10. H. G. E. Lloyd, I. Spense, and G. A. R. Johnston, *Brain Res.*, **462**, 391-395 (1988).
11. M. T. Piasik, P. L. Wisler, C. L. Jonson, and J. D. Potter, *J. Biol. Chem.*, **255**, 4176-4181 (1980).
12. J. P. Robinson and D. A. Kendall, *J. Neurochem.*, **52**, 690-698 (1989).
13. Y. Salomon, in: *Advances in Cyclic Nucleotide Research*. G. Brocker *et al.* (Eds.), Vol. 10, New York (1979), pp. 35-55.
14. H. Shuntoh, K. Taniyama, H. Fukuzaki, and C. Tanaka, *J. Neurochem.*, **51**, 1565-1572 (1988).
15. E. Susunni, W. T. Manders, D. R. Knight, *et al.*, *Circ. Res.*, **65**, 1145-1150 (1989).

Effect of KLN-93 on Ventricular Fibrillation Induced by Reperfusion and Electrical Stimulation in Cats

N. B. Leonidov, P. A. Galenko-Yaroshevskii,*
S. B. Fitilev, V. A. Knyazhev, and V. V. Gatsura

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 8, pp. 135-137, August, 1997
Original article submitted September 28, 1996

In experiments devoted to modeling of reperfusion ventricular fibrillation and determination of the electric threshold of fibrillation, a protective effect of KLN-93 (a para-aminobenzoic acid ester derivative) is compared with that of lidocaine. It is shown that KLN-93 in doses stopping reperfusion fibrillation is 2-4-fold less toxic than the isoeffective doses of lidocaine. In a dose of 0,4 mg/kg (2.5% LD_{50}) KLN-93 increases the fibrillation threshold 4.5-fold, while isotoxic dose of lidocaine increases this parameters approximately 2-fold. These data suggest that KLN-93 is an effective antifibrillatory agent.

Key Words: *antifibrillatory agents; lidocaine; crystallographycally homogeneous substances; conformers of local anesthetics*

Previous studies showed that crystallographycally homogeneous substance of the para-aminobenzoic acid ester derivative KLN-93 elicits a more potent antiarrhythmic effect in coronary arrhythmias in dogs and cats than isoeffective doses of lidocaine [4].

Similar results were obtained in aconitine-induced arrhythmia in rats and barium chloride-induced arrhythmia in rabbits [4]. Taking into account the lower toxicity of isoeffective doses of KLN-93 in comparison with lidocaine and its prolonged (to 40 min) effect after intravenous administration in arrhythmias reproduced as described previously [3], it seems very important to test KLN-93 as a potential antifibrillatory agent.

Bioeffect State Research Institute, Ministry of Science and Technological Strategy of the Russian Federation, Moscow; *Kuban Medical Academy, Krasnodar

MATERIALS AND METHODS

Acute experiments were carried out on 93 narcotized cats. Quantitative parameters of different experimental series are listed in Tables 1 and 2.

Early occlusion and reperfusion arrhythmias in narcotized cats were induced by intraperitoneal injection of 40 mg/kg Nembutal. Temporary occlusion of the anterior interventricular branch of the left coronary artery was made at the level of the lower edge of the auricle. The ligature was removed after 30 min, which induced reperfusion arrhythmia usually transformed into ventricular fibrillation (VF). KLN-93 (0.125% solution) and a reference agent lidocaine (1% solution, Egis, Hungary) were injected intravenously slowly in isotoxic doses constituting 1, 2.5, 5, 10, and 15% of LD_{50} (for rats upon intravenous administration) 5-7 min before coronary occlusion.

0007-4888/97/0008-0738$18.00

TABLE 1. Comparative Effectiveness of KLN-93 and Lidocaine in Reperfusion Ventricular Fibrillation in Cats

Agent	Dose, mg/kg (% LD_{50})	n	Early postocclusion arrhythmias (number of animals with arrhythmia)	Reperfusion arrhythmias (number of animals)	
				with arrhythmia	with VF
Control		15	9	15	10
KLN-93	0.08 (1)	6	5	5	4
	0.2 (2.5)	6	0*	1*	0*
	0.4 (5)	7	0*	0*	0*
Lidocaine	0.7 (2.5)	6	3	5	3
	1.4 (5)	11	7	6	5
	2.8 (10)	6	0*	2	0*
	4.2 (15)	7	0*	0*	0*

Note. Here and in Table 2: *$p<0.05$ in comparison with the control.

Effects of test drugs on the threshold of electrical VF were studied in experiments on cats narcotized with Nembutal (40 mg/kg intravenously) as described elsewhere [2]. The animals were transferred to jet ventilation and, after left thoraco- and pericardiotomy, silver bipolar electrodes were fixed on the right ventricle. The threshold of electrical VF was determined 30 min after fixation of the electrodes by scanning the electrically unstable period of the cardiac cycle by a series of 20 square pulses of increasing intensity (4 msec pulse duration, 50 pulses/sec frequency) until VF developed. The VF threshold was evaluated by the minimal current intensity (in mA) inducing VF. A 215/11 HSE electrical stimulator was used in the experiments. Blood pressure was recorded using electromanometers (Elema-Siemens) and ECG in II standard lead was recorded on an Attack-350 analyzer (Nihon Kohden). Defibrillation was made by capacitor discharge using a DI-3 apparatus. KLN-93 (0.4 and 0.8 mg/kg, 0.125% solution) and reference drugs lidocaine (1.4 mg/kg, 1% solution) and bonnecor (1 mg.kg, 0.125% solution) were injected intravenously slowly at a constant rate using a Seringer pump 355 (Sage Instruments). The data were processed statistically using the Student's t and χ^2 tests [1].

RESULTS

As follows from Table 1, acute reperfusion arrhythmias was observed in all control animals and transformed into VF in 67% cats.

KLN-93 in a dose of 0.08 mg/kg (1% LD_{50}) had no effect on the occurrence of VF. After increasing the dose to 0.2 and 0.4 mg/kg no cases of VF were observed.

Lidocaine in isotoxic doses of 0.7 and 1.4 mg/kg (2.5 and 5% LD_{50}) exhibited no antifibrillatory effect and only after increasing the dose to 2.8 and 4.2 mg/kg (10 and 15% LD_{50}) it prevented the development of VF in 83 and 100% cats, respectively.

These findings suggest that KLN-93 exerted a more pronounced protective effect in modeled reperfusion VF than isotoxic doses of lidocaine.

In cats with intact myocardium, KLN-93 in a dose of 0.4 mg/kg increased the threshold of electrical VF more than 4.5 fold, the effect persisting for more than 1 h (Table 2).

When KLN-93 was used in a dose of 0.8 mg/kg, the threshold of VF increased by more than 7.5-fold and the effect persisted for more than 1 h.

It was shown that KLN-93 is superior to the reference drug lidocaine (1.4 mg/kg) in intensity and duration of its effect; it induces a more pronounced and significant elevation of VF threshold in cats with intact myocardium (Table 2).

It should be noted that in experiments with proximal ligature of the anterior interventricular branch of the left coronary artery, KLN-93 (1.43 mg/kg, 17.84% LD_{50}), similar to lidocaine (5 mg/kg,

TABLE 2. Effect of KLN-93 and Lidocaine on Electrical Threshold of VF in Cat Heart (mA)

Time of observation, min	KLN-93		Lidocaine, 1.4 mg/kg (n=5)
	0.4 mg/kg (n=6)	0.8 mg/kg (n=5)	
Control	2.76±0.69	2.52±0.58	2.26±0.47
5	12.98±2.89*	19.5±0.48*	5.0±1.21
10		18.0±0.67*	4.89±1.05
30		11.1±2.04*	2.84±0.75
60		8.0±1.61*	2.24±0.49
90		3.0±0.75	

17.84% LD_{50}), completely prevented the development of VF [4].

The antifibrillatory effect of KLN-93 correlates with its protective effect under conditions of arrhythmia induced by aconitine or barium chloride. However, KLN-93 in a wide range of concentrations (5-25% LD_{50}) exerted practically no protective effect against strophanthin-, adrenaline-, and calcium chloride-induced arrhythmias. In strophanthin-induced arrhythmias this drug exhibited even a proarrhythmic activity, which attested to a considerable specificity of the mechanism of its antiarrhythmic effect.

This assumption is confirmed by the facts that KLN-93 (3×10^{-5}-3×10^{-6} M) exerts a more pronounced inhibitory effect on automatism of pacemaker cells in the atrioventricular funnel of the frog heart, possesses a higher affinity for sodium channels, and is characterized by a 12-fold lower toxicity of isoeffective concentrations, judging from prolongation of the refractory period of guinea pig myocardium.

These data on a pronounced antifibrillatory effect of KLN-93 in arrhythmias induced by reperfusion and electric stimulation in combination with lower toxicity of its isoeffective doses in comparison with lidocaine (2-4-fold) suggest that this agent is a promising candidate for developing a new drug for the treatment of VF.

REFERENCES

1. M. L. Belen'kii, *Quantitative Evaluation of a Pharmacological Effect* [in Russian], Leningrad (1963).
2. S. Yu. Berdyaev, "Search and pharmacological study of drugs improving electrical stability of the heart," Author's Synopsis of Doct. Med. Sci. Dissertation [in Russian], Moscow (1991).
3. A. S. Harris, *Circulation*, **1**, No. 6, 1318 (1950).
4. N. B. Leonidov, V. V. Gatsura, P. A. Galenko-Yaroshevskii, *et al.*, *J. Mol. Cell. Cardiol.*, **28**, No. 5, A57 (1996).