DEMONSTRATION OF ANTINOCICEPTIVE ACTION OF SOME ANTIDEPRESSANTS USING MODELS OF ACUTE NOCICEPTION IN MICE

DEMONSTRAREA ACTIVITĂȚII ANTINOCICEPTIVE A UNOR ANTIDEPRESANTE FOLOSIND MODELE DE NOCICEPTIE ACUTĂ LA ȘOARECI

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Abstract

During the last few years, the relationship between pain and depression has become a frequent topic for the specialists in the field. In order to achieve a rational pain therapy, it was sought to demonstrate the antinociceptive potential of some drugs that do not belong to the classical analgesic group. In the present study, we demonstrated the antinociceptive action of nortriptyline, doxepin and clomipramine (tricyclic antidepressants), based on the ability of these agents to influence some mediators involved in pain processes (noradrenaline (NA), serotonin (5-HT). The antinociceptive potency decreases, for the inflammatory pain model, in the following order: nortriptyline, clomipramine, doxepine. For the thermal stimulus model, potency decreases as follows: clomipramine, nortriptyline, doxepine. The demonstration of these antinociceptive actions suggests that these substances modulate pain and may be used as adjuncts in its therapy.

Rezumat

Relația dintre durere și depresie a devenit în ultimii ani o temă de actualitate pentru specialiștii în domeniu. Pentru realizarea unei terapii raționale a durerii s-a căutat a se demonstra potențialul antinociceptiv a unor substanțe medicamentoase care nu fac parte din grupa analgezicelor clasice. În studiul de față s-a demonstrat acțiunea antinociceptivă a nortriptilinei, doxepinei si clomipraminei (antidepresive triciclice) având la bază capacitatea acestor substanțe de a influența unii mediatori implicați în procesele algice (noradrenalina (NA), serotonina (5-HT). Potența antinociceptivă scade in ordinea: nortriptilina, clomipramină, doxepină, pentru modelul de durere inflamatorie. Pentru modelul cu stimul termic potența scade astfel: clomipramină, nortriptilină, doxepină. Demonstrarea acțiunii antinociceptive sugerează că aceste substanțe modula durerea și ar putea fi adjuvante în tratamentul acesteia.

1. Introduction

During the last years, the role of antidepressants in algic states has been steadily demonstrated.

Also, in clinical settings, it has been tried to establish connections between pain and depression.

The questions that has been often addressed by the workgroups in the literature was: "Is pain a cause of depression or depression is a cause of pain?".

Thus, in the literature has been demonstrated the efficacy of antidepressants in neuropathic pain, chronic pain or other hyperalgesic situations [1].

The models for chronic nociception rely frequently on the serotoninergic or dopaminergic system [2].

Taking into account the fact that the antidepressants can influence the neuromediators, noradrenaline (NA), 5-OH triptamine (5-HT) or dopamine (DA) through the inhibition of the re-uptake, receptor blocking or presynaptic agonists, the analgesic action of these compounds might be explained through the action mechanisms characteristic to each substance [3].

In the present study, we propose the evaluation of the antinociceptive potential of several antidepressants using models of acute nociception in mice.

2. Materials and Method

The investigations of the present study were made using male Swiss mice, weighing 18-22 g. The animals were housed in plexiglas cages with drippers kept in conditions of temperature of 21 ± 2°C and controlled humidity, with a light/darkness cycle 7:00 AM/7:00PM and received food and water *ad libitum*.

All experimental procedures were made in agreement with the IASP (International Association for the Study of Pain) and the actual legislation [4].

The following substances were used: Doxepin (Sigma), Nortriptyline (Sigma), Clomipramine (Sigma), Sodium Carboxymethyl Cellulose (NaCMC), Zymosan A (Sigma).

The substances for investigation were administrated orally, suspended in NaCMC using dose sequences in geometric progression.

Nociceptive agent Zymosan A was administrated intraperitoneally as saline suspension.

The nociceptive models used were:

- Zymosan-induced abdominal constrictive response (writhing test) (quantal interpretation) and
- the hot plate test (thermal nociception model) (gradual interpretation).

The test of the abdominal constrictive response induced with Zymosan A is a model of chemical nociception with specificity on inflammatory pain and uses the method of Siegmund, the technique of Koster [5, 6].

The test is made by the intraperitoneal administration of a suspension of Zymosan A, 40 mg/kg/bw and recording a characteristic response called abdominal constrictive response (writhing) during 12 minutes from the moment of the administration of the chemical agent.

The interpretation of the effect is quantal, characterized by the presence or the absence of the response.

The hot plate test implies the use of a specialized device called the hot plate Ugo Basile model 7280 and represents the placing of the animal on a surface which is heated at 52.5 ± 0.2°C during 30 seconds.

The characteristic responses are noted: licking/shaking of the posterior paws, jumping. The interpretation of the effect is gradual, calculating a maximum possible effect (MPE %) obtained.

For determining the efficacy of the substances taken in study the ED50 value of each substance for each model was calculated [6].

3. Results and Discussions

After the administration of a dose sequence of nortriptyline 0.25-2 mg/kg.bw orally (table 1, fig. 1, 2) the following results were obtained:

Table 1

Results after the administration of a dose sequence of nortriptyline 0.25 – 2 mg/ kg.bw orally

Writhing test			Hot plate test		
Dose mg/kg	**MPE %**	**Value ED50 mg/kg**	**Dose mg/kg**	**MPE %**	**Value ED50 mg/kg**
2.00	83.33	0.615±0.193 Y=5.43+2.05*X R:980	2.00	63.240	0.547 ± 0.091 Y = 56.91 + 26.41*X R:0.961 TLC (0.041, 1300)
1.00	66.66		1.00	57.340	
0.5	50.00		0.5	52.920	
0.25	14.66		0.25	38.22	

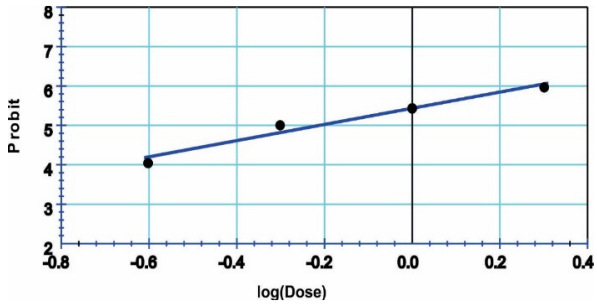

Figure 1. Regression line for nortriptyline using the writhing test

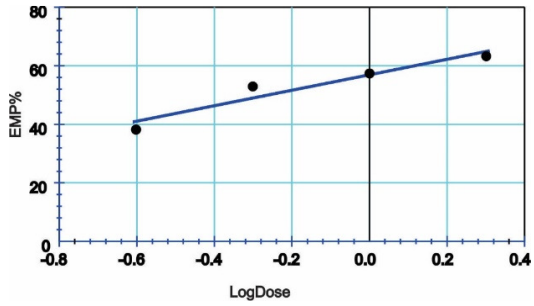

Figure 2. Regression line for nortriptyline using the hot plate test

By administrating dose sequences of doxepin 2.5 – 20 mg/kg bw, respectively 5 – 20 mg orally (table 2, fig. 3, 4) the following results were obtained:

Table 2

Values registered after dose sequences of doxepin 2.5 – 20 mg/kg bw, respectively 5 – 20 mg orally

Writhing test			Hot plate test		
Dose mg/kg	**MPE %**	**Value ED50 mg/kg**	**Dose mg/kg**	**MPE %**	**Value ED50 mg/kg**
20.00	83.33	8.13 ± 2.56 Y= 3.13+2.05*X R:980	20.00	59.88	14.55 ± 4.131 Y = -4.55 + 46.90*X R: 0.923 TLC (17.468, 5.569)
10.00	50.00		10.00	35.53	
5.00	33.33		5.00	31.64	
2.50	16.66				

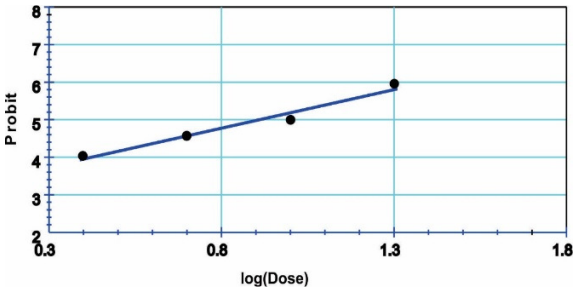

Figure 3. Regression line for doxepin using the writhing test

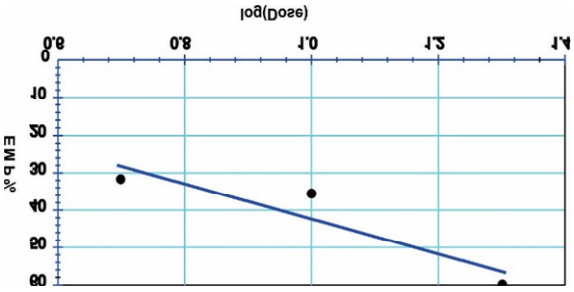

Figure 4. Regression line for doxepin using the hot plate test

By administrating dose sequences of clomipramine 0.187 – 3 mg/kg bw, respectively 0.187– 0.750 mg orally (table 3, fig. 5, 6) the following results were obtained:

Table 3

Values registered after dose sequences of clomipramine 0.187 – 3 mg/kg bw, respectively 0.187– 0.750 mg orally

Writhing test			Hot plate test		
Dose mg/kg	MPE %	Value ED50 mg/kg	Dose mg/kg	MPE %	Value ED50 mg/kg
3.00	66.66	1.51 ± 0.79 Y= 4.77+ 1.27*X R:976	0.750	64.03	0.486 ± 0.085 Y = 68.25 + 59.09*X R: 963 TLC (0.658 0.204)
1.50	50.00		0.375	37.59	
0.75	33.33		0.87	28.37	
0.375	16.66				
0.187	16.66				

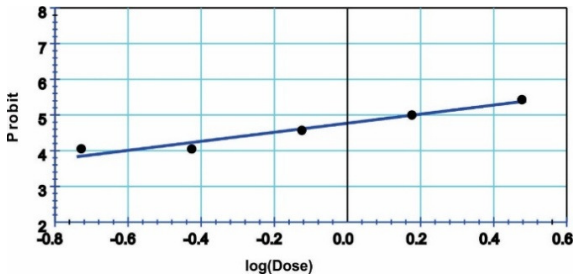

Figure 5. Regression line for clomipramine using the writhing test

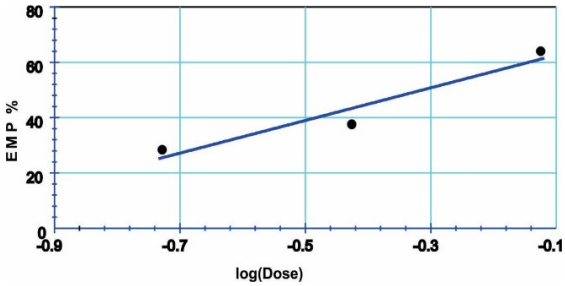

Figure 6. Regression line for clomipramine using the hot plate test

For all the substances taken in study we could demonstrate de ED50 for the nociception models used.

Doxepin, nortriptyline and the clomipramine belong from the group of tricyclic antidepressants which are non-selective inhibitors for noradrenaline and serotonin re-uptake.

Analyzing the ED50 values obtained for the nociception model with chemical stimulus we observed that the antinociceptive potency decreases in the order nortriptyline, clomipramine, doxepin. For the nociception test using thermal stimulus the potency decreases in the order: clomipramine, nortriptyline, doxepin (thus clomipramine and nortriptyline have comparable potency).

The potency of the inhibition of the monoamine neurotransmitter re-uptake is variable: the nortriptyline has a maximal potency for NA re-uptake inhibition of 4.35 comparative with 18.5 for serotonin and 1140 for dopamine which offers it a selectivity of 4.25 in favor of NA; similarly the doxepin demonstrates a potency of 29.4 for NA, 66.7 for 5HT and 12200 for DA which gives it a minimal selectivity of 2.27 in favor of NA; finally the clomipramine, as an agent with selectivity for 5HT has a potency of 37 for NA, 0.28 for 5HT and 2200 for DA which gives it a selectivity of 132 for 5HT [7].

A hypothesis which might explain the antinociceptive action of the three substances could be the inhibition of re-uptake in various degrees of NA and 5HT at the level of the inhibitory noradrenergic and serotoninergic pain control pathways with midbrain-spinal direction. The phenomenon was observed by Jones et al in 2004 using duloxetine as nonselective inhibitor of noradrenergic and serotoninergic re-uptake [8].

Taking into account the chances for the appearance of metabolites which might influence the results, a useful endeavor might be the investigation of the plasma concentration of the substances using a high accuracy method and also the identification and measuring of potential

metabolites of the substance taken in study. While the literature presents such assessments using human and rat blood with detection limits in the order of nanograms, the following step would be to identify and quantify those substances in mouse.

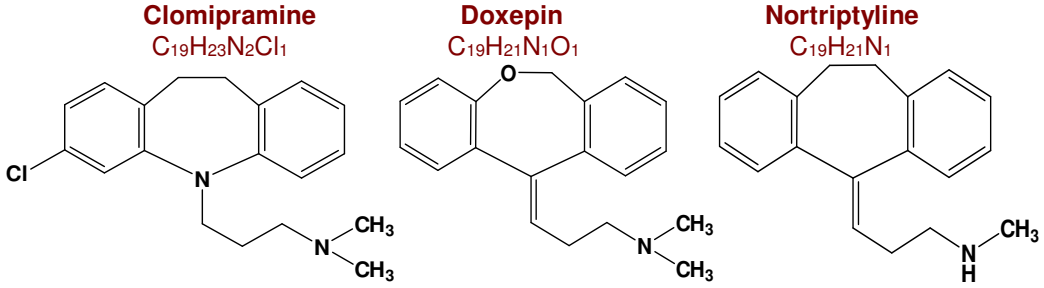

Figure 7. Molecular formulas and plane structure formulas for clomipramine, doxepin and nortriptyline

The tertiary amino- moieties of clomipramine and doxepin as well as the secondary amino-moiety of nortriptyline can be protonated in an acid environment. Thus, these molecules can be analyzed in a positive ionization mode using a mass spectrometer equipped with a source of gentle ionization like MALDI or ESI.

The molecular ions which are formed [clomipramine + H]$^+$, [doxepin + H]$^+$ and [nortriptyline + H]$^+$ can be observed at values of m/z of 315,163,280,170 and respectively 264,175.

Nozawa et al published in 2015 a study in which they have demonstrated the analysis of tricyclic antidepressant drugs and their metabolites using the MALDI-QTOF on samples of human blood plasma [9].

Yuan Shen et al published in 2010 a study in which they have shown the assay of amitriptyline and nortriptyline from rat plasma. The calibration curves were linear in the concentration domain 10-1000 ng/ml for nortriptyline. The quantification limit was 10 ng/ml [10].

The present study demonstrated an antinociceptive action on both nociception models, for all substances taken in study. Thus we can conclude that re-uptake inhibitors of NA and 5HT might have also a clinical efficacy in pain modulation, alone or as adjuvants for classical analgesic therapy.

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