7. A. L. Zabludovskii and V. V. Zhulin, Byull. Eksp. Biol. Med., 99, No. 5, 545 (1985).
8. M. Ya. Maizelis, A. L. Zabludovskii, and S. N. Shikhov, Neirokhimiya, 2, No. 3, 228 (1983).
9. M. Ya. Maizelis, A. L. Zabludovskii, and S. N. Shikhov, Zh. Nevropatol. Psikhiat., 83, No. 3, 394 (1983).
10. E. L. Abel, Pharmacol. Biochem. Behav., 10, No. 2, 239 (1979).
11. N. W. Bond, Psychol. Rep., 41, No. 3, 1267 (1977).
12. A. K. Rawat, Res. Commun. Chem. Pathol. Pharmacol., 12, No. 4, 723 (1975).

EFFECT OF ANTHRACYCLINE ANTIBIOTICS ON IONIC CURRENTS AND ON Na^+/Ca^{++}-EXCHANGE-RELATED CONTRACTION OF FIBERS OF THE FROG ATRIUM

T. I. Bukei, A. K. Filippov,
L. A. Vasilets, and V. I. Porotikov

UDC 615.33.017:615.277.3].065:[616.125-008.3+616.12-008.923.3]-092.9].07

KEY WORDS: anthracycline antibiotics; Na^+/Ca^{++}-exchange; ionic currents; cardiotoxicity; myocardium of cold-blooded animals.

The use of anthracycline antibiotics as antitumor agents has been delayed by their cardiotoxicity and, in particular, by the development of cardiomyopathy, heart failure, and fatal arrhythmias [1, 8, 15]. The toxicity of drugs of this class is linked with the formation of semiquinone radicals of anthracyclines and activation of a chain of intracellular free-radical reactions, leading to oxidative damage of the cell structures [10, 14]. Previous investigations, aimed at studying the effects of the cumulative cardiotoxicity of anthracycline derivatives, showed that their action leads to inhibition of contractivity of isolated papillary muscles, to a change in the parameters of action potentials (AP), and to a steady decline of Na-conductance, confirming that anthracyclines act on the cell systems involved in coupling of electrical and mechanical activity [1, 3].

In the investigation described below, a method of simultaneous recording of AP, ionic currents, and contractions [6] was used to study the effect of rubomycin and its less toxic nitroxyl analog, emoxyl (ruboxyl) [7] on the pathways of entry of Ca^{++} ions into the heart cells and the possible role of these changes in the mechanism of the cardiotoxic action of anthracyclines.

EXPERIMENTAL METHOD

Experiments were carried out on atrial trabeculae of *Rana ridibunda*. The heart fibers used in the experiments had a diameter of 0.1-0.15 mm and a length of 3.5-5 mm. The fibers were perfused with standard Ringer's solution (in mM): NaCl — 110, KCl — 2.5, $CaCl_2$ — 1.8, $MgCl_2$ — 1.0, $NaHCO_3$ — 2.4, glucose — 5.5 (pH 7.4-7.5). Rubomycin* was obtained from "Khimfarmreaktiv" and emoxyl was synthesized at the Institute of Chemical Physics, Academy of Sciences of the USSR; both were used in a concentration of 100 mg/liter.

Ionic currents were investigated under voltage clamp conditions by the double sucrose gap method, using a four-electrode circuit for current clamping [11, 12]. Contraction of the trabeculae were measured at the same time, using an optical recording method [6] (the amplitude of contractions was normalized relative to the maximum). Ionic currents were measured by the use of standard circuits. The Ca current (I_{si}) was measured by testing voltage steps 70 msec in duration and 40-130 mV in amplitude immediately after preliminary membrane depolarization by 35-40 mV for 150 msec, causing inactivation of the fast Na-current. The Ca-current was

*Alternative name daunorubicin — translator.

Research Institute of Therapeutic Substances, Kupavna. Chernogolovka Branch, Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow Oblast. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 10, pp. 456-458, October, 1988. Original article submitted August 21, 1987.

 0007-4888/88/0010-1450$12.50

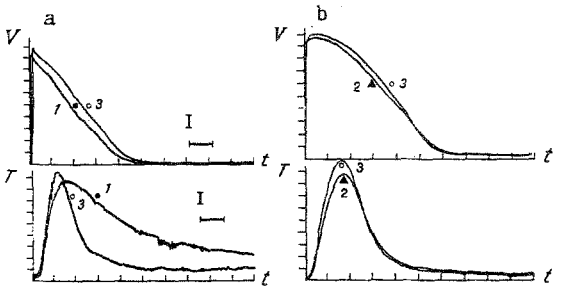

Fig. 1. Effect of rubomycin (a) and emoxyl (b) on AP (V) and contraction of fibers (T) of frog atrium. 1) Rubomycin, 2) emoxyl (concentration of antibiotics 100 mg/liter, for 7 min), 3) control. Calibration: top traces 10 mV, 50 msec; bottom traces 0.1 relative unit, 200 msec.

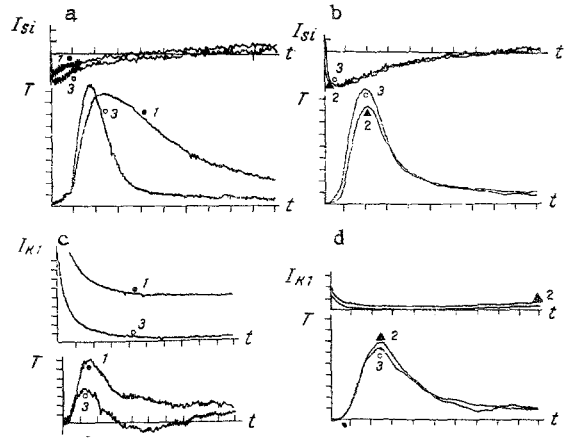

Fig. 2. Action of rubomycin (a, c) and emoxyl (b, d) on ionic currents and on phasic and tonic components of contraction. 1) Rubomycin, 2) emoxyl (100 mg/liter, 7 min), 3) control. a, b) Inward Ca-currents (I_{si}, top traces) and phasic contractions (bottom traces). For I_{si}: abscissa, time (each division is 7 msec); ordinate, current (each division is 30 nA in a) and 20 nA in b)); c, d) outward background currents (I_{K1}, top traces) and tonic contractions (bottom traces). For I_{K1}: abscissa, time (each division is 30 msec); ordinate, current (each division 0.1 μA). For phasic and tonic contractions: abscissa, time (each division 200 msec); ordinate, amplitude (each division 0.1 relative unit).

measured from the peak to the value established at the end of the testing pulse. To investigate tonic contraction the optical signal arising in response to a square pulse of voltage with an amplitude of +140 mV and a duration of 300 msec was measured. At such voltages the Ca-current (I_{si}) was close to zero and contraction was activated by Ca entering the cell by a mechanism of Na^+/Ca^{++}-exchange. The experiment was conducted and the data processed by an automated system using the SM-3 computer, coupled to the experimental apparatus through a KAMAK module [5].

EXPERIMENTAL RESULTS

Data on the effect of rubomycin and emoxyl on AP and on associated contraction of trabeculae of the frog atrium are given in Fig. 1. The duration of AP usually varied from one preparation to another, due to differences in the initial values of the Ca- and K-currents. Rubomycin (Fig. 1a) reduced the duration of AP at levels of both 50% (by 29.3 ± 7.7%) and 10% of the amplitude of AP (by 13.7 ± 3.2%). Rinsing in normal Ringer's solution was ineffective. Similar results were obtained in another two experiments. Unlike rubomycin, emoxyl had no

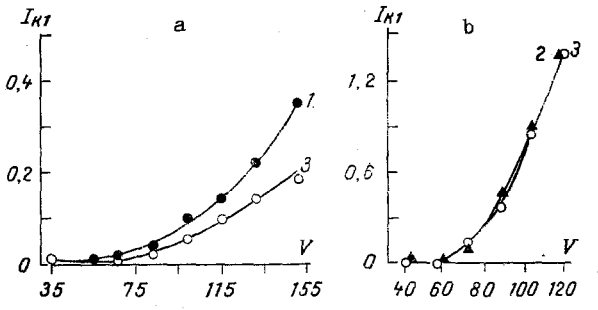

Fig. 3. Effect of rubomycin (1) and emoxyl (2) on current-voltage characteristic curves of outward background current (I_{K1}). Concentration of antibiotics 100 mg/liter (7 min). Empty circles (3) indicate control. Reference potential = resting potential. Abscissa, voltage shift on membrane V (in mV); ordinate, outward backward current I_{K1} (in μA).

significant (within 5% limits) effect on AP or on the duration of relaxation of the fibers, in all seven experiments (Fig. 1b).

Experiments with voltage clamping showed that neither substance had any appreciable effect over the whole period of investigation (7-10 min) on the inward Ca^{++}-ion current (I_{si}) entering the cell through the Ca-channels of the membrane (Fig. 2a, b). Rubomycin, however, doubled the relaxation time of the fibers, unlike emboxyl, which had comparatively little effect on the characteristics of contraction (Fig. 2a, b). Moreover rubomycin almost doubled the amplitude of tonic contraction (Fig. 2c) which, as was shown previously [6, 11, 12], is associated with a change in Ca^{++}-ion transport by a mechanism of Na^+/Ca^{++} exchange. An increase in tonic contraction in the absence of any positive inotropic effect of rubomycin and slowing of relaxation suggests that under the influence of this compound the outflow of Ca^{++} from the myoplasm by a mechanism of Na^+/Ca^{++} exchange is delayed. Emoxyl virtually had no effect on tonic contraction (Fig. 2d). Meanwhile differences were observed in the action of the antibiotics on the outward background current (I_{K1}), which included changes in the outward K-current and a leakage component. Rubomycin irreversibly increased I_{K1}, whereas emoxyl did not change it (see Fig. 2c, d, top traces; Fig. 3).

The results reveal significant differences in the action of rubomycin and its nitroxyl derivative emoxyl on parameters of excitation and contraction of frog heart fibers. The action of rubomycin can be characterized as a whole as toxic. Its principal manifestation is an increase in the duration of relaxation and in the amplitude of tonic contraction of the fibers. This effect is evidence that rubomycin significantly reduces the rate of evacuation of Ca^{++} ions from the cytoplasm of poikilothermal animal cells. The sarcoplasmic reticulum in the frog heart fibers is feebly developed compared with that in homoiothermal animals and relaxation of the muscle takes place mainly by a mechanism of Na^+/Ca^{++}-exchange [4]. Thus slowing of relaxation under the influence of rubomycin reflects inhibition of the Ca^{++} outflow through Na^+/Ca^{++} exchange. An increase in the Ca^{++} concentration in the cytoplasm in turn can induce overloading of the cell with these ions, induce activation of phospholipases and intensification of lipid peroxidation, and can lead to irreversible changes in the cell membranes. In fact, an increase in the background outward current under the influence of rubomycin can be interpreted as an increase of leakage through the membrane, resulting in partial membrane depolarization.

Unlike rubomycin, its nitroxyl analog emoxyl, as the results of the present experiment show, has a much weaker toxic action on the poikilothermal myocardium, for its effect on the electrophysiological characteristics and contraction of the frog atrial fibers is much weaker than that of rubomycin. This fact is in agreement with data obtained previously relating to the action of these antibiotics on the homoiothermal myocardium when administered over a long period in vivo [1, 3]. Since the rat myocardium, on which the investigation cited was conducted, has a well developed sarcoplasmic reticulum and since Na^+/Ca^{++}-exchange in these animals does not make a significant contribution to the outflow of Ca^{++} ions, the delayed phase of relaxation on the development of toxic effects of rubomycin was not observed in these experiments. In other experiments on the guinea pig myocardium, which has a comparatively less highly developed system of sarcoplasmic reticulum, the direct action of rubomycin, just as in

the frog, caused slowing of relaxation and the appearance of frequency-dependent contracture [2]. This is evidence that inhibition of Na^+/Ca^{++} exchange, found in isolated vesicles of the sarcolemma under the influence of the cardiotoxic antibiotic dexorubicin, which closely resembles rubomycin in its structure [9], is a universal mechanism of the cardiotoxicity of the anthracyclines, common to the myocardium of both poikilothermal and homoiothermal animals.

LITERATURE CITED

1. L. A. Vasilets, T. I. Guseva, and V. P. Mokh, Farmakol. Toksikol., No. 6, 125 (1985).
2. L. A. Vasilets and T. I. Guseva, Proceedings of the 2nd All-Union Conference on Comparative Cardiology [in Russian], Syktyvkar (1985), p. 37.
3. L. A. Vasilets and L. Kh. Ganieva, Byull. Éksp. Biol. Med., No. 8, 61 (1987).
4. V. V. Nesterenko and L. V. Rozenshtraukh, Byull. Vses. Kardiol. Nauch. Tsent., No. 1, 99 (1984).
5. V. I. Porotikov, V. G. Litvinov, A. K. Filippov, et al., Automation of Biological Research [in Russian], Pushchino (1982), pp. 55-67.
6. A. K. Filippov, R. V. Plotnikov, and V. I. Porotikov, Biofizika, 29, No. 5, 886 (1984).
7. N. M. Émanuél', N. P. Konovalova, R. F. D'yachkovskaya, et al., Current Problems in Experimental Chemotherapy of Tumors [in Russian], Sverdlovsk (1982), pp. 126-128.
8. E. Bachman, E. Weber, and G. Zbinden, Agents Actions, 5, 385 (1975).
9. P. Caroni, F. Villani, and E. Carafoli, FEBS Lett., 30, 180 (1981).
10. J. H. Doroshov, Cancer Res., 43, 4543 (1983).
11. A. K. Filippov and V. I. Porotikov, Gen. Physiol. Biophys., 2, 95 (1983).
12. M. Horackova, Canad. J. Physiol. Pharmacol., 62, 874 (1984).
13. C. E. Myers, W. P. McGuire, R. H. Liss, et al., Science, 197, 165 (1977).
14. J. W. Peters, G. R. Gordon, D. Kashiwase, et al., Biochem. Pharmacol., 35, 1309 (1986).
15. G. Zbinden and E. Brandle, Cancer Chem. Rep., 59, 707 (1975).

MODIFICATION OF THE CYTOGENETIC EFFECT OF FOTRIN ON INDUCTION OF A METABOLIC SYSTEM BY PHENOBARBITAL

V. S. Zhurkov, L. L. Sycheva, and N. P. Burmantova

UDC 615.277.4.015.4:/615.916.4.24:547.845.4/.015.4

KEY WORDS: fotrin; phenobarbital; chromosomal aberrations; microsomal mono-oxygenase system.

Evidence has now been obtained to show that the level of mutagenic effects depends on the state of systems of the body responsible for the biotransformation of chemical compounds. The writers' previous investigations showed that even against the background of weak (1.5-fold) induction of the microsomal mono-oxygenase system (MMS), activating many indirect mutagens, the effect of cyclophosphamide is potentiated; the modification, moreover, is observed also in the case of long-term induction [2, 5]. Such levels of induction of MMS, incidentally, are observed under real external environmental conditions in the case of pollution by discharges from factories, automobiles, pesticide application, and so on.

The aim of this investigation was to study whether the effect of the direct-action mutagen fotrin can be modified against a background of different levels of long-term MMS induction.

EXPERIMENTAL METHOD

Experiments were carried out on 157 noninbred male rats weighing 200-250 g. The direct-action mutagen fotrin, containing 5 ethylenimine groups in its structural formula, was used.

Laboratory for Hygienic Evaluation of Late Effects of Exposure to Environmental Factors and Laboratory of Biochemistry, A. N. Sysin Research Institute of General and Communal Hygiene, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR G. I. Sidorenko.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 10, pp. 458-460, October, 1988. Original article submitted July 13, 1987.