Acta Poloniae Pharmaceutica – Drug Research, Vol. 65 No. 1 pp. 159–164, 2008 ISSN 0001-6837
Polish Pharmaceutical Society

CORTISOL LEVEL IN MEN WITH MAJOR DEPRESSIVE DISORDER TREATED WITH FLUOXETINE OR IMIPRAMINE

JADWIGA PIWOWARSKA[1*], KAROLINA DRYLL[1], WALDEMAR SZELENBERGER[2] and JAN PACHECKA[1]

[1] Department of Biochemistry and Clinical Chemistry, Medical University, 1 Banacha Street, 02-097 Warsaw, Poland
[2] Department of Psychiatry, Medical University, 27 Nowowiejska Street, 00-665 Warsaw, Poland

Abstract: The aim of this research was to find out whether increased plasma cortisol levels appear in unipolar or bipolar patients with major depressive disorder (MDD) and whether the effective antidepressant treatment by imipramine and fluoxetine leads to regulation of the cortisol level. Cortisol levels were studied in two groups of patients with major depressive disorder: unipolar and bipolar patients treated with fluoxetine (doses: 20-60 mg/day). This group included 5 patients (age 29-46 yr); unipolar and bipolar subjects treated with imipramine (50-150 mg/day), this group included 5 patients (aged 24-70 yr). Cortisol and fluoxetine or imipramine plasma levels were assessed using HPLC methods: before treatment, after 3, 6 and 24 h of drug administration as well as in the 2nd, 4th, 6th, and 8th week of antidepressant treatment. HPLC methods were previously validated. The research conducted and the clinical data may be useful for proving the essential role of enhanced HPA axis activity for the pathogenesis and depressive disorder proceedings.

Keywords: cortisol, fluoxetine, imipramine, HPA, depression.

Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis is thought to play a role in the etiology of major depressive disorder (MDD). In depression we deal with irregularity of the HPA axis, effective antidepressant treatment should thus lead to normalization of the HPA axis activity.

In recent decades many researchers tried to investigate a relation between HPA axis and depression (1-6). These observations were mostly focused on the role of cortisol – stress hormone – in the etiology of depression. Some authors of the studies were particularly interested in the change of stress mediators' action: from protective to damaging one. They drew a conclusion that stress mediators release a number of intracellular reactions, whose aim is to protect the organism and to facilitate its survival. Activation of the stress centres, such as HPA axis, dopamine neurons in mesencephalon and noradrenergic neurons of the locus coeruleus enhances psychomotor functions and diminishes temporarily irrelevant functions, such as immunological, reproductive or anabolic processes (2). However, prolonged and too intense stress reaction has negative results. It has been found that in men with depressive disorders mechanisms breaking stress reaction are impaired, presumably due to disrupted cortisol negative feedback. There are many similarities between chronic stress and depression disorders. Pathologically enhanced HPA axis activities, as well as impaired monoaminergic neurotransmission are the main biochemical changes observed in patients with MDD. Studies have reported relationship between HPA axis abnormalities and severity, endogenicity, psychotic features, anxiety, length of the current depressive episode, chronicity and number of previous episodes (6). Supposing that in depression, HPA axis functions abnormally, effective antidepressant treatment should normalize its activity. This thesis is supported by the results of numerous works, for example by the research of Paul Linkowski et al. (4). According to the authors, during the acute phase of the illness, depressed patients had hypercortisolism, early timing of the nadir of the cortisol rhythm, and diurnal hypersecretion of GH. After successful treatment these neuroendocrine abnormalities were corrected. The researchers have also confirmed that plasma cortisol levels returned to normal during clinical remission.

* Corresponding author: Tel./fax: +48-22-5720-735, E-mail address: piwowarska.jadwiga@sezam.pl

Some more details concerning the mechanisms of antidepressants' action are given by Pariante et al. (5). They assume that patients with major depression exhibit hyperactivity of the HPA axis, caused by impaired negative feedback. This hyperactivity is associated with elevated circulating levels of cortisol and it is glucocorticoid receptor (GR) which plays an important role in the regulation of the HPA when endogenous levels of cortisol are high. During depression periods the function of GR is reduced and antidepressants reverse these GR changes. Tricyclic antidepressants, like imipramine induce upregulation of brain GR, MR or both. Studies examining selective serotonin reuptake inhibitor (SSRI) antidepressants, like fluoxetine have found that chronic treatment with these chemicals upregulates MR expression, while it has no effect on GR expression. The authors also claim that antidepressants exert their action thanks to inhibition of membrane steroid transporters, which are localized at the blood-brain barrier (BBB) and thus they increase the access of cortisol to the brain. In turn, antidepressant-caused GR and MR activation leads to enhanced negative feedback of the HPA axis by circulating glucocorticoid hormones and, as a result, to resolution of glucocorticoid hypersecretion.

The aim of this work was to examine whether in the group of patients with unipolar or bipolar affective disorder elevated plasma cortisol levels appear, as well as to find out if the antidepressant therapy using fluoxetine or imipramine leads to normalization of the cortisol level.

SUBJECTS AND METHODS

Experimental subjects

Patients treated with fluoxetine and imipramine who were qualified for the therapeutic drug monitoring were subjects of this treatment. They were treated in the Department of Psychiatry of Medical University of Warsaw, donors of the plasma for this research.

In the group of fluoxetine treated patients there were 5 subjects: 4 women and 1 man, aged 29-46 yr, all of them with diagnosis of unipolar and bipolar endogenous depression. The duration of the disease ranged from 0.5 to 5 yr, number of hospitalizations from 1 to 6. Fluoxetine was administered to subjects p.o. with a gradually increasing dose of Bioxetine from 20 mg/day to 40 mg/day. In one case the maximum daily dose of fluoxetine was 60 mg/day. No subjects were on chronic medication.

Imipramine, fluoxetine and cortisol plasma levels in subjects of this group were assessed before treatment and after 3 and 24 h and 2,4,6 and 8 weeks.

The group of patients treated with imipramine included 6 subjects: 4 women and 2 men (age 24-70 yr), diagnosed with unipolar (3 subjects) or bipolar (3 patients) endogenous depression. Patients were treated with imipramine, administered orally, starting with a daily dose of 50 mg and reaching eventually 75, 125 or 150 mg/day.

The severity of depression was assessed using the 21-item Hamilton Depression Rating Scale. Before treatment, fluoxetine–treated patients had a Hamilton score between 23 and 34, while in imipramine–treated group this score ranged from 25 to 34. Patients psychological condition was also evaluated with the use of Hamilton Anxiety Rating Scale. The development of side-effects was assessed using SERS (Side-Effects Rating Scale).

The blood was collected under normal conditions, that is at 8 a.m. For the control reasons the plasma was collected from the group of healthy volunteers as well (there were 24 subjects: 8 men and 16 women, 20 to 81 years old.

METHODS

HPLC determination of fluoxetine and its metabolite as well as of imipramine was the subject of theses carried out within the cooperation between the Department of Psychiatry of Medical University of Warsaw and the Clinic of Psychiatry of Medical University of Warsaw, while conducting the joint research project of the monitored therapy.

Plasma cortisol levels were determined by HPLC, using 6-α-methylprednisolone as an internal standard (IS). The chromatographic system from Shimadzu consisted of a LC-10 AS pump, equipped with a 100 µL injection loop, a programmable SPD-10AV UV-Vis absorbance detector. The separation was performed by a Waters C 18 precolumn and a Waters Symmetry C 18, 5 µm, 150 × 4,6 mm ana-

Table 1. Validation parameters.

Linearity	25-1000 [ng/mL]
Intermediate precision	CV < 10%
Repeatability	CV < 15%
Recovery	89.55 %
Accuracy	CV < 10%
DL	11.87 [ng/mL]
QL	21.58 [ng/mL]
Sample stability	CV < 15%

Table 2. Clinical characteristics and plasma cortisol and fluoxetine levels before and after treatment. C – cortisol concentration [ng/mL], F – fluoxetine concentration [ng/mL], D – fluoxetine daily dose [mg], HDRS – Hamilton score.

Time of sample collection		Subjects				
		1 F	2 F	3 F	4 F	5 F
0	**C**	**131.16**	**0**	**0**	**177.01**	**52.7**
	F	10.38	10.38	0	0	0
	D	0	0	0	0	0
	HDRS	35	32	28	28	35
6 h	**C**	**103.86**	**52.27**	**56.28**	**151.01**	**41.9**
	F	14.4	17.82	0	0	1.03
	D	20	20	20	20	20
	HDRS	35	35	28	28	35
24 h	**C**	**186.14**	**58.37**	**125.14**	**158.15**	**50.95**
	F	0	12.24	0	0	4.4
	D	20	20	20	20	20
	HDRS	35	35	28	28	35
2 weeks	**C**	**70.01**	**100.16**	**171.29**	**222.47**	**42.6**
	F	40.24	83.86	51.64	45.12	28.24
	D	20	40	20	20	20
	HDRS	19	28	26	26	28
4 weeks	**C**	**40.8**	**61.27**	**215.31**	**131.9**	**143.18**
	F	95.58	82.77	140.2	62.04	23.64
	D	20	40	20	40	20
	HDRS	7	22	15	25	26
6 weeks	**C**	**112.87**	**72.81**	**no data**	**156.33**	**37.73**
	F	135.85	99.52	no data	65.55	34.6
	D	20	40	no data	20	40
	HDRS	13	22	no data	25	21
8 weeks	**C**	**52.48**	**131.79**	**no data**	**97.99**	**74.78**
	F	67.89	175.63	no data	70.19	86.8
	D	20	60	no data	20	40
	HDRS	12	14	no data	21	13

lytical column. Data recording was carried out by CHROMAX-2004 Software. For the cortisol determination the following chromatographic conditions were recognized as optimal:

- the mobile phase: methanol-water-tetrahydrofuran (110:100:2.5 v/v/v) degassed by a vacuum pump. The mobile phase used was prepared just before the assays
- the flow-rate was 1.0 mL/min.
- the eluate was monitored by UV absorbance at 252 nm.

Under these conditions the following average retention time (R_t) parameters were obtained: 8.132 min. for cortisol and 13.800 min. for 6-α-methylprednisolone (IS).

The best recovery for both, cortisol and internal standard, 6-α-methylprednisolone, was achieved under the following extraction procedure: 500 µL of plasma was mixed with 50 µL of internal standard (5 µg/mL 6-α-methylprednisolone in methanol-water (1:1 v/v)), 250 µL of 0,1 M sodium hydroxide and 50 µL of methanol.

Table 3. Clinical characteristics and plasma cortisol and imipramine levels before and after treatment. C – cortisol concentration [ng/mL], I – imipramine concentration [ng/mL], D – imipramine daily dose [mg], HDRS – Hamilton score

Time of sample collection		Subjects			
		1 I	2 I	3 I	4 I
0	**C**	**123.6**	**122.82**	**16.56**	**333.68**
	I	0	no data	89.46	40.36
	D	0	0	0	0
	HDRS	25	25	28	28
3 h	**C**	**124.01**	**142.6**	**108.69**	**137.51**
	I	61.35	no data	60.44	73.49
	D	50	50	50	50
	HDRS	25	25	28	28
24 h	**C**	**237.91**	**136.19**	**188.22**	**248.89**
	I	36.99	44.47	no data	29.6
	D	75	75	75	50
	HDRS	25	25	28	28
2 weeks	**C**	**2855.22**	**136.36**	**117.34**	**235.99**
	I	60.71	41.91	52.5	54.32
	D	75	125	150	75
	HDRS	23	24	26	26
4 weeks	**C**	**0.96**	**103.04**	**124.14**	**151.48**
	I	102.96	85.53	no data	59.8
	D	75	125	150	100
	HDRS	18	24	25	24
6 weeks	**C**	**129.41**	**91.26**	**103.55**	**119.68**
	I	64.18	51.22	46.93	94.38
	D	75	125	150	150
	HDRS	16	18	20	22
8 weeks	**C**	**179.83**	**94**	**53.3**	**187.51**
	I	63.9	51.31	132.89	98.94
	D	75	125	150	150
	HDRS	16	17	18	18

After addition of 2 mL of dichloromethane, the samples were shaken for 10 min and afterwards frozen. The organic layer was subsequently transferred in a glass tube and evaporated under a stream of nitrogen at 45°C. The residue was reconstituted in 500 µL of methanol-water (1:1 v/v). A 100 µL aliquot of this sample was injected onto the HPLC system.

The HPLC method of cortisol determination was validated. Table 1 shows the parameters of this validation.

RESULTS

The level of cortisol in the blood plasma of healthy subjects (the control group, N = 24) ranges between 7 and 281 ng/mL (x = 165.56 ng/mL, CV = 64.95%) and is compliant with the common standards.

One of the tasks focused on was the analysis of the changes of plasma cortisol in patients with MDD during 8 weeks of fluoxetine or imipramine treatment. Cortisol concentration in blood was calculated using peak areas of extracted plasma samples.

As a point of reference for the altering cortisol blood concentrations the initial hormone levels were taken – assessed just before the administration of the first medicine dose. The initial cortisol concentrations in subjects treated with fluoxetine are presented in Table 2 and in imipramine-treated patients – in Table 3.

Cortisol plasma level in subjects was assessed before the commencement of the therapy, then after 6 or 3 h ('fluoxetine' or 'imipramine' group, respectively), 24 h and after 2,4,6 and 8 weeks of the start of treatment. A few additional assessments were also done.

Before drug administration, in the examined population of subjects elevated cortisol level appears in 1/9 patients only; in 4/9 patients the hormone concentration is within the established range and in another 4 out of 9 subjects it is too low.

The results of our experiment cannot be interpreted unambiguously. We observed a decrease of the cortisol level in 3/9 subjects, whereas in 2/9 cases increasing hormone concentration was noted. The rest 4/9 subjects demonstrated variable cortisol levels.

Another subject of our research was the analysis of the relationship between the plasma cortisol level and patient's psychical condition. This condition was assessed using the following kind of scales: HDRS (Hamilton Depression Rating Scale), HARS (Hamilton Anxiety Rating Scale), and SERS (Side-Effects Rating Scale), but our analysis concerned HDRS only. 50% reduction in a psychometric scale score is considered as clinical state improvement. In a group of 5 patients treated with fluoxetine, 2 patients accomplished recovery in the 4th week of the therapy, another 2 subjects – in the 8th week; 1 patient did not achieve recovery, according to the HDRS score reduction. Psychological recovery seems to be related with fluoxetine therapeutic blood concentrations in 4/5 subjects. In 1/5 subjects (5F) psychological recovery was observed despite a drug concentration that was not within the 'therapeutic window'. Probably in this case HDRS reduction might be associated with fluoxetine metabolites.

None of the patients treated with imipramine demonstrated 50% or more HDRS score reduction. This fact is presumably connected with too low imipramine concentrations – exceeding the therapeutic range.

Only in 1 subject (1F) psychological recovery appeared simultaneously with the reduction of the cortisol level during the treatment. 3 out of 9 patients achieved recovery together with an increase of the hormone level. The same number of subjects (3/9) performed an opposite relation: cortisol level decreased without subsequent clinical condition improvement. In the rest 2 patients changeable cortisol levels and no significant HDRS score reduction was observed.

DISCUSSION

The role of the HPA axis in the pathogenesis and in the proceedings of the depressive disorder is a material one, what is confirmed by the data obtained from the research conducted on animals, as the clinical data are sparse.

The results of numerous studies concerning cortisol secretion in MDD patients are inconsistent. Well established is the opinion that people with endogenous depression demonstrate elevated cortisol levels in plasma, urine and cerebrospinal fluid, as well as erratic circadian rhythm of the hormone secretion (1, 3, 5, 7-10). Long-term research proved numerous irregularities in cortisol secretion. Apart from its elevated levels, disturbances such as a flattened circadian curve, a reduction in the length of the nocturnal quiescent period, an earlier nadir and an elevated nadir have been observed (4, 6).

Some scientists are sceptical about the view that hypercortisolism is the characteristic of the depression disorder (6). Peeters et al. findings suggest that erratic cortisol secretion may be more characteristic feature of the HPA axis dysregulation in MDD than hypercortisolism (6). This hypothesis is supported by our results. According to our data only 1/9 subjects exhibited pathologically elevated cortisol concentration, which is not in agreement with the opinion that hypercortisolism is a core characteristic of depression. The results of presented research suggest more likely that in the examined population of patients with endogenous depression cortisol levels are normal or too low. Peeters et al. (6) did not find elevated cortisol levels in depressed patients either. What is more, they paid attention to erratic circadian cortisol secretion. Unfortunately, we were not able to verify this hypothesis, as from the period before the commencement of the therapy only single specimens were accessible. Data from our work illustrate solely cortisol levels falling on 8.00. However, circadian cortisol secretion could not have been assessed, because significant aberrations could have occurred anytime during day and night. Many laboratories determine cortisol level routinely at 8.00 and/or 17.00, which may give a false evaluation of the cortisol level in patient (10). For more reliability of our findings it would be

advisable to measure plasma cortisol at short (15 min) intervals for 24 h in each subject before the beginning of the fluoxetine or imipramine therapy.

CONCLUSION

In the examined population of depressed patients no elevated cortisol levels were observed before the commencement of the therapy.

No relation – either between plasma medicine level or therapeutic effect (presented as HDRS score reduction) and plasma cortisol level was found.

Unfortunately, hitherto obtained results of our research have not given the unambiguous results, probably due to the difficult methodology and the low number of subjects under research. In this situation it seems that further research on this subject is justified.

REFERENCES

1. Abreu Feijo de Mello A., Feijo de Mello M., Carpenter L.L., Price L.H.: Rev. Bras. Psiquiatr. 25, 231 (2003).
2. Bilkiewicz A., Pużyński S., Rybakowski J., Wciórko J.: Psychiatry (in Polish), pp. 148, 155, 179, 591, Elsevier Urban & Partner, Wrocław 2002.
3. Budziszewska B.: Pol. J. Pharmacol. 54, 343 (2002).
4. Linkowski P., Mendlewicz J., Kerkhofs M., et al.: J. Clin. Endocrinol. Metab. 65, 141 (1987).
5. Pariante C.M., Thomas S.A., Lovestone S., Makoff A., Kerwin R.W.: Psychoneuroendocrinology 29, 423 (2004).
6. Peeters F., Nicolson N.A., Berkhof J.: Psychiatr. Res. 126, 1 (2004)
7. Duncan G.E., Knapp D.J., Carson S.W., Breese G.R.: J. Pharmacol. Exp. Ther. 285, 579 (1998).
8. Kostowski W.: Psychiatria 1, 63 (2004).
9. Kudoh A., Ishihara H., Matsuki A.: J. Clin. Anaesth. 12, 383 (2000).
10. Sher L.: Med. Hypotheses 62, 198 (2004).