Study Regarding the Phototoxicity of Some Tricyclic Antidepressants

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Objective: Our main objective was the development of a research methodology in order to assess the phototoxic potential on in vitro erythrocytes of five frequently used tricyclic antidepressants derivates.
Methods: The hemolytic effect of imipramine, clomipramine, amitryptiline, nortryptiline, doxepine hydrochloride was studied on erythrocytes after irradiation with UV light.
Results: The studied substances exhibited phototoxic effects on erythrocytes in the presence of UV light, causing their lysis to a greater extent than the one observed in a saline erythrocytes solution irradiated with UV light.
Discussions: The differences between the effects of the studied antidepressants on erythrocyte lysis in UV light are noticeable, the most pronounced effect being observed in the case of clomipramine hydrochloride and the lowest being observed in the case of doxepin hydrochloride.
Conclusions: The molecular structure influences significantly the phototoxic character of the studied substances. The molecule photosensitivity is not directly proportional with the phototoxic potential of the tricyclic antidepressants.

Keywords: tricyclic antidepressants, phototoxicity, erythrocytes, hemolysis

Introduction

With the increasing use of drugs and cosmetic products along with UV light exposure, the problem of phototoxicity exacerbated lately [1–3]. It is a well-established fact that many medicinal substances exhibit phototoxic effects. These effects represent a problem, as phototoxic products formed in the patient's skin can cause unwanted effects because of their ability to transfer energy to cellular components. This explains the citotoxicity of some photodegradation products on erythrocytes, capable to produce their hemolysis [1,2].

There are several reports of photoallergic and phototoxic responses regarding substances with topic or systemic administration. Along with thiazidic diuretics, non-steroid antiinflammatory drugs and quionolones, the group of tricyclic antidepressants is also mentioned in the literature as susceptible to phototoxicity [1,2,4–6].

Regarding the phototoxic properties of tricyclic antidepressants, numerous studies have been conducted, most of them targeting the product protriptyline, well-known for its effect of photosensitisation of the human skin [1,2,4,5]. Phototoxic reactions, vary considerably in the intensity and incidence of their side effects, depending on the substance [1,2,7–9].

Hemolysis under the action of UV light in the presence of tricyclic antidepressants has been investigated in several studies [1,2].

Our objective was to develop a phototoxicity research methodology in order to assess the phototoxic potential on in vitro erythrocytes of five frequently used tricyclic antidepressants. Investigations have pursued to establish an order in terms of the degree of phototoxicity of the studied substances.

Materials and method

The five tricyclic antidepressants were purchased from the following sources: imipramine hydrochloride, clomipramine hydrochloride, amitriptyline hydrochloride, nortriptyline hydrochloride from Sigma-Aldrich, Germany and doxepin hydrochloride from Dipharma, Italy, and were of pharmaceutical grade. During the experimental work the following reagents were used: sodium dihydrogen phosphate ($NaH_2PO_4*2H_2O$) and disodium hydrogen phosphate ($Na_2HPO_4*2H_2O$) (Merck, Germany). All reagents were of chemical purity.

Buffer solution preparation

PBS 0.01M solution (PBS – Phosphate Buffer Solution) with a pH 7.4 was prepared from 81 ml $Na_2HPO_4*2H_2O$ 0.1 M and 19 ml $NaH_2PO_4*2H_2O$ 0.1 M, by dilution to 1000 ml with purified water isotonized with NaCl (0.135 M).

Erythrocytes preparation

A volume of 2 ml of human blood was mixed with 3 ml of 0.01 M phosphate buffer pH 7.4 and was submitted to centrifugation for 15 minutes at 2500 rotations/min. After centrifugation the supernatant was removed and another 3 ml of phosphate buffer was added to the solution. If it was necessary, the washing process was repeated until the supernatant became colorless.

Sample preparation

1 ml of packed erythrocytes was mixed with 19 ml buffer solution to obtain the erythrocytes stock solution. To obtain the solutions for irradiation a solution of 100 ng/ml with the studied substances and a saline solution (NaCl

Table I. Primary data of the photohemolytic study in the presence of imipramine hydrochloride

Time	Physiological saline solution with erythrocyte		Irradiated physiological saline solution with erythrocyte		Imipramine solution 100 ng/ml with erythrocyte		Irradiated imipramine solution 100 ng/ml with erythrocyte		Imipramine solution 100 ng/ml previously irradiated	
	RBC*10.8	Degr%	RBC*10.8	Degr%	RBC * 10.8	Degr%	RBC * 10.8	Degr%	RBC *10.8	Degr%
0 min	1.892	0.000	1.892	0.000	1.892	0.000	1.892	0.000	1.892	0.000
30 min	1.890	0.128	1.891	0.090	1.890	0.122	1.891	0.114	1.892	0.005
60 min	1.886	0.318	1.890	0.131	1.886	0.305	1.890	0.128	1.891	0.054
90 min	1.881	0.555	1.812	4.210	1.882	0.544	1.337	29.310	1.889	0.139
120 min	1.879	0.672	0.029	98.493	1.878	0.727	0.023	98.789	1.889	0.174

Table II. Primary data of the photohemolytic study in the presence of clomipramine hydrochloride

Time	Physiological saline solution with erythrocyte		Irradiated physiological saline solution with erythrocyte		Imipramine solution 100 ng/ml with erythrocyte		Irradiated imipramine solution 100 ng/ml with erythrocyte		Imipramine solution 100 ng/ml previously irradiated	
	RBC*10.8	Degr%	RBC*10.8	Degr%	RBC * 10.8	Degr%	RBC * 10.8	Degr%	RBC *10.8	Degr%
0 min	1.892	0.000	1.892	0.000	1.892	0.000	1.892	0.000	1.892	0.000
30 min	1.890	0.085	1.890	0.090	1.890	0.095	1.889	0.180	1.891	0.064
60 min	1.888	0.192	1.886	0.343	1.890	0.132	1.813	4.162	1.891	0.073
90 min	1.884	0.428	1.843	2.580	1.884	0.413	0.462	75.557	1.891	0.061
120 min	1.880	0.634	0.040	97.909	1.883	0.484	0.023	98.798	1.890	0.081

0.9%) was prepared. The concentration value of 100 ng/ml was chosen taking into account the plasma concentration achieved during drug treatment with these substances. Three solutions were prepared:

- 7 ml erythrocytes stock solution was mixed with 63 ml physiological saline solution;
- 7 ml erythrocytes stock solution was mixed with 63 ml solution containing 100 ng/ml medicinal substance dissolved in physiological saline solution;
- 100 ml solution containing 100 ng/ml medicinal substance dissolved in physiological saline solution.

Solution irradiation

50 ml of each of the three solutions were submitted to irradiation from a distance of 40 cm at 25°C. Room temperature was chosen because there was no significant difference to the analysis at 37°C. The solutions were continuously mixed using a mechanical agitator. 4 ml samples were prelevated at well-defined time intervals at 0, 30, 60, 90, 120, 180 min.

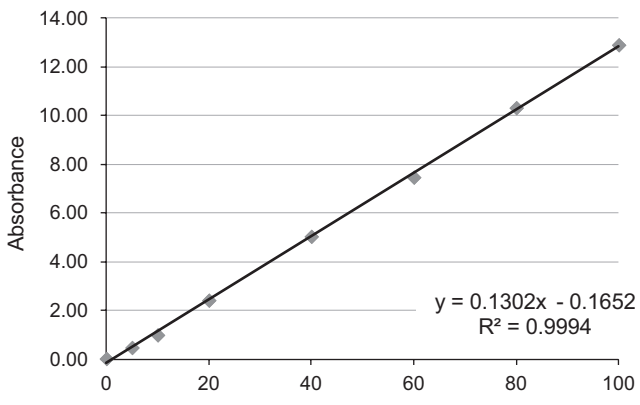

Fig. 1. Calibration curve of the photohemolytic process

Erythrocytes number determination

For the determination of antidepressants' phototoxicity on erythrocytes, human blood containing RBC (red blood cells) of 4.73 * 10^6/µl was used. In the samples the calculated erythrocyte content was 1.892*10^8.

Determination of hemolysis grade

Samples were centrifuged for 10 minutes at 2500 rotations/minute. The solutions were measured spectrophotometrically at 410 nm. To determine the degree of hemolysis of erythrocytes, we worked with erythrocytes in saline solution. In this solution the content of erythrocytes was considered 100% and from this solution we prepared dilutions of 5%, 10%, 20%, 40%, 60% and 80%. 50 ml of each solution was irradiated under conditions similar to the investigated solutions. The irradiation time was 2 hours, because during this period total erythrocyte hemolysis occurs. After irradiation the solutions were centrifuged and their absorbance was measured. Using the appropriate data, the calibration curve was drawn, in order to determine the degree of hemolysis of erythrocytes (Figure 1).

To verify data and demonstrate statistically significant differences the non-parametric test multicomponent DUNN was used.

Results

a) Imipramine hydrochloride

Erythrocytes in physiological saline solution, imipramine solution 100 ng/ml and in previously irradiated imipramine solution 100 ng/ml exhibit a good stability at room temperature. A more emphasized degradation of erythrocites by radiation in the presence of imipramine hydrochloride can be observed, basically 90 minutes after irradiation erythorycites undergo a 4.21% lysis in the case of

Table III. Primary data of the photohemolytic study in the presence of amitriptyline hydrochloride

Time	Physiological saline solution with erythrocyte		Irradiated physiological saline solution with erythrocyte		Imipramine solution 100 ng/ml with erythrocyte		Irradiated imipramine solution 100 ng/ml with erythrocyte		Imipramine solution 100 ng/ml previously irradiated	
	RBC*10.8	Degr%	RBC*10.8	Degr%	RBC * 10.8	Degr%	RBC * 10.8	Degr%	RBC *10.8	Degr%
0 min	1.892	0.000	1.892	0.000	1.892	0.000	1.892	0.000	1.892	0.000
30 min	1.888	0.206	1.890	0.130	1.889	0.147	1.891	0.050	1.891	0.068
60 min	1.886	0.297	1.879	0.680	1.888	0.194	1.828	3.365	1.891	0.035
90 min	1.881	0.595	1.799	2.594	1.861	1.640	0.775	59.019	1.890	0.088
120 min	1.878	0.721	0.025	98.686	1.886	0.324	0.029	98.450	1.889	0.153

Table IV. Primary data of the photohemolytic study in the presence of nortriptyline hydrochloride

Time	Physiological saline solution with erythrocyte		Irradiated physiological saline solution with erythrocyte		Imipramine solution 100 ng/ml with erythrocyte		Irradiated imipramine solution 100 ng/ml with erythrocyte		Imipramine solution 100 ng/ml previously irradiated	
	RBC*10.8	Degr%	RBC*10.8	Degr%	RBC * 10.8	Degr%	RBC * 10.8	Degr%	RBC *10.8	Degr%
0 min	1.892	0.000	1.892	0.000	1.892	0.000	1.892	0.000	1.892	0.000
30 min	1.888	0.206	1.891	0.130	1.891	0.050	1.892	0.000	1.891	0.032
60 min	1.886	0.298	1.890	0.681	1.889	0.165	1.887	0.253	1.891	0.065
90 min	1.881	0.595	1.812	2.611	1.887	0.277	1.315	30.490	1.889	0.136
120 min	1.878	0.722	0.029	98.772	1.886	0.315	0.029	98.442	1.890	0.127

Table V. Primary data of the photohemolytic study in the presence of doxepine hydrochloride

Time	Physiological saline solution with erythrocyte		Irradiated physiological saline solution with erythrocyte		Imipramine solution 100 ng/ml with erythrocyte		Irradiated imipramine solution 100 ng/ml with erythrocyte		Imipramine solution 100 ng/ml previously irradiated	
	RBC*10.8	Degr%	RBC*10.8	Degr%	RBC * 10.8	Degr%	RBC * 10.8	Degr%	RBC *10.8	Degr%
0 min	1.892	0.000	1.892	0.000	1.892	0.000	1.892	0.000	1.892	0.000
30 min	1.889	0.184	1.891	0.040	1.890	0.098	1.890	0.132	1.892	0.025
60 min	1.885	0.366	1.884	0.436	1.886	0.323	1.886	0.332	1.890	0.092
90 min	1.880	0.636	1.808	4.409	1.884	0.412	1.483	21.628	1.891	0.065
120 min	1.878	0.756	0.031	98.820	1.845	0.461	0.036	98.095	1.889	0.135

saline solution and a 29.31% lysis for imipramine solution (Table I).

b) Clomipramine hydrochloride

A significant lysis of erythrocytes cannot be noticed, after two hours only 0.64%, respectively 0.48% decomposition can be observed (Table II). Also, in the clomipramine solution of 100 ng/ml previously irradiated and mixed with erythrocytes the decomposition rate is even lower than in the saline solution (Table I).

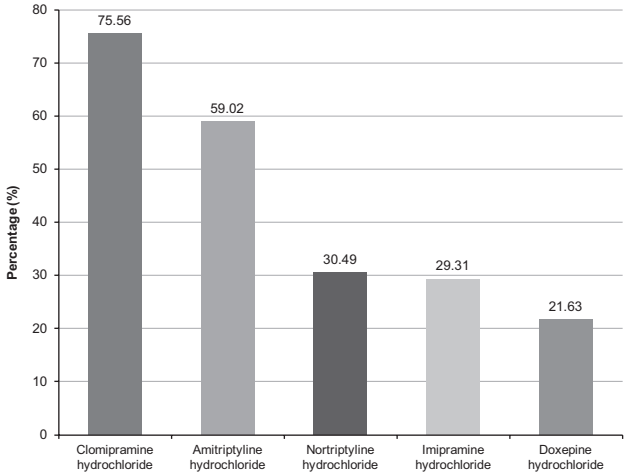

Fig. 2. Erythrocyte hemolysis after 90 min in the presence of the investigated substances

Erythrocytes irradiated in the saline solution and in the solution of clomipramine 100 ng/ml undergo a pronounced decomposition, after two hours a total lysis occurs in both solutions, the solution becoming red, without sediment after centrifugation.

A difference between the two irradiated solutions is also noticeable, as in the clomipramine solution the decomposition is more pronounced, after one-hour erythorycites undergo a 4.17% lysis in comparison with 0.34% in the saline solution.

After a 90 minutes irradiation degradation of red blood cells is 75.56% compared to 2.58% (Table II). After 2 hours the hemolysis is almost complete, in every solution subjected to radiation.

c) Amitryptiline hydrochloride

Similar with the study conducted on clomipramine hydrochloride, we found an insignificant hemolysis with the saline solution (0.72%), amitriptyline solution (0.32%) and with the previously irradiated amitriptyline solution (0.15%) in the absence of irradiation (Table III).

It can be noted that after an hour of irradiation erythrocytes undergo 3.36% degradation for the amitriptyline solution and 0.68% degradation in saline solution. After a 90 minutes irradiation the values are 59.02% for the amitriptyline solution, and 2.59% in saline solution.

d) Nortryptiline hydrochloride

Erythrocytes are stable in the absence of irradiation, their lysis being insignificant with saline solution (0.72%), nortriptyline solution (0.31%) and with the previously irradiated nortriptyline solution (0.12%) (Table IV).

Irradiation with UV light in the presence of nortriptyline hydrochloride, leads to faster lysis of erythrocytes, although degradation after 90 minutes for nortriptyline hydrochloride is lower (30.49%) than for amitriptyline hydrochloride (59.02%) (Table III, IV).

e) Doxepine hydrochloride

Just like for the other studied substances hemolysis in saline solution (0.76%), doxepin solution (0.46%) and in previously irradiated doxepin solution (0.14%) is insignificant.

Lysis is significantly increased by irradiation in the presence of doxepine hydrochloride in comparison with the saline solution. Hemolysis is 21.63% in the presence of doxepin hydrochloride, comparing it with 4.41% in the saline solution (Table V).

Discussions

Erythrocytes exhibit a good stability in saline solution, as after 3 hours the hemolysis didn't exceed 0.8%. Antidepressant solutions with a concentration of 100 ng/ml exhibit also a favorable environment for erythrocytes, lysis of red blood cells being below 0.8%. Using the statistical test DUNN, we demonstrated that there is no significant difference between lysis of erythrocytes in saline solution and in the antidepressants solutions (Q values are below the critical value Q 2.7996).

Erythrocytes exhibited a good stability even in the antidepressant solutions with a concentration of 100 ng/ml, which previously were irradiated under UV light, the lysis being very close to that recorded in saline solution. The DUNN test showed no significant differences to the results obtained with saline solution (Q values are also below the critical Q 2.7996).

Erythrocyte irradiation with UV light, both in saline solution and in the antidepressants solution leads to hemolysis, the values obtained indicating a significant difference from non-irradiated solutions, demonstrated statistically (test DUNN), the critical value of the parameter Q being exceeded in all the studied cases.

Regarding lysis of erythrocytes irradiated in the presence of saline solution and the one in the presence of antidepressant solutions, we found a statistically significant difference between the two levels of hemolysis. Lysis in the presence of the studied antidepressants was significantly higher than that in saline solution.

Differences between the effects of the studied antidepressant substances on erythrocytes lysis can be observed, the most pronounced effect being observed in the case of clomipramine hydrochloride and the lowest for doxepin hydrochloride. Although previous studies have shown that the two substances suffer significant degradation in solution by exposure to UV light [11–13], clomipramine cau-ses an accentuated red cell lysis (75.56%, after 90 minutes), while the effect of doxepin is three times lower (21.63%, after 90 minutes) (Figure 2). This difference in the intensity of hemolysis can be explained assuming a differential photodegradation of the two substances in terms of the degradation product's toxicity. This can explain the fact that data from the literature point out the phototoxic effect of clomipramine, but there is no data regarding doxepin, although the latter undergoes increased degradation after exposure to light. Also, it can be stated, that a possible higher photostability of a molecule does not necessarily mean a lower phototoxic potential. Previous studies have shown that amitriptyline hydrochloride has a much higher photostability than doxepin hydrochloride, however, it presents an almost 3 times higher phototoxicity on erythrocytes compared to doxepin.

Differences between the effect of antidepressant substances on red blood cell lysis in the presence of UV light can be observed, lysis occurring in the following descending order: clomipramine hydrochloride, amitriptyline hydrochloride, nortriptyline hydrochloride, imipramine hydrochloride and doxepin hydrochloride.

Results are correlated with in vivo photosensitizing potential of these antidepressants, the literature data suggesting that photohemolysis occurs through the involvement of biological membranes.

Conclusions

The study demonstrates the in vitro phototoxic property of the studied substances on biological systems.

All these data indicate that red cell lysis occurs faster in the case of irradiation in the presence of tricyclic antidepressants than in simple saline solution, but is not determined by the tricyclic antidepressants or their stable degradation products, but rather by intermediate photodecomposition products, which act probably through a radical mechanism. Molecular structures influence significantly the photoxic character of the studied substances.

The developed method can probably be also applied to assess the photohemolytic potential of other medicinal substances.

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