Toxic Interactions between Fluconazole and Disopyramide in Chick Embryos

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Received July 9, 2004; accepted September 7, 2004

The present study evaluated the effect of fluconazole on the heart, as well as and the toxic interactions between fluconazole and disopyramide 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. Fluconazole 0.4 mg/egg, 0.8 mg/egg, 1.2 mg/egg alone or disopyramide 0.3 mg/egg alone was injected into the air sac of each fertilized egg. And fluconazole 0.4 mg/egg with disopyramide 0.3 mg/egg was injected into the air sac of each fertilized egg. Electrocardiograms (ECGs) were recorded 0 to 60 min 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 fluconazole 0.4 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 fluconazole 0.8 mg/egg and 1.2 mg/egg. The heart rate was also significantly decreased by the administration of fluconazole 0.4 mg/egg together with disopyramide 0.3 mg/egg. In addition, an arrhythmia was produced by fluconazole and disopyramide. These findings indicate that the interaction between fluconazole and disopyramide has a marked influence on the heart rate in chick embryos.

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

Fluconazole is an effective triazole antifungal agent which is related to the imidazole. A long half-life permits once daily dosing. The mechanism of action of fluconazole is similar to that of other imidazole and triazole antifungal agents, specifically, the inhibition of cytochrome P-450-dependent ergosterol synthesis. The drug is effective when administered orally and intravenously for a variety of fungal infections, especially cryptococcosis in acquired immunodeficiency syndrome patients.

Case reports have described QT prolongation and torsades de points associated with fluconazole. The concurrent administration of Class I antiarrhythmic agents and agents that prolong the QT interval, such as fluconazole, may increase the risk of cardiotoxicity.

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 to that in mice, rats and humans, and also has a similar atrioventricular system. Chick embryos have been widely used in pharmacologic and toxicologic experiments for evaluating drug action on the fetus. 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. To develop alternative methods, we have studied the biologic effects of drugs on the cardiovascular system of chick embryos using physiologic techniques. And 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.

Drug drug interactions have been demonstrated for a variety of drugs, including disopyramide and propranolol, in the heart failure patients. We have evaluated the toxic interactions between propranolol and disopyramide in chick embryos. Toxic interaction between fluconazole and disopyramide may result in additive effects on QT prolongation. The present study evaluated the effect of fluconazole on the heart and the toxic interactions between fluconazole 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.

Fluconazole (Pfizer Japan, Inc., Tokyo, Japan) and disopyramide preparation (Chugai Pharmaceutical, Tokyo, Japan) were used for the treatment. Fluconazole 0.4 mg/egg, 0.8 mg/egg, 1.2 mg/egg alone or disopyramide 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. The following doses were chosen based on data from previous studies. Fluconazole at 0.4 mg/egg and disopyramide at 0.3 mg/egg were 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. 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 (mp 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

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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.

RESULTS

The heart rates of chick embryos before each drug injection were as follows; fluconazole 0.4 mg/egg alone: 230±9 beats/min, 0.8 mg/egg alone: 233±13, 1.2 mg/egg alone: 234±15 beats/min, disopyramide 0.3 mg/egg alone: 236±15 beats/min. Moreover, the body weight of chick embryos gradually increased with the day of incubation. After the administration of fluconazole 0.4 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 fluconazole 0.8 mg/egg and 1.2 mg/egg (Fig. 2). The heart rate was also significantly decreased by the administration of fluconazole 0.4 mg/egg with disopyramide 0.3 mg/egg (Fig. 3). In addition, an arrhythmia was produced by amitriptyline and disopyramide.

DISCUSSION

Fluconazole is a triazole antifungal agent and is used for the treatment of serious systemic candidal infections. It has been reported that a prolonged QT interval and torsades de points were caused in a 59-year-old female with Candida albicans peritonitis following intravenous fluconazole 400 to 800 mg daily for 5 weeks followed by intraperitoneal fluconazole 150 mg daily for 2 d. The authors conclude that QT prolongation was a direct effect of fluconazole, due to the time course of events and the fact that the patient was receiving no other QT-prolonging agents.

The cardiotoxicity of fluconazole was demonstrated in chick embryos. After the administration of fluconazole 0.4 mg/egg, the heart rate was not different compared with control. However, the heart rate was significantly decreased by the administration of 0.8 mg/egg or 1.2 mg/egg fluconazole. In addition, arrhythmia was produced by the high dosing of fluconazole. Fluconazole led to QTc interval prolongation in the ECGs. 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.

The present results indicate that our ECG recording system using chick embryos may be used as an animal testing alternative.

In addition, toxic interactions between antiarrhythmic drugs were demonstrated in chick embryos. 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. The concurrent administration of Class I antiarrhythmic agents and agents that prolong the QT interval, such as fluconazole, is not recommended. An increased risk of cardiotoxicity may be caused by the additive effects on QT prolongation.

After the administration of fluconazole 0.4 mg/egg alone, the heart rate of the chick embryos was not different compared with the control. Toxic interactions between fluconazole and disopyramide were demonstrated in chick embryos. The combination with disopyramide modified the pharmacological effects of the fluconazole in chick embryos and led to an arrhythmia of the ECGs.

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.

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 fluconazole and disopyramide.

Acknowledgments The authors express their gratitude to Ms. Tomomi Ikeda and Ms. Michiko Shoiri for their assistance in this study.

This study was supported in part by the Japanese Society of Alternatives to Animal Experiments (JSAAE).

REFERENCES