ORIGINAL ARTICLE

Toxic Interactions between Miconazole and Disopyramide in Chick Embryos

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Abstract
The toxic interactions between miconazole and disopyramide were studied in chick embryos. Chick embryos have been widely used in pharmacologic and toxicologic experiments for evaluating drug action. Fertilized eggs of White Leghorns were incubated and investigated. Miconazole 1 mg/egg, 5 mg/egg, 10 mg/egg alone or disopyramide 0.3 mg/egg alone was injected into the air sac of each fertilized egg. Miconazole 1 mg/egg with disopyramide 0.3 mg/egg was injected into the air sac of each fertilized egg. Electrocardiograms (ECGs) were recorded after the drug injection, and heart rate was determined from ECG wave cycles. Changes in heart rate were expressed as mean-percent changes of the drug-treated groups to the matched control. After the administration of miconazole 1 mg/egg alone, the heart rate did not differ compared with that of the controls. However, the heart rate was significantly decreased with the administration of miconazole 5 mg/egg and 10 mg/egg. The heart rate was also significantly decreased by the administration of miconazole 1 mg/egg together with disopyramide 0.3 mg/egg. In addition, an arrhythmia was produced by miconazole and disopyramide. These findings indicate that the interaction between miconazole and disopyramide has a marked influence on the heart rate in chick embryos.

Key words: toxic interaction; miconazole; disopyramide; chick embryo; electrocardiogram

Introduction
Miconazole is an effective antifungal agent when used topically or systemically and has successfully eradicated various fungal infections (Benson and Nahata, 1988).

The mode of action by which miconazole exhibits fungicidal activity in vitro against Candida species is the alteration of the permeability of the mycotic cell resulting in the leakage of essential constituents and the inhibition of glucose utilization of the cell (Swamy et al, 1976).

Arrhythmias, and fatal bradyarrhythmias have also been associated with or without rapid intravenous injection of miconazole, and should be used with caution in patients with pre-existing heart disease (Coley and Crain, 1997, Heel et al, 1980).

The toxicological and pharmacological effects of cardiovascular drugs are usually studied in mammals and the results obtained are extrapolated to humans.

Chick embryonic heart develops through a similar process as in mice, rats and humans and also has a similar atrioventricular system (Bulter and Juurlink, 1987). Chick embryos have been widely used in pharmacological and toxicological experiments for evaluating drug action on the fetus (Paff and Boucek, 1975, Rajala et al, 1984, Tazawa, 1992). 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. In order to develop alternative methods, we have studied the biological
effects of drugs on the cardiovascular system of chick embryos using physiological techniques (Yoshiyama et al, 2004b, 2004c, 2003a, 2003b, Saito et al, 1990, Miyazaki et al, 1994). We have also reported that the chick embryonic model of hypothyroidism produced by treatment with thiamazole can be used to examine the pharmacological and toxicological effects of cardiovascular drugs (Sugiyama et al, 2000).

Drug drug interactions have been demonstrated for a variety of drugs, including disopyramide and propranolol, in the heart failure patients (Ellrodt and Singh, 1980, Podrid et al, 1980). We have evaluated the toxic interactions between propranolol and disopyramide in chick embryos (Yoshiyama et al, 1997, Yoshiyama et al, 2001). These data indicate that our recording system in ECG of chick embryos may be useful for investigating the toxic interactions. Toxic interaction between miconazole and disopyramide may result in additive effects on arrhythmias. The present study evaluated the effect of miconazole on the heart and the toxic interactions between miconazole and disopyramide 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.

Miconazole (Mochida Pharmaceutical, Tokyo, Japan) and disopyramide preparation (Chugai Pharmaceutical, Tokyo, Japan) were used for the treatment. Miconazole at 1 mg/egg, 5 mg/egg, 10 mg/egg alone or disopyramide at 0.3 mg/egg alone was injected into the air sac of each fertilized egg on the 16th day of incubation, and the heart rate was measured after each drug injection. Miconazole at 1 mg/egg with disopyramide at 0.3 mg/egg was injected into the air sac of each fertilized egg on the 16th day of incubation.

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

Electrocardiograms (ECGs) were recorded 0 to 60 min after drug injection, and heart rate was determined based on R-R intervals. In this system, we recorded good ECG tracing of chick embryos and continued by 60 min (Sugiyama et al, 1996). Changes in heart rate were expressed as mean percentage changes in the drug-treated groups compared with the matched control.

Four small holes were made at 90-degree intervals on "the equator," as well as one small hole on "the south pole," and one small hole on "the north pole" of each fertilized egg using an electric drill, and there were all sealed with paraffin (m.p. 60 °C). Specially designed needle electrodes were inserted into the appropriate holes of the equator and the south pole. Two needles on the equator were used as a bipolar lead from the embryonic heart, and the needle on the south pole was used as a ground lead. These needles were connected to a memory oscilloscope (VC-11, Nihon Koden Co., Tokyo, Japan). ECGs were recorded as bipolar waves between two needles on a recorder (PowerLab System, ADInstruments Japan Co., Tokyo, Japan) (Fig. 1).

The data were analyzed using 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-tailed, was used as the criterion to determine significance.
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Fig. 2. Heart rate of chick embryo after administration of miconazole.

Miconazole 1 mg/egg alone (Open circle), miconazole 5 mg/egg alone (Open triangle) or miconazole 10 mg/egg alone (Open square) was injected into the air sac of fertile eggs on the 16th day of incubation. Heart rates were presented as the mean percent changes of drug-treated groups over the time-matched control. Each point represents the mean and S.D. (bar) of 6 eggs. *Significantly different from miconazole 1 mg/egg alone group, p<0.05.

Fig. 3. Heart rate of chick embryo after administration of miconazole in combination with disopyramide.

Miconazole 1 mg/egg alone (Open circle), disopyramide 0.3 mg/egg alone (Open rhomboid) or miconazole 1 mg/egg plus disopyramide 0.3 mg/egg (Closed circle) was injected into the air sac of fertile eggs on the 16th day of incubation. Heart rates were presented as the mean percent changes of drug-treated groups over the time-matched control. Each point represents the mean and S.D. (bar) of 6 eggs. *Significantly different from miconazole 1 mg/egg alone group, p<0.05.

Results
The heart rates of chick embryos before each drug injection were as follows; miconazole 1 mg/egg alone: 233±9 beats/min, 5 mg/egg alone: 234±15, 10 mg/egg alone: 230±15 beats/min, disopyramide 0.3 mg/egg alone: 235±13 beats/min. Moreover, the body weight of chick embryos gradually increased with the day of incubation. After the administration of miconazole 1 mg/egg alone, the heart rate did not differ compared with that of the controls. However, the heart rate was significantly decreased with the administration of miconazole 5 mg/egg and 10 mg/egg (Fig. 2). The heart rate was also significantly decreased by the administration of miconazole 1 mg/egg with disopyramide 0.3 mg/egg (Fig. 3). In addition, an arrhythmia was produced by miconazole and disopyramide. (Fig. 4)
Discussion

Miconazole is an antifungal agent and is used for the treatment of various fungal infections (Benson and Nahata, 1988). Arrhythmias, including fatal bradyarrhythmias, tachycardia, ectopic atrial rhythms, and delayed intraventricular conduction, are described with the administration of miconazole (Coley and Crain, 1997, Heel et al, 1980).

The cardiotoxicity of miconazole was demonstrated in chick embryos. After the administration of miconazole 1 mg/egg, the heart rate was not different compared with the control. However, the heart rate was significantly decreased by the administration of 5 mg/egg or 10 mg/egg miconazole. In addition, arrhythmia was produced by the high dosing of miconazole. Miconazole is known to alter myocardial function manifested by electrocardiogram changes. We have evaluated the cardiotoxicity of fluconazole in chick embryos (Yoshiyama et al, in press). After the drug was injected into the air sac of each fertilized egg, it accumulated in the eggshell. 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 reported that toxic interaction between antiarrhythmic drugs were demonstrated in chick embryos (Yoshiyama et al, 2004a). The combination with disopyramide modified the pharmacological effects of the propranolol in chick embryos and led to an arrhythmia of the ECGs. Toxic interactions between disopyramide and other antiarrhythmic agents may result in potentially serious adverse reactions, particularly in patients with intraventricular conduction disturbances (Ellrodt and Singh, 1980).

The concurrent administration of Class I antiarrhythmic agents and miconazole is not recommended. An increased risk of cardiotoxicity may be caused in patients with pre-existing heart disease. After the administration of miconazole 1 mg/egg alone, the heart rate of the chick embryos was not different compared with the control. Toxic interactions between miconazole and disopyramide were demonstrated in chick embryos. The combination with disopyramide modified the pharmacological effects of the miconazole in chick embryos and led to an arrhythmia of the ECGs.

Figure 4 ECG tracing in chick embryos after administration of miconazole in combination with disopyramide.

A: Before injection of miconazole in combination with disopyramide, B: Arrhythmia was shown at 60 minutes after injection of miconazole 1 mg/egg plus disopyramide 0.3 mg/egg.

Nayler has shown that antiarrhythmic drugs inhibit 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, thereby resulting in myocardial depression (Nayler, 1966).

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 miconazole and disopyramide.

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