Toxic Interactions between Disopyramide and Propranolol in Chick Embryos

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The toxic interaction between disopyramide and propranolol were studied in chick embryos. Fertilized eggs of White Leghorns were incubated and investigated. Disopyramide with and without propranolol was injected into the air sac of a fertilized egg on the 16th day of incubation. Electrocardiograms (ECGs) were recorded 0 to 60 min after the injection. After each drug injection alone, the heart rate was not different compared with control. However, the heart rate was significantly decreased by combination with disopyramide and propranolol. In addition, arrhythmia was produced by disopyramide 1.0 mg/egg alone and in combination with propranolol. These findings indicate that the interaction between disopyramide and propranolol has a marked influence on the heart rate in chick embryos.

Key words toxic interaction; disopyramide; propranolol; chick embryo; electrocardiogram

Recently, many antiarrhythmic drugs have been put on the market in Japan and classified into subcategories. Such classification of antiarrhythmic mechanisms should prove of value in predicting the therapeutic as well as toxic effects of different agents, and may be particularly important in planning combination therapy for resistant arrhythmias.

Drug–drug interactions have been demonstrated for a variety of drugs, including disopyramide and propranolol, in the heart failure patients.1,2 With the recent concern for animal rights, experimental studies using mammals have been limited in number and methods. Thus, based on social acceptance, experimental studies using chick embryos have drawn attention. Chick embryos are used extensively in biological studies.3–5 We have used chick embryos as an experimental model to evaluate the chronopharmacology and chronopharmacokinetics of drugs.6–7 Chick embryonic heart develops through a similar process as in mice, rats and humans and also has a similar atrioventricular system.8 In order to develop alternative methods, we have studied the biological effects of drugs on the cardiovascular system of chick embryos using physiological techniques.9–11

The present study evaluated the toxic interactions between disopyramide and propranolol in chick embryos.

MATERIALS AND METHODS

Fertilized eggs of White Leghorns (Omiya Poultry Laboratory, Saitama, Japan) were incubated at 37.5 ± 0.2°C at a relative humidity of about 65%, turned automatically every hour, and candled daily for viability.

Disopyramide preparation (Chugai Pharmaceutical, Tokyo, Japan) and propranolol preparation (Sumitomo Pharmaceutical, Tokyo, Japan) were used for the treatment. Disopyramide at 0.3 and 1.0 mg/egg and propranolol at 0.1 mg/egg were injected into the air sac of each fertilized egg on the 16th day of incubation.

After each drug injection alone or in combination, the values of heart rate were measured.

Electrocardiograms (ECGs) were recorded 0 to 60 min after the drug injection, and heart rate was determined from R–R intervals. Changes in heart rate were expressed as mean-percent changes of the drug-treated groups to the matched control.

Four small holes every 90 degrees on “the equator” and one small hole on “the south pole” were made on each fertilized egg by an electric drill and sealed with paraffin (m.p. 60°C). Specially designed electro-needles were inserted into the appropriate holes of “the equator” and “the south pole”. The two needles on “the equator” were used as a bipolar lead of the embryonic heart, and the needle on “the south pole” was used as a ground lead. These needles were connected to the electrocardiograph system (Nihon Koden AVB-21, Tokyo, Japan). ECGs were recorded as bipolar waves between two needles on a thermal array recorder (Nihon Koden PTA-1100M, Tokyo, Japan) with a paper speed of 25 mm/s.

The data were analyzed by one way analysis of variance. If there was a significant difference among the groups, a multiple comparison test was conducted (Tukey’s test). The fiducial limit of 0.05, two-tails, was used as the criterion for significance.

RESULTS

The body weight of chick embryos gradually increased with the day of incubation. After each drug injection alone, the values of heart rate were not different compared with the control (Fig. 1). However, the heart rate was decreased dose- and time-dependently at 0.3 and 1 mg/egg disopyramide in the presence of propranolol (Fig. 2). In addition, an arrhythmia was produced by disopyramide 1.0 mg/egg alone and in combination with propranolol.

DISCUSSION

It is surprising that propranolol, which has long been noted for its negative inotropic myocardial action, has been reported to cause little heart failure in patients.23 After each drug injection alone, the heart rate of the chick embryos was not different compared with the control.

Toxic interactions between disopyramide and other
Fig. 1. Changes in Heart Rate of Chick Embryo after Administration of Propranolol Alone or Disopyramide Alone

Propranolol 0.1 mg/egg alone (○), disopyramide 0.3 mg/egg alone (▲) or disopyramide 1.0 mg/egg alone (□) was injected into the air sac of fertile eggs on the 16th day of incubation. Changes in heart rate were presented as mean percent changes of drug-treated groups over the time-matched control.

The heart rates of chick embryos before each drug injection; propranolol 0.1 mg/egg alone: 230 ± 9 beats/min, disopyramide 0.3 mg/egg alone: 246 ± 15 beats/min, disopyramide 1.0 mg/egg alone: 248 ± 12 beats/min.

Fig. 2. Changes in Heart Rate of Chick Embryo after Administration of Propranolol in Combination with Disopyramide

Propranolol 0.1 mg/egg alone (○), propranolol 0.1 mg/egg plus disopyramide 0.3 mg/egg (▲) or propranolol 0.1 mg/egg plus disopyramide 1.0 mg/egg (■) was injected into the air sac of fertile eggs on the 16th day of incubation. Changes in heart rate were presented as mean percent changes of drug-treated groups over the time-matched control. * Significantly different from propranolol 0.1 mg/egg alone group, p < 0.05. # Significantly different from propranolol 0.1 mg/egg plus disopyramide 0.3 mg/egg group, p < 0.05.

The heart rates of chick embryos before each drug injection; propranolol 0.1 mg/egg alone: 230 ± 9 beats/min, propranolol 0.1 mg/egg plus disopyramide 0.3 mg/egg: 244 ± 15 beats/min, propranolol 0.1 mg/egg plus disopyramide 1.0 mg/egg: 237 ± 5 beats/min.
antiarrhythmic agents may result in potentially serious adverse reactions, particularly in patients with intraventricular conduction disturbances.\(^1\)

Toxic interactions between disopyramide and propranolol were demonstrated in chick embryos. The combination of propranolol modified the pharmacological effects of the disopyramide in chick embryos and led to a Q–T\(_c\) interval prolongation of the ECGs.

After the drug was injected into the air sac of each fertilized egg, it accumulated in the egg shell. Therefore, the heart rate may be decreased time-dependently. This time-dependent effect of the drug on the heart rate should be investigated further.

We have demonstrated in this report that our recording system for an electron cardiogram system using chick embryo may be applied as an animal test alternative.

Accordingly, this toxic interaction could be clearly analyzed in chick embryos, as in mammals, especially the decrease in heart rate, namely, prolongation of the R–R interval, accompanied by prolongation of the Q–T\(_c\) interval in the chick embryos, as was observed in humans. The clinical use of disopyramide in conjunction with other antiarrhythmic agents, such as propranolol, led to Q–T\(_c\) interval prolongation of the ECGs.\(^1\) This indicates that developing chick embryos are appropriate as an alternative experimental animal rather than traditional mammals.

Nayler has shown that propranolol inhibits the lipid-facilitated transport of calcium from an aqueous to a lipid-solvent phase. Such an interaction may inhibit or impede the transport of calcium from the sarcoplasmic reticulum through lipid membranes and cause a reduced concentration of myoplasmic calcium that is inadequate for the proper initiation of contraction and thereby results in myocardial depression.\(^2\)

Although the exact mechanism underlying the influence of the interaction on the pharmacological effects of the drug remains to be clarified, the interaction seems to enhance the toxicity of the drug in chick embryos.

In conclusion, our in ovo recording system for ECG of chick embryos may be useful for investigating the toxic interactions of cardiovascular drugs.

REFERENCES