LACK OF A MODULATORY EFFECT OF IMIPRAMINE ON GLUCOCORTICOID-INDUCED SUPPRESSION OF INTERFERON- AND INTERLEUKIN-10 PRODUCTION *IN VITRO*

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Antidepressant drugs have been shown to reverse some changes evoked by glucocorticoids or stress. In the present study we attempted to find out whether imipramine, one of the most frequently used antidepressant drugs, interfered with glucocorticoids, modulating the production of IFN- and IL-10, pro-inflammatory and anti-inflammatory cytokines, respectively. We observed a significant inhibitory effect of hydrocortisone, dexamethasone and the glucocorticoid receptor agonist RU 28362, used at doses of 10^{-6} and 10^{-5} M, on the production of IFN- and IL-10 by whole blood cells stimulated by mitogens. Imipramine at doses of 10^{-6} and 10^{-5} M did not modulate IFN- or IL-10 production, whereas at a dose of 10^{-5} M it increased the production of IL-10 and decreased that of IFN- , those results being statistically insignificant, though. A combination of imipramine and dexamethasone or hydrocortisone at doses of 10^{-6} or 10^{-5} M significantly suppressed the production of IFN- and IL-10, the level of inhibition being similar to that observed for glucocorticoids alone. The classic antidepressant imipramine was not able to modulate the suppressive effect of “stress” doses of hydrocortisone on the production of cytokines.

Key words: *imipramine, glucocorticoids, interferon- , interleukin-10*

The hyperactivity of the hypothalamic-pituitary-adrenocortical (HPA) axis is, besides disturbance of the monoaminergic transmission, the main biochemical change observed in major depression. A high incidence of depression in Cushing's syndrome, as well as antidepressant effects of adrenocortical enzyme inhibitors support the hypothesis that the hyperactivity of the HPA axis may be involved in the pathogenesis of depression [12, 22]. The above-mentioned hyperactivity is usually corrected during a clinically effective therapy with antidepressant drugs [8]. Recent data suggest that antidepressant drugs can enhance, *via* an increase in the concentration of glucocorticoid receptors in the CNS, a negative feedback mechanism that controls the HPA axis activity [24]. Apart from an action on the feedback mechanism, these drugs can block some effects induced by stress or corticosterone administration. Moreover, some changes observed after glucocorticoids (GCs) or stress are similar to alterations observed in depression, but do not resemble effects induced by antidepressant drugs [20]. For example, the corticosterone-induced changes in serotonin receptors (decreases in the level and function of 5-HT_{1A} receptors; increases in the 5-HT_{2A} receptor level) are similar to alterations observed in depression, and dissimilar to the effects induced by antidepressant drugs [17, 21, 30]. Antidepressant drugs inhibit (1) the stress-induced decrease in the level of brain-derived neurotrophic factor (BDNF) in the rat hippocampus [23]; (2) the chronic stress- or corticosterone-induced neurodegenerative changes in the rat hippocampus [25, 32]; (3) the GC-elevated TRH concentration in a culture of hypothalamic neurons [11]. In accordance with the above data, we found previously that antidepressant drugs inhibited the production of pro-inflammatory cytokines in rats subjected for 8 weeks to unpredictable stressors in a chronic mild stress model of depression [15], and suppressed the glucocorticosteroid receptor-mediated gene transcription in a culture of fibroblast cells [4].

GCs exert potent regulatory effects on the synthesis of cytokines. Used at pharmacological doses, they inhibit the production of both pro- and anti-inflammatory cytokines [13]. On the other hand, a number of data show that antidepressants enhance *in vitro* the production of the anti-inflammatory cytokine IL-10 and/or suppress that of the pro-inflammatory cytokine IFN- γ , and reduce the IFN- γ /IL-10 production ratio [14, 18, 34]. The aim

of our study was to find out whether imipramine, one of the most frequently used antidepressant drugs, could modulate the effect of GCs on the synthesis of IFN- γ and IL-10. We chose those two cytokines for our study, because it had been suggested that the elevated concentration of IFN- γ and/or the decreased level of IL-10 might play some role in the pathogenesis of depression [18].

Blood samples for IL-10 and IFN- γ assays were collected from 5 volunteers. The mean age and the male/female ratio were 29.2 ± 8.3 years and 3/2, respectively. All the subjects were free of any chronic medical illnesses, acute infections or allergic reactions, as well as of drugs known to modify the immune and endocrine functions for at least one month before blood sampling. Blood for those assays was taken between 2 and 3 p.m. The impact of GCs and imipramine on the cytokine production was examined by stimulating whole blood, diluted four times, with PHA (1 μ g/ml; Murex Diagnostics Ltd, Dartford, England) and LPS (5 μ g/ml, Sigma, Belgium). A total of 750 μ l of the RPMI-1640 medium with L-glutamine (Gibco BRL), supplemented with 100 IU/ml of penicillin (Sigma), 100 mg/ml of streptomycin (Sigma), and PHA + LPS was transferred onto 24-well cell culture plates (Falcon 3047, Becton Dickson). Imipramine was dissolved in sterile water, whereas GCs were dissolved in a 20% 2-hydroxypropyl- β -cyclodextrin solution (Research Biochemicals International, Natick, USA). The 100 μ l portions of the drug solution were added to the wells and gently mixed with the medium. The 10^{-7} M hydrocortisone (CORT) concentration employed in the experiment was chosen on the basis of the data on physiological plasma concentrations of that agent, whereas its higher concentrations (10^{-6} and 10^{-5} M) were selected on the basis of experiments showing such concentrations immediately after therapeutic application of the agent in question, or as a physiological response to some stressors [7, 9]. Dexamethasone (DEX) (Sigma) and the GC receptor agonist RU 28362 (Roussel UCLAF, France) were added to the culture wells at the same final concentrations as CORT. Imipramine concentrations tested in the experiment were chosen on the basis of literature data. Thus, the 10^{-6} M concentration used in the study was at a therapeutic range of plasma concentrations obtained during drug administration in clinical practice, whereas the higher concentration corresponded to that usually employed by other researchers

in *ex vivo* experiments aimed at examining the effect of drugs on isolated monocytes and lymphocytes. The samples were incubated for 48 h in a humidified atmosphere containing 5% CO₂ at 37°C. Supernatants were taken off carefully and kept at +4°C. All the assays of IFN- or IL-10 were carried out two days later by an ELISA method (Eurogenetics, Tessenderlo, Belgium) using a monoclonal-monoclonal antibody pair and a biotin-streptavidin amplification system. The intra-assay CV values for both those analyses were less than 8%. In our laboratory, detection limits were 0.9 U/ml for IFN- and 10 pg/ml for IL-10. The viability of cells was checked with trypan blue.

Statistical significance of differences was determined using a one-way analysis of variance (ANOVA) followed by Dunnett's test.

In the present study, we observed a significant inhibitory effect of high doses (10⁻⁶ and 10⁻⁵ M) of each of the used GCs on IFN- and IL-10 production by whole blood cells stimulated by mitogens (groups 3, 4, 6, 7, 9, 10; Tab. 1). Also a lower dose (10⁻⁷ M) of synthetic, but not endogenous, GCs inhibited IL-10 production in a statistically signifi-

cant manner. Imipramine at a dose of 10⁻⁵ M increased IL-10 production by about 30%, but that result was not statistically significant. Co-incubation of whole blood cells with a combination of imipramine and CORT or DEX at doses of 10⁻⁶ or 10⁻⁵ M each significantly suppressed the production of IFN- and IL-10. The level of inhibition was similar to that observed for GCs alone.

The present results confirm the potent suppressive effect of high doses of steroids on IFN- and IL-10 production. It is a well-established fact that GCs at pharmacological doses suppress the production of cytokines by T lymphocytes, macrophages and other cells [13, 16]. Another well-known fact is the atrophic effect of high GCs exposure on the thymus and thymocyte maturation [33].

In the present study, we observed that high (10⁻⁶ M and 10⁻⁵ M) doses of all the three GCs tested inhibited more potently the production of IFN- than that of IL-10. For example, production of IL-10 was reduced by about 70%, but production of IFN- was reduced almost 90% after whole blood incubation in 10⁻⁵ M RU 28362.

Table 1. The effect of glucocorticoids and imipramine on intrferon- (IFN-) and interleukin 10 (IL-10) production by human whole blood cells stimulated by mitogens

Group	Drug	n	IFN- (pg/ml)	IL-10 (IU/ml)
1	none (control)	10	1192 ± 211	2800 ± 521
2	DEX 10 ⁻⁷ M	4	656 ± 381	560 ± 133*
3	DEX 10 ⁻⁶ M	10	164 ± 46**	396 ± 47**
4	DEX 10 ⁻⁵ M	10	101 ± 31**	555 ± 84**
5	CORT 10 ⁻⁷ M	4	858 ± 178	2044 ± 564
6	CORT 10 ⁻⁶ M	10	251.8 ± 63**	611 ± 130**
7	CORT 10 ⁻⁵ M	10	203 ± 73**	598 ± 108**
8	RU-28362 10 ⁻⁷ M	4	715 ± 242	812 ± 226*
9	RU-28362 10 ⁻⁶ M	4	154 ± 8*	672 ± 170*
10	RU-28362 10 ⁻⁵ M	4	131 ± 43*	896 ± 178*
11	IMI 10 ⁻⁶ M	6	1010 ± 243	3505 ± 434
12	IMI 10 ⁻⁵ M	6	859 ± 211	3614 ± 187
13	DEX 10 ⁻⁶ M + IMI 10 ⁻⁶ M	6	144 ± 40 ^{##} vs gr. 11, ns vs gr. 3	525 ± 98** vs gr. 11, ns vs gr. 3
14	DEX 10 ⁻⁵ M + IMI 10 ⁻⁵ M	6	183 ± 42 ^{##} vs gr. 12, ns vs gr. 4	420 ± 22 ^{##} vs gr. 12, ns vs gr. 4
15	CORT 10 ⁻⁶ M + IMI 10 ⁻⁶ M	6	224 ± 72 ^{##} vs gr. 11, ns vs gr. 6	725 ± 130 ^{##} vs gr. 11, ns vs gr. 6
16	CORT 10 ⁻⁵ M + IMI 10 ⁻⁵ M	6	98 ± 27 ^{##} vs gr. 12, ns vs gr. 7	637 ± 63 ^{##} vs gr. 12, ns vs gr. 7

All results are shown as means ± SE. Experiments were conducted in duplicates and each result was taken for a statistical analysis. * p < 0.05; ** p < 0.01 in comparison with the control, ns – non significant, # p < 0.05; ## p < 0.01 in comparison with the indicated groups

IL-10 is secreted by a few different cell types, including Th1, Th2, and Th0 cells, macrophages, monocytes and B cells [10]. The expression of IL-10 is not confined to a particular T cell subset, but has been traditionally associated with Th2 cells, due to its antagonistic effect on Th1 cells. IFN- γ is produced exclusively by Th1 and NK cells. The stronger suppressive effect of high doses of GCs on Th1 cytokine production than on Th2 cytokine production is in agreement with the results obtained by other authors [31]. In mice, it has been shown that DEX preferentially suppresses IL-2, but not IL-4, products of Th1 and Th2 cells, respectively [6].

In the present study, a dose of 10^{-7} M of DEX decreased IL-10 production by 80%, whereas Visser et al. [31] observed only a 20% reduction of IL-10 production by whole blood cells for similar doses of that drug. In the latter authors' study, only macrophages were stimulated to produce IL-10, whereas in the present experiment we stimulated macrophages and lymphocytes. It may thus be speculated that lymphocytes are more sensitive to the suppressive effect of DEX than macrophages. Also Cupps and Fauci [5] suggested that lymphocytes were the subpopulation of leukocytes that was most powerfully affected by GCs treatment.

The present study has shown that synthetic GCs are more efficient in inhibiting the production of cytokines than the endogenous GC cortisol. A dose of 10^{-7} M of DEX or RU-23 inhibited IL-10 and IFN- γ production to a similar extent by over 70% (IL-10) and over 40% (IFN- γ), respectively. Cortisol at a dose of 10^{-7} M decreased the production of either cytokine to a significantly smaller extent, i.e. by less than 30%. A similar result has also been reported by other authors, and is in line with differences in the affinity of these steroids for the GC receptor [27].

An original finding of this paper is that the antidepressant drug imipramine does not modify the inhibitory effect of GCs on cytokine production. We showed earlier that imipramine inhibited GR-mediated gene transcription in fibroblast cells. In that case, however, imipramine affected the action of corticosterone *via* the GC-responsive element (GRE), a DNA sequence specific to GCs. The suppressive effect of GCs on cytokine synthesis is probably connected with their action *via* the negative influence of GRE and the protein-protein interaction with transcription factors binding to AP-1 [2]. Their suppressive effect on cytokine produc-

tion is also connected with their inhibitory effect on the transcription factor NF- κ B, *via* an increase in the transcription and protein synthesis of its cytoplasmic inhibitor I κ B [1, 19, 28]. Hence, imipramine can inhibit the action of corticosterone exerted *via* GRE (our previous study), but has no effect on GCs-mediated action on other DNA sequences (present paper).

Our previous papers showed that antidepressants increased the production of IL-10 and decreased the IFN- γ /IL-10 ratio. Moreover, some of them decreased the production of IFN- γ [14, 18]. The inhibitory effect of antidepressants on the IFN- γ /IL-10 ratio is probably connected with their stimulatory effect on cAMP production. It has been shown that the elevated cellular level of cAMP induces an increase and a decrease in IL-10 and IFN- γ production, respectively [3, 29]. On the other hand, some experiments demonstrated that an *in vitro* exposure of rat CD4 T lymphocytes to low concentrations of DEX elevated the mRNA level of IL-10 and other Th2 cytokines, such as IL-4 and IL-13, while under those conditions the production of IFN- γ and TNF- α (Th1 cytokines) mRNA was inhibited [26]. Moreover, after both an *in vitro* and *in vivo* exposure to DEX, murine splenocytes reduced the elevated level of IL-4 and lowered that of IL-2 and IFN- γ [6]. Considering the latter observation, we expected that imipramine would alleviate the inhibitory effect of high doses of GCs on IL-10 production and increase the suppressive effect of GCs on IFN- γ production.

The lack of a modulatory effect of antidepressants on the inhibitory action of GCs probably stems from the very potent inhibition of cytokine production by high doses (10^{-6} and 10^{-5} M) of DEX and CORT, used in the present study. It may be worthwhile to examine the effect of a combination of antidepressants and lower doses of GCs on cytokine production. On the other hand, a dose of 10^{-6} M of cortisol used in present paper can be regarded as a "stress" dose of GCs, because that level of cortisol was observed in a stress response. Therefore, it may be concluded that the classic antidepressant imipramine is not capable of reversing the suppressive effect of "stress" doses of GCs on the production of cytokines.

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REFERENCES

1. Auphan N., Didonato J.A., Rosette C., Helmberg A., Karin M.: Immunosuppression by glucocorticoids: inhibition of NF- κ B activity through induction of I κ B synthesis. *Science*, 1995, 270, 286–289.
2. Barnes P.J., Adcock I.: Anti-inflammatory actions of steroids: molecular mechanisms. *Trends Pharmacol. Sci.*, 1993, 14, 436–441.
3. Benbernou N., Esnault S., Shin H.C., Fekkar H., Gueunounou M.: Differential regulation of IFN- γ , IL-10 and inducible nitric oxide synthase in human T cells by cyclic AMP-dependent signal transduction pathway. *Immunology*, 1997, 91, 361–368.
4. Budziszewska B., Jaworska-Feil L., Kajta M., Lasoń W.: Antidepressant drugs inhibit glucocorticoid receptor-mediated gene transcription – a possible mechanism. *Brit. J. Pharmacol.*, 2000, 130, 1385–1393.
5. Cupps T.R., Fauci A.S.: Corticosteroid-mediated immunoregulation in man. *Immunol. Rev.*, 1982, 65, 133–155.
6. Daynes R.A., Araneo B.A.: Contrasting effects of glucocorticoids on the capacity of T cells to produce growth factors interleukin-2 and interleukin-4. *Eur. J. Immunol.*, 1989, 19, 2319–2325.
7. Dodt C., Kern W., Fehm H.L., Born J.: Antimineralocorticoid canrenoate enhances secretory activity of the hypothalamus-pituitary-adrenocortical (HPA) axis in humans. *Neuroendocrinology*, 1993, 58, 570–574.
8. Heuser I.J.E., Schweiger U., Gotthardt U., Schmider J., Lammers C-H., Dettling M., Yassouridis A., Holsboer F.: Pituitary-adrenal system regulation and psychopathology during amitriptyline treatment in elderly depressed patients and normal comparison subjects. *Amer. J. Psychiat.*, 1996, 153, 93–99.
9. Holsboer F., von Bardeleben U., Heuser I., Steiger A.: Human corticotropin-releasing hormone challenge tests in depression. In: *The Hypothalamic-Pituitary-Adrenal Axis: Physiology, Pathophysiology, and Psychiatric Implications*. Eds. Schatzberg A.F., Nemeroff C.B., Raven Press, Ltd., New York, 1988, 79–100.
10. Howard M., O'Garra A.: Biological properties of IL-10. *Immunol. Today*, 1992, 13, 198–200.
11. Jackson I.M., Luo L.G.: Antidepressants inhibit the glucocorticoid stimulation of thyrotropin releasing hormone expression in cultured hypothalamic neurons. *J. Invest. Med.*, 1998, 46, 470–474.
12. Jeffcoate W.J., Silverstone J.T., Edwards C.R.W., Besser G.M.: Psychiatric manifestations of Cushing's syndrome: response to lowering of plasma cortisol. *Quart. J. Med.*, 1979, 191, 465–472.
13. Kelso A., Munck A.: Glucocorticoid inhibition of lymphokine secretion by alloreactive T lymphocyte clones. *J. Immunol.*, 1984, 133, 784–791.
14. Kubera M., Lin A.-H., Kenis G., Bosmans E., van Bockstaele D., Maes M.: Anti-inflammatory effects of antidepressants through suppression of the interferon- γ /interleukin-10 production ratio. *J. Clin. Psychopharmacol.*, 2001, in press.
15. Kubera M., Symbirtsev A., Basta-Kaim A., Borycz J., Roman A., Papp M., Claesson M.: Effect of chronic treatment with imipramine on interleukin 1 and interleukin 2 production by splenocytes obtained from rats subjected to a chronic mild stress model of depression. *Pol. J. Pharmacol.*, 1996, 48, 503–506.
16. Kunicka J.E., Talle M.A., Denhardt G.H., Brown M., Prince L.A., Goldstein G.: Immunosuppression by glucocorticoids: inhibition of production of multiple lymphokines by in vivo administration of dexamethasone. *Cell. Immunol.*, 1993, 149, 39–49.
17. Kuroda Y., Mikuni M., Ogawa T., Takahashi K.: Effect of ACTH, adrenalectomy and the combination treatment on the density of 5-HT $_2$ receptor binding sites in neocortex of rat forebrain and 5-HT $_2$ receptor-mediated wet-dog shake behaviors. *Psychopharmacology*, 1992, 108, 27–32.
18. Maes M., Song C., Lin A.H., Bonaccorso S., Kenis G., de Jongh R., Bosmans E., Scharpe S.: Negative immunoregulatory effects of antidepressants: inhibition of interferon- γ and stimulation of interleukin-10 secretion. *Neuropsychopharmacology*, 1999, 20, 370–379.
19. Marx J.: How the glucocorticoids suppress immunity. *Science*, 1995, 270, 232–233.
20. McCormick C.M., Smythe J.W., Sharma S., Meaney M.J.: Sex-specific effects of prenatal stress on hypothalamic-pituitary-adrenal responses to stress and brain glucocorticoid receptor density in adult rats. *Develop. Brain Res.*, 1995, 84, 55–61.
21. Mendelson S.D., McEwen B.S.: Autoradiographic analyses of the effects of adrenalectomy and corticosterone on 5-HT $_{1A}$ and 5-HT $_{1B}$ receptors in the dorsal hippocampus and cortex of the rat. *Neuroendocrinology*, 1992, 55, 444–450.
22. Murphy B.E.P.: Antigluco-corticoid therapies in major depression: a review. *Psychoneuroendocrinology*, 1997, 22, Suppl. 1, S125–S132.
23. Nibuya M., Morinobu S., Duman R.S.: Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J. Neurosci.*, 1995, 15, 7539–7547.
24. Peiffer A., Veilleux S., Barden N.: Antidepressant and other centrally acting drugs regulate glucocorticoid receptor messenger RNA levels in rat brain. *Psychoneuroendocrinology*, 1991, 16, 505–515.
25. Przegaliński E., Budziszewska B.: The effect of long-term treatment with antidepressant drugs on the hippocampal mineralocorticoid and glucocorticoid receptors in rats. *Neurosci. Lett.*, 1993, 161, 215–218.
26. Ramirez F., Fowell D.J., Puklavec M., Simmonds S., Mason D.: Glucocorticoids promote a Th2 cytokine response by CD4 $^+$ T cells in vitro. *J. Immunol.*, 1996, 156, 2406–2412.
27. Rupprecht R., Reul J.M.H.M., van Steensel B., Spengler D., Soder M., Berning B., Holsboer F., Damm K.: Pharmacological and functional characterization of human mineralocorticoid and glucocorticoid receptor ligands. *Eur. J. Pharmacol.*, 1993, 247, 145–154.

28. Scheinman R.I., Cogswell P.C., Lofquist A.K., Baldwin A.S. Jr.: Role of transcriptional activation of I β in mediation of immunosuppression by glucocorticoids. *Science*, 1995, 270, 283–286.
29. Shin H.C., Benbernou N., Fekkar H., Esnault S., Guenounou M.: Regulation of IL-17, IFN- γ and IL-10 in human CD8(+) T cells by cyclic AMP-dependent signal transduction pathway. *Cytokine*, 1998, 10, 841–850.
30. Stahl S.: 5HT1A receptors and pharmacotherapy. *Psychopharmacol. Bull.*, 1994, 30, 39–43.
31. Visser J., van Boxel-Dezaire A., Methorst D., Brunt T., de Kloet E. R., Nagelkerken L.: Differential regulation of interleukin-10 (IL-10) and IL-12 by glucocorticoids in vitro. *Blood*, 1998, 91, 4255–4264.
32. Watanabe Y., Gould E., Daniels D.C., Cameron H., McEwen B.S.: Tianeptine attenuates stress-induced morphological changes in the hippocampus. *J. Pharmacol.*, 1992, 222, 157–162.
33. Wyllie A.H.: Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature*, 1980, 284, 555–556.
34. Xia Z., DePierre J., Nassberger L.: Tricyclic antidepressants inhibit IL-6, IL-1, and TNF- α release in human blood monocytes and IL-2 and interferon- γ in T cells. *Immunopharmacology*, 1996, 34, 27–37.

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