DOI 10.1007/s11094-014-1152-1
Pharmaceutical Chemistry Journal, Vol. 48, No. 9, December, 2014 (Russian Original Vol. 48, No. 9, September, 2014)

MOLECULAR BIOLOGY PROBLEMS OF DRUG DESIGN AND MECHANISM OF DRUG ACTION

EFFECTS OF 3-HYDROXYPYRIDINE AND SUCCINIC ACID DERIVATIVES ON RESISTANCE TO ACUTE ADRENALINE INTOXICATION AND FORMALIN EDEMA IN MICE

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Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 48, No. 9, pp. 3 – 6, September, 2014.

Original article submitted April 16, 2014.

We report here a comparative study of the effects of Russian-produced derivatives of 3-hydroxypyridine and succinic acid (emoxypine, Reamberin, and Mexidol) on the resistance of mice to acute adrenaline intoxication and formalin edema. The corresponding effects of α-lipoic acid (α−LA) were also studied - this has previously been used as a reference agent in assessments of the antihypoxic activity of 3-hydroxypyridine and succinic acid derivatives. Single doses of emoxypine, Reamberin, Mexidol, and α−LA at the optimum doses, corresponding to the therapeutic range for humans, were found to increase the duration of life in mice dying from acute adrenaline intoxication. α−LA was found to decrease formalin edema, while emoxypine and Mexidol increased its severity. The protective effects of all compounds tested against adrenalin intoxication were found to increase in direct proportion to their ability to increase formalin edema.

Keywords: 3-hydroxypyridine and succinic acid derivatives, adrenaline intoxication, formalin edema, Reamberin.

The original Russian drugs emoxypine, Reamberin, and Mexidol constitute a unique series of 3-hydroxypyridine and succinic acid derivatives and have been registered as drugs and approved for use as medicines in Russia. The 3-hydroxypyridine components of this series (emoxypine, Mexidol) have the same cation (2-ethyl-6-methyl-3-hydroxypyridine) but different anions – Cl^- for emoxypine and succinate for Mexidol. The anionic component of Reamberin is succinate, forming a salt with the non-3-hydroxypyridine pharmacophore meglumine (N-(1-deoxy-D-glucitol-1-yl)-N-methylammonium). These chemical structural features allow Mexidol to be regarded as a drug which is both a 3-hydroxypyridine derivative and a succinate derivative, while emoxypine is a 3-hydroxypyridine derivative only and Reamberin is a succinic acid derivative only. Emoxypine, Reamberin, and Mexidol have significant therapeutic potential, illustrating the value of including these drugs in schemes for the complex treatment of socially important and common diseases in humans. This is evidenced by the effective use of 3-hydroxypyridine and succinic acid derivatives in cerebrovascular and cardiovascular pathologies, diabetic and compression neuropathies, withdrawal syndromes, pulmonary tuberculosis, and primary open-angle glaucoma [1 – 6]. The high clinical efficacy of emoxypine, Reamberin, and Mexidol in a great variety of diseases and syndromes may be related to the ability of these drugs to correct stress-related and inflammatory reactivity, which is directly linked to the development of most pathological states. The adrenaline intoxication test, which reproduces endocrine changes in acute stress [7], is useful in experimental studies of this possibility, as is formalin edema, which models acute aseptic inflammation [7]. The present article reports a comparative analysis of the effects of 3-hydroxypyridine and succinic acid derivatives on the resistance of mice to acute adrenaline intoxica-

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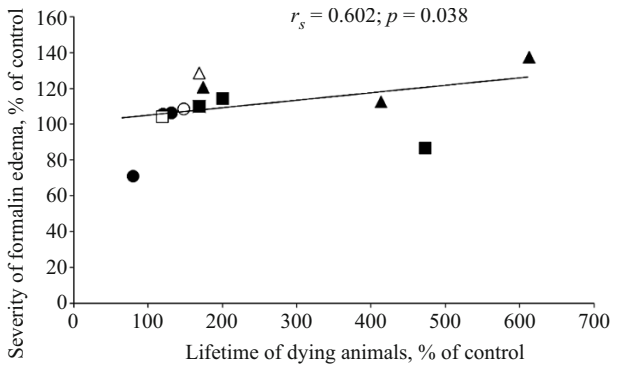

Fig. 1. Relationship between protective action of study drugs in acute adrenaline intoxication and their effects on formalin edema: triangles show 1/2EMTD, circle show EMTD, and squares show 2EMTD; empty symbols show emoxypine; black symbols show Reamberin; gray symbols with black outlines show Mexidol; gray symbols without outlines show α-lipoic acid.

tion and formalin edema. The corresponding effects of α-lipoic acid (α−LA) were also studied; this has previously been used as a reference compound for assessment of the antihypoxic activity of 3-hydroxypyridine and succinic acid derivatives [8].

EXPERIMENTAL SECTION

The study included two series of experiments and used 579 adult mongrel mice of both genders, weighing 18 – 25 g. The experiments were organized in compliance with the ethical principles expressed in Russian and international normative declarations of rights regulating animal experiments [9]. Study drugs in both experimental series were given as a single i.p. doses 30 min before modeling acute adrenaline intoxication (series I) or formalin edema (series II). Each drug was used at three doses extrapolated from the individual doses in the human therapeutic range, allowing for differences in relative body surface area [7]. The smallest dose in the study range was half the calculated equivalent mean therapeutic dose (EMTD). The maximum dose was double the EMTD. Emoxypine (2-ethyl-6-methyl-3-hydroxypyridine hydrochloride, FGUP Moscow Endocrine Factory, Russia) was used at doses of 12.5, 25, and 50 mg/kg. Study doses of 1.5% Reamberin (N-(1-deoxy-D-glucitol-1-yl)-N-methylammonium sodium succinate, OOO "NTFF POLISAN," Russia) solution were 25, 50, and 100 ml/kg. Mexidol (2-ethyl-6-methyl-3-hydroxypyridine succinate, OOO "NPK Farmsoft," Russia) was used at doses of 25, 50, and 100 mg/kg. α−LA (Berliton, Berlin-Chemie/Menarini Pharma GmbH, Germany) was used at doses of 50, 100, and 200 mg/kg (1/2EMTD, EMTD, and 2EMTD respectively). All doses of study drugs were given in a final volume of 100 ml/kg (diluted in 0.9% NaCl solution when necessary. Mice of the control group received the same volume of isotonic saline.

Acute adrenaline intoxication was modeled in experiments of series I as described in [10] and modified as described in [7]. Mice received 0.056% adrenaline hydrochloride solution s.c. at a dose of 14 mg/kg. Deaths among the animals were monitored over the next 60 min. Results were expressed as lethality in mice and the duration of life of animals dying within 1 h of adrenaline administration.

Formalin edema was modeled in experiments of series II by administration of 0.05 ml of 3.3% neutral formalin solution beneath the footpad aponeurosis [11]. Mice were sacrificed by cervical dislocation 1 h after formalin injection, both ankle joints were separated and a torsion balance was used to determine the weights of the inflamed and intact feet and the increase in the weight of the inflamed foot was calculated as a percentage of the weight of the intact foot.

Statistical analysis was run on SPSS-17.0. Data on the latency of deaths in mice with adrenaline intoxication and formalin edema were processed by descriptive statistical methods and are presented as means (Me) and the range between the "lower" (LQ, 25th percentile) and "upper" (UQ, 75th percentile) quartiles. Significant intergroup differences were identified using the Mann-Whitney U test. The statistical significance of differences in measures of lethality after adrenaline administration were assessed using Fisher's exact test. Interactions between results from experimental series I and II, standardized in relation to the controls, were assessed by calculating the Spearman correlation coefficient (r_s). Statistical hypotheses were verified at a critical significance level of $p = 0.05$.

RESULTS AND DISCUSSION

The results from experiments of series I showed that the study drugs had no effect on measures of lethality in mice in acute adrenaline intoxication but did produce significant prolongation of the lifetimes of animals dying within 1 h of adrenaline administration (Table 1). This effect was most clearly apparent on the background of administration of Reamberin, which was the only agent significantly increasing the latency of the animals' death over the whole dose range. The marked protective activity of this succinic acid derivative in acute adrenaline intoxication followed a U-shaped relationship with dose, with the strongest effects at the minimal (1/2EMTD) and maximal (2EMTD) doses. The protective action of Reamberin may be linked with realization of the NAD-independent substrate function of succinic acid, which plays a central role in supporting aerobic energy metabolism in the ischemia which consistently develops on the background of adrenaline intoxication [12]. It is entirely probable that this mechanism of the protective action of Reamberin is mediated at the level of the heart, which is the primary target of the harmful effects of toxic doses of adrenaline [13]. The correctness of this suggestion is illustrated by the positive inotropic action of Reamberin in patients with diabetes mellitus [1], which is characteristic of significant activation of the sympathoadrenal system [14].

TABLE 1. Effects of Emoxypine, Reamberin, Mexidol, and α-Lipoic Acid on the Resistance of Mice to Acute Adrenaline Intoxication and Formalin Edema.

Group (drug dose)	Acute adrenaline intoxication		Extent of formalin edema, %
	Lethality	Lifetime of dying mice, min	
Emoxypine			
Control	19/34 (55.88 %)	29.0 (23.0 – 38.0)	38.0 (31.6 – 53.0) (n = 10)
1/2EMTD (12.5 mg/kg)	17/31 (54.84 %)	49.0* (43.0 – 55.5)	48.9* (46.0 – 56.2) (n = 10)
EMTD (25 mg/kg)	19/31 (61.29 %)	43.0* (32.0 – 52.0)	41.2 (34.6 – 43.9) (n = 10)
2EMTD (50 mg/kg)	22/31 (70.97 %)	34.5 (27.3 – 41.3)	39.6 (30.2 – 45.9) (n = 10)
Reamberin			
Control	19/34 (55.88 %)	29.0 (23.0 – 38.0)	42.6 (35.4 – 47.9) (n = 10)
1/2EMTD (25 mg/kg)	18/31 (58.06 %)	50.5* (35.8 – 54.5)	51.4 (45.6 – 53.2) (n = 10)
EMTD (50 mg/kg)	13/31 (41.94 %)	38.0* (33.0 – 47.0)	45.3 (39.9 – 49.1) (n = 10)
2EMTD (100 mg/kg)	11/31 (35.48 %)	49.0* (45.0 – 56.0)	46.8 (44.6 – 52.7) (n = 10)
Mexidol			
Control	30/51 (58.82 %)	7.5 (3.8 – 25.0)	38.0 (31.6 – 53.0) (n = 10)
1/2EMTD (25 mg/kg)	17/26 (65.38 %)	46.0* (8.0 – 55.0)	52.2* (44.0 – 56.3) (n = 10)
EMTD (50 mg/kg)	21/32 (65.63 %)	9.0 (3.5 – 40.0)	40.2 (30.9 – 48.6) (n = 10)
2 EMTD (100 mg/kg)	19/26 (73.08 %)	15.0 (5.0 – 53.0)	43.5 (32.1 – 48.6) (n = 10)
α-Lipoic acid			
Control	30/51 (58.82 %)	7.5 (3.8 – 25.0)	42.6 (35.4 – 47.9) (n = 10)
1/2EMTD (50 mg/kg)	18/26 (69.23 %)	31.0 (4.8 – 50.3)	47.9 (35.5 – 53.8) (n = 10)
EMTD (100 mg/kg)	19/32 (59.38 %)	6.0 (3.0 – 46.0)	30.3* (22.8 – 36.1) (n = 10)
2 EMTD (200 mg/kg)	14/26 (53.85 %)	35.5* (5.8 – 51.5)	36.9 (32.2 – 49.8) (n = 10)

Notes. Absolute values for lethality are shown as fractions, where the numerator is the number of mice dying within 60 min of s.c. administration of adrenaline hydrochloride (14 mg/kg) and the denominator is the total number of animals in the group. Lethality levels were no different from those in controls ($p > 0.05$, Fisher's exact test, in all cases); the lifetime of dying mice (in the first 1 h after adrenaline administration) and the severity of formalin edema are shown as medians and interquartile ranges – [Me (LQ-UQ)]; group sizes in terms of measures of the lifetime of dying mice with acute adrenaline intoxication correspond to the numerator in the lethality column; group size in the experiment with formalin

The protective activity of 3-hydroxypyridine derivatives (emoxypine and Mexidol) had a significantly different dose-response relationship from that of Reamberin (Table 1). The protective action of emoxypine decreased progressively with increases in dose and reached statistical significance only when relatively low doses were used (1/2EMTD and EMTD). A similar pattern was seen on administration of Mexidol, which had a significant protective effect only at the lowest dose. The decrease in the protective activity of emoxypine and Mexidol with increases in dose (Table 1) is consistent with the previously published observation that decreases in antioxidant activity in vitro are accompanied by increases in the concentrations of these 3-hydroxypyridine derivatives [15]. It remains possible that the known "concentration inversion of the antioxidant effect" [16] is directly related to the decrease in the protective effect of emoxypine and Mexidol when used at relatively high doses. It should be emphasized that the fatal cardiotoxic effects of adrenaline are largely linked with its prooxidant action [13]. Attention should be drawn to the fact that Mexidol, which is a derivative of both 3-hydroxypyridine and succinic acid, was less active than the compound which is a 3-hydroxypyridine derivative only (emoxypine) in terms of the dose distribution of the protective action (Table 1). This may be associated with the indirect prooxidant action of the succinate anion in the structure of Mexidol, as the end product of the mitochondrial oxidation of succinic acid is H_2O_2, which has a well-known role in inducing free-radical oxidation [16]. We would add that the presence of succinic acid in Mexidol significantly decreases both the antioxidant potential of this agent and the positive inotropic activity of its 3-hydroxypyridine component in diabetes mellitus [1, 15]. It is also impossible to exclude the possibility that the indirect prooxidant action of succinic acid in Reamberin to some extent restricts its protective action in adrenaline intoxication and is the cause of the nonlinear dose relationship of this effect.

The reference agent (α−LA), in contrast to emoxypine, Reamberin, and Mexidol, prolonged the lifetimes of mice dying after administration of adrenaline only at the maximum dose (Table 1). This is evidently due to the fact the this this dose of α−LA had the most marked antihypoxic activity [8]. It remains possible that the antihypoxic activities of emoxypine and Mexidol, which were most marked at the lower dose, also make some contribution to the protective actions of these 3-hydroxypyridine derivatives in acute adrenaline intoxication. This suggestion does not apply to

Reamberin, which had no effect on resistance to acute hypoxic hypoxia [8].

The results of experiments from series II identified a significant anti-inflammatory action only with the reference agent (α–LA), which decreased formalin edema at the intermediate dose (EMTD) (Table 1). Reamberin had on effect on resistance to the phlogogenic effect of formalin, while 3-hydroxypyridine derivatives (emoxypine and Mexidol) significantly increased the severity of formalin edema when used at minimal doses. This phenomenon may be due to the antiaggregant action of 3-hydroxypyridine derivatives [17], such that emoxypine and Mexidol prevent thrombosis in the microcirculatory bed, increasing hyperemia and concomitant edema in the zone of formalin inflammation. It is also possible that 3-hydroxypyridine derivatives enhance the edematogenic action of formalin by potentiating GABAergic vasodilation mechanisms in the tissue damage zone. This possibility is illustrated by the increase in postischemic cerebral hyperemia on the background of pre-ischemia administration of Mexidol, whose vasodilatory action is blocked by bicuculline [18]. The clear dose relationships of the protective actions of 3-hydroxypyridine derivatives in adrenaline intoxication and their potentiating effects on formalin edema (Table 1) should particularly be emphasized. standardization of these data in terms of the medians for the control group followed by correlation analysis established the universal nature of this pattern for all the drugs studied. As shown (Fig. 1), the protective effects of all the study agents in adrenaline intoxication increased in direct relation to their abilities to increase formalin edema.

The protective actions of the study agents in acute adrenaline intoxication, like their potentiating influence on formalin inflammation, shows a significant dependence on the extent of the vasodilatory action in tissue damage zones.

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