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Experimental pharmacological research regarding the potential antidepressant activity induced by some newly synthesised dibenzo-[a,d]-cycloheptene compounds

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Abstract

Background. Depression is a relatively frequent encountered mental disorder in the general population, affecting both the quality of the individual life and its ability to perform the social tasks; it is generally accepted that new studies related to this pathologic condition are further necessary, in order to identify more adequate, efficient and accessible therapeutic compounds.

Materials and methods. This study was performed on a sample of 60 white male mice, NMRI strain, who were divided into 6 groups of 10 animals and treated with 4 new derivatives of dibenzo [a, d] cycloheptene, amitriptyline as a reference substance, or with distilled water for the control group. The animals were tested in respect to the forced swim test, both before and at 2 hours after administration of the mentioned substances. It was determined thus the time of immobilization.

Results. The study showed the fact that only two of the four investigated compounds presented a relatively similar antidepressant effect with the reference substance.

Conclusions. Minor structural changes, such as modifications of some substitutes from the basic chemical core of the reference substance can decisively influence the conservation or loss of the antidepressant properties.

Introduction

Currently, depression tends to become the most common mental disorder, being present in more than 350 million people of all ages, in both women and men from all communities (1). According to a study conducted by the World Health Organization, it has been estimated that about 3-15% of the general population present a depressive episode every year, of which about 5% suffer from severe depressive episodes. Just in Europe for example, 58 subjects from a sample of 1000 adults are affected by major depressive disorder, which means a total of 33.4 million affected people (1, 2).

In 2011, statistics performed by the Ministry of Health revealed that 2739 cases of mental illness for 100.000 people have been recorded in Romania, 80% of which were represented by: anxiety, depression, neurosis, etc. All of these worrying results justified the fact that depression became currently one of the most studied psychiatric disorders. Although there are known several relative efficient therapeutic procedures for depression, the access to treatment still remains a problem for many countries. According to WHO, in some countries less than 10% of the persons who suffer from depression receive an adequate treatment (1, 2).

Depression is a complex mental disorder with severe implications, in respect not only to the patient but also to family and society. The physio-pathologic mechanisms of depression are not fully understood, existing however a bio-psycho-social model for depression that is widely accepted by researchers and clinicians. This model is based on the idea that the onset of depression is influenced by multiple factors such as: the disruption of the biochemical mediators in the brain, psychological imbalances of persons with fragile personalities, and possible social factors such as daily stress linked to negative events of life, environmental hostility and so on. In almost a quarter of cases the first depressive

episode occurs as a reaction to a negative life event, and it may happen again later apparently without any reason. It is also documented that depression may be favoured by some neurological disorders such as epilepsy, endocrine diseases, infection cases implying HIV/ AIDS, mononucleosis, pneumonia, tuberculosis or some cancer types associated with prolonged pain (3, 4).

Materials and Methods

Depression is usually a temporary and qualitative/ subjective mental disorder, characterized by a complex of emotional-affective events that are difficult to be objectively quantified (5). The desperation test is a specific way to investigate preclinical the antidepressant action of various compounds, and consists in determination of the immobilization time of a mouse which is put in the situation of forced swimming. In essence, through this manoeuvre it is intended to induce a similar state (from a biochemical perspective) with the state of depression characterised by adreno-serotonergic hypofunction.

Although the Porsolt test is useful in the evaluation of antidepressant action, however it is not very objective (6, 7). The test can give false positive results if it is performed using inadequate containers, or if the amount of water is incorrectly calculated. This could imply that the mice can cling with the anterior paws to the lateral sides or can reach with the hind paws to bottom, or can jump out. To avoid these possibilities it is recommended to use cylinders with smooth walls and having 30 cm in diameter, the depth of the water column to be at least 10 cm, and the distance from the water to the edge of the cylinder should not be lesser than 10 cm.

Materials

- Mice, NMRI (Naval Medical Research Institute), weighing between 18-25 g;

- standardized nutritional grains from cereals;

- substances to be tested: derivatives of dibenzo [a, d] cycloheptene (8), synthesized in the Department of Organic Chemistry at the "Carol Davila" Faculty of Pharmacy, Bucharest (C1, C2, C3, C4), reference substance (amitriptyline) (9), and control (distilled water);

- transparent cages from Plexiglas, lined with wood shavings;

- transparent glass cylinder (30 cm diameter, 24 cm height) containing water (at 22˚C, until a height of 15 cm);

- electronic balance; syringes

Methods

The animals were transported from the designed department of "Carol Davila University" with 5 days prior the onset of the experiment, in order to allow their acclimatisation to the new environment (receiving food and water "ad libitum", the ambient temperature was 22-23˚C, and the animals were exposed to a standard cycle of light/ dark).

Animals were brought into the room where the experiment was performed with 60 minutes prior to the start of it, for adjustment. The study deployed on groups of 10 animals, relatively similar in terms of biometric characteristics (mass, age, sex).

The initial tests were performed, leading to delineation of animals on distinct lots, according to the times of immobilization registered in this stage. The animals were weighed, and received the substances (C1, C2, C3, C4, amitriptyline, control), orally 60 minutes before the test.

The study was performed with 6 groups of 10 mice, as follows:

Sample 1: control (distilled water), at a dose of 0.1ml / 10g orally

Sample 2: Reference-amitriptyline susp. 1%, at a dose of 100 mg / kg orally

Sample 3: C1 substance susp. 1%, at a dose of 100 mg / kg orally

Sample 4: C2 substance susp. 1%, at a dose of 100 mg / kg orally

Sample 5: C3 substance susp. 1%, at a dose of 100 mg / kg orally

Sample 6: C4 substance susp. 1%, at a dose of 100 mg / kg orally

The animals were placed within cylinders and followed by the same observer for 6 minutes: during the last four minutes the total duration of immobilization was recorded, being obtained by summing the periods of time during which the animal stands still; the movements made by animal to maintain the head above the water were not taken into account. Data obtained from the treated groups were statistically analysed and compared with control and reference groups.

All procedures were performed respecting the rules of bioethics in research on experimental animals for scientific purposes, in accordance with Law 43/2014 on the protection of animals used for scientific purposes and Directive 2010/63 / EU of the European Parliament and of the Council of Europe - September 22, 2010 on protection of animals used for scientific purposes.

The overall interpretation of the results was carried out by applying the statistical t test - Student to compare two columns with normal distribution, and ANOVA to compare more than two columns with normal distribution. The statistical test ANOVA was followed by Dunnett's post-test, for the event of a possible statistical significance. Normal or abnormal distribution of the data was determined by the D`Agostino – Pearson test. All these tests were performed using Graph Pad Prism software version 5.01, and for the graphics Microsoft Office Excel, 2007 version was used.

The effect (%) over time of immobilisation was calculated as the difference between the initial and final time of restraint, in rapport to the initial time of immobilization, as follow:

$$Ef\,\% = \frac{Tf - Ti}{Ti} \times 100$$

Where: Ef% - effect on the time of immobilization, %

Ti - initially time of immobilization

Tf – final time of immobilization

Results

Table 1. Evolution of immobilization time for despair test obtained in the control group

No.	Initial time of immobilization (s)	Final time of immobilization (s)
1	45	51
2	42	54
3	65	70
4	74	76
5	56	62
6	80	60
7	76	71
8	46	55
9	66	80
10	80	74
Mean ± EMS	63 ± 4,695	65,3 ± 3,229
DS	14,85	10,21
Normal distribution (D`Agostino – Pearson test)	Yes	Yes
Effect (%) vs. Initial data	-	-3,65%
t-Student test	p > 0,05	

Table 2. Evolution of immobilization time for despair test obtained in the group C1

No.	Initial time of immobilization (s)	Final time of immobilization (s)
1	55	42
2	86	66
3	81	41
4	77	37
5	76	42
6	62	55
7	49	43

8	78	35
9	84	69
10	69	48
Mean ± EMS	71,7 ± 3,978	47,8 ± 3,726
DS	12,58	11,78
Normal distribution (D`Agostino – Pearson test)	Yes	Yes
Effect (%) vs. Initial data	-	-33,33%
t-Student test	p < 0,001	

Table 3. Evolution of immobilization time for despair test obtained in the group C2

No.	Initial time of immobilization (s)	Final time of immobilization (s)
1	76	72
2	59	51
3	64	59
4	56	45
5	51	46
6	80	77
7	53	61
8	42	58
9	57	42
10	56	64
Mean ± EMS	59,4 ± 3,597	57,5 ± 3,68
DS	11,37	11,64
Normal distribution (D`Agostino – Pearson test)	Yes	Yes
Effect (%) vs. Initial data	-	-3,2%
t-Student test	p > 0,05	

Table 4. Evolution of immobilization time for despair test obtained in the group C3

No.	Initial time of immobilization (s)	Final time of immobilization (s)
1	76	57
2	56	45
3	42	47
4	67	79
5	48	62
6	50	54
7	74	81
8	53	49
9	69	81
10	54	47
Mean ± EMS	58,9 ± 3,71	60,2 ± 4,685
DS	11,73	14,82
Normal distribution (D`Agostino – Pearson test)	Yes	Yes
Effect (%) vs. Initial data	-	+2,21%
t-Student test	p > 0,05	

Table 5. Evolution of immobilization time for despair test obtained in the group C4

No.	Initial time of immobilization (s)	Final time of immobilization (s)
1	69	62
2	74	47
3	48	35
4	66	53
5	58	41
6	67	54
7	71	50
8	78	54

9	77	49
10	76	64
Mean ± EMS	68,48 ± 2,971	50,9 ± 2,767
DS	9,395	8,749
Normal distribution (D`Agostino – Pearson test)	Yes	Yes
Effect (%) vs. Initial data	-	-25,67%
t-Student test	p < 0,001	

Table 6. Evolution of immobilization time for despair test obtained in the reference group

No.	Initial time of immobilization (s)	Final time of immobilization (s)
1	60	36
2	78	48
3	51	33
4	69	29
5	71	20
6	55	45
7	63	40
8	57	32
9	48	22
10	53	26
Mean ± EMS	60,5 ± 3,056	33,1 ± 2,949
DS	9,664	9,327
Normal distribution (D`Agostino – Pearson test)	Yes	Yes
Effect (%) vs. Initial data	-	-45,29%
t-Student test	p < 0,0001	

Table 7. Evolution of immobilization time for despair test obtained in the control group

No.	Initial time of	Initial time	Initial time	Initial time	Initial time	Initial time of

	immobiliz control	of immobiliz C1	of immobiliz C2	of immobiliz C3	of immobiliz C4	immobiliz reference
Mean ± EMS	63 ± 4,695	71,7 ± 3,978	59,4 ± 3,597	58,9 ± 3,71	68,48 ± 2,971	60,5 ± 3,056
DS	14,85	12,58	11,37	11,73	9,395	9,664
Normal Distribution (D`Agostino Pearson test)	Yes	Yes	Yes	Yes	Yes	Yes
Effect% vs. control	-	+13,81%	-5,71%	-6,51%	+8,7%	-3,97%
ANOVA test	p > 0,05					
Dunett post test	-	NS	NS	NS	NS	NS

Table 8. Evolution of final immobilization time for despair test obtained in the control group

No.	Final time of immobiliz control	Final time of immobiliz C1	Final time of immobiliz C2	Final time of immobiliz C3	Final time of immobiliz C4	Final time of immobiliz reference
Mean ± EMS	65,3 ± 3,229	47,8 ± 3,726	57,5 ± 3,68	60,2 ± 4,685	50,9 ± 2,767	33,1 ± 2,949
DS	10,21	11,78	11,64	14,82	8,749	9,327
Normal Distribution (D`Agostino Pearson test)	Yes	Yes	Yes	Yes	Yes	Yes
Effect% vs. control	-	-26,8%	-11,95%	-7,81%	-22,05%	-49,31%
ANOVA test	p < 0,0001					
Dunett post test	-	**	NS	NS	*	***

Figure 1

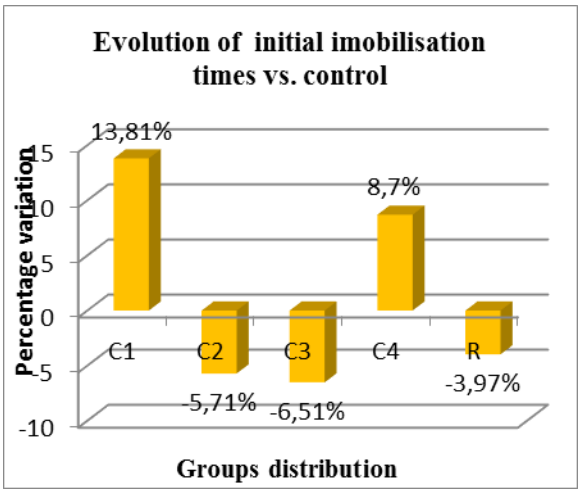
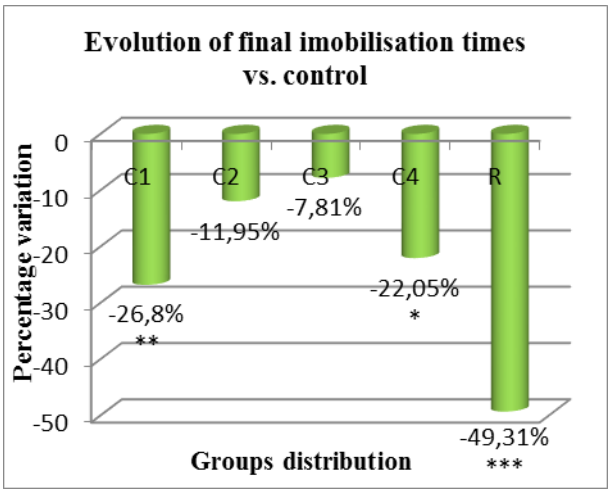


Figure 2



Discussions

Depression is a mental disorder that has implication not only in cognitive- behavioural processes, but also in the functioning of the body as a whole (10). It can be defined as a declining of the general/ basal well-being, often reiterating unpleasant, sad and threatening feelings. Depressive syndrome is characterized by absence of the good mood, slowing of the thought processes, psychomotor slowness and, in addition, clinical expression of a various auxiliary somatic symptoms (5).

The biochemical theories in respect to the etiopathogeny of depression usually refer especially to the model of endogenous depression (that is the primary depression major), and present several hypotheses:

The monoamine deficiency theories suggest that there is a deficiency on the transmission line of one of the cerebral neurotransmitters, referring either to noradrenaline (NA) or the serotonin (5-HT). These theories take into considerations only the quantitative deficiency of the neuromodulators implied, leading to a division of the endogenous/ biochemical depression into two distinct subgroups: through deficiency of serotonin, or through deficiency of norepinephrine; the dosage for each of these neurotransmitters metabolites shows low levels in the depressed patients (1, 3).

Dopamine (DA) has also been incriminated in some types of depression, an idea supported by an increased frequency of depressive episodes occurring during the evolution of Parkinson's disease. However, the dopamine deficiency may be invoked as a pathogenic mechanism only to a limited group of patients, presenting dopamine-dependent depression (1, 3).

The imbalance theory between the transmission pathways of various neuromodulators assumes that there is an imbalance between NA and 5-HT, the level of NA decreasing while the level of serotonine increasing. Such disruption could result from a possible competitive action of the two precursors at the blood brain barrier, namely tyrosine (for NA) and tryptophan (for 5-HT). The implications of different cerebral neurotransmitters in clinical manifestations of depression are the main etiopathogenic arguments supporting the psychopharmacological models (4).

From a pharmacological perspective, antidepressants that are used currently can be classified in the following groups based on their mechanism of action (11):

► adrenaline, noradrenaline and/ or serotonin reuptake inhibitors

► adrenergic and serotonergic receptor antagonists

► monoaminoxidaze inhibitors

1. The reuptake inhibitors of synaptic transmission are:

● non-selective reuptake inhibitors of norepinephrine and serotonine (amitriptyline, nortriptyline, imipramine, doxepin)

● non-selective reuptake inhibitors of serotonin and dopamine (venlafaxine)

● non-selective reuptake inhibitors of noradrenaline and dopamine (amfebutamona)

● selective noradrenaline reuptake inhibitors (maprotiline oxaprotiline, dezipramina, reboxetine

- selective serotonin reuptake inhibitors (sertraline, fluoxetine, paroxetine, citalopram, fluvoxamine)

2. The adrenergic and serotonergic receptor antagonists are:

- α2 presynaptic receptor antagonists (mirtazapine)
- selective 5HT2 receptor antagonists, α2 and H1 (mianserin)

3. The monoaminoxidaze inhibitors can be:

- non-selective and irreversible inhibitors (nialamide, phenelzine)
- selective and reversible inhibitors of monoamine oxidase A (moclobemide)

The compounds investigated in this study presents a dibenzo [a, d] cycloheptene chemical core, which is structurally similar to amitriptyline. It was studied here if/ how the minor structural variations of the mentioned chemical core can influence the antidepressant action.

Data obtained here showed that the administration of the investigated compounds leads to different periods for immobilization time. The compounds C1 and C4 induced a shorter immobilization time compared to initial time –33.33%, respectively –25.67% (statistically significant for Student-t test). Compared to control, decreases were –26.8% for C1, respectively –22.05% for C4. The compounds C2 and C3 lead to minor changes for immobilization time compared with initial time, namely –3.2%, respectively +2.21% (statistically insignificant for Student-t test). Compared to control, a decreasing of immobilization time with –11.95% for C2, respectively with –7.81% for C3 was recorded.

Analysing the initial immobilization times for all groups, a statistically insignificant variation of ANOVA test was observed which in fact an expression of the normal inter-individual variability is. The ANOVA test applied in order to compare the final immobilization times obtained after administration of

investigational compounds with the immobilization time of the control, highlight the statistical significance of the decreasing periods reordered after administration of C1 and C4.

Conclusion

According to our data, only two of the four studied compounds maintain their antidepressant properties. Due to the fact that all compounds incorporates a dibenzo [a, d] cycloheptene chemical core, it can be concluded that their substituents have a decisive role in preserving or losing the antidepressant properties.

New studies are necessary in order to further highlight possible advantages (in terms of pharmacokinetics and pharmacotoxicology) of the four compounds investigated here, in comparison with the already existing compounds on the pharmaceutical market.

Disclosure

No authors involved in the production of this article have any commercial associations that might pose or create a conflict of interest with information presented herein.

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