INFLUENCE OF HYPOPHYSDISM INDUCED BY THIAMAZOLE ON THE TOXICITY OF FLUCONAZOLE IN CHICK EMBRYOS

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SYNOPSIS
The effect of hypothyroidism induced by thiamazole on the toxicity of fluconazole was studied in chick embryos. To our knowledge, no information is now available on the effects of thyroid gland function on the regeneration and differentiation of tissue. Fertilized eggs of White Leghorns were incubated and investigated. 1.2 mg/0.2 mL/egg of thiamazole was injected into the albumen of fertilized eggs on the 9\(^{th}\) day of incubation. Fluconazole at 0.4 mg/egg was injected into the air sac of fertilized eggs on the 16\(^{th}\) day of incubation. Electrocardiograms were recorded 0 to 60 min after the injection. After the injection of fluconazole into the thiamazole-treated eggs, the heart rate was significantly decreased compared with the untreated eggs. These findings indicate that hypothyroidism induced by thiamazole has a marked influence on the toxicity of fluconazole in chick embryos.

Key words: hypothyroidism, cardiototoxicity, chick embryo

INTRODUCTION
With regard to the use of experimental animals for research and education, alternative methods for animal testing came to be discussed on account of criticisms made on animal welfare and humanitarian grounds as well as awareness among scientists.\(^1\) 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.\(^2\)\(^-\)\(^6\)

We have evaluated the toxic interactions between amitriptyline and fluconazole in chick embryos.\(^7\) Periodontology and oral surgery each have their own stock of research in regenerative dentistry. Antifungal agents are used at periodontics and operative dentistry. Fluconazole is an effective triazole antifungal agent which is related to the imidazole.\(^6\) The drug is effective when administered orally and intravenously for a variety of fungal infections, especially cryptococcosis in acquired immunodeficiency syndrome patients.\(^8\)\(^,\)\(^9\) Case reports have described QT prolongation and torsades de points associated with fluconazole.\(^10\)\(^,\)\(^11\) The concurrent administration of Class I antiarrhythmic agents and agents that prolong the QT interval, such as fluconazole, may increase the risk of cardiototoxicity.
An experimental animal model with heart disease originating from abnormalities of the thyroid gland in chick embryos has been produced by treatment with thiamazole and the pharmacological and toxicological effects of cardiotonics were examined using this model. To our knowledge, no information is now available on the effects of thyroid gland function on the regeneration and differentiation of tissue. The pharmacological and toxicological activities of thiamazole have characteristics in common with that of thiourea. It has been reported that when thiourea derivatives were injected into the albumen of eggs, the time of the injection strongly affected in the body weight and thyroid gland weight from the 9th to 12th day of incubation. In addition, they showed that that a state of hypothyroidism could be produced in chick embryos by injection of these drugs.

The present study evaluated the effect of hypothyroidism induced by thiamazole on the toxicity of fluconazole in chick embryos.

**MATERIALS AND METHODS**

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

Thiamazole (Chugai Pharmaceutical, Tokyo, Japan) and fluconazole (Pfizer Japan, Inc., Tokyo, Japan) were used for the treatment. 1.2mg/0.2 mL/egg of thiamazole was injected into the albumen of fertilized eggs on the 9th day of incubation. Fluconazole at 0.4 mg/egg, 0.8 mg/egg or 1.2 mg/egg was injected into the air sac of the thiamazole untreated eggs on the 16th day of incubation (six eggs in each group). Fluconazole at 0.4 mg/egg was injected into the air sac of the thiamazole-treated eggs or the untreated eggs on the 16th day of incubation (six eggs in each group).

After the injection of fluconazole into the eggs, the heart rate values 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 in "the equator," as well as one small hole in "the south pole," and one small hole in "the north pole" of each fertilized egg using an electric drill, and then they 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). ECGs were recorded as bipolar waves between two needles on a recorder (PowerLab System, ADInstruments Japan Co., Tokyo) (Fig. 1).

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 to determine significance.

**RESULTS**

The body weight of chick embryos gradually increased with the day of incubation. After the administration of fluconazole 0.4 mg/egg, the heart rate did not differ compared with that of controls. However, the heart rate was significantly decreased by the administration of 0.8 mg/egg and 1.2 mg/egg fluconazole (Fig. 2). After the injection of
fluconazole into the thiamazole-treated eggs, the heart rate was significantly decreased compared with the untreated eggs (Fig. 3).

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 days. 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. 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 in a time-dependent manner. 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 actions on the fetus.

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.

In the present study, the effects of hypothyroidism induced by thiamazole on the toxicity of fluconazole were investigated in chick embryos, and it was found the hypothyroidism induced by thiamazole modified the toxicity of fluconazole in the chick embryos.

The antithyroid drugs thiamazole and propylthiouracil have been widely used as therapeutic drugs in patients with hyperthyroidism. Toxicological studies have shown that antithyroid drugs at overdose levels cause functional and morphological changes in the thyroid in rats and dogs. In addition, it has been reported that functional abnormalities of the thyroid gland are often accompanied by heart disease and can show unexpected responses to cardiotonics, such as digoxin. Therefore, to predict the effects of cardiovascular drugs, experimental animals with a very sensitive heart condition such as hypothyroidism or hyperthyroidism should be used. A convenient thyrotoxic model would be of great benefit for evaluating the side effects and toxicity of cardiovascular drugs. Although the exact mechanism underlying the effects of thyroid gland function on the regeneration and differentiation of tissue remains to be clarified, a further investigation using our in ovo recording system for ECG of chick embryos is necessary to clarify the relationship with the regeneration mechanism and thyroid gland function.
Fig. 2. Heart rate of chick embryo after administration of fluconazole. Fluconazole 0.4 mg/egg alone (Open circle), fluconazole 0.8 mg/egg alone (Open triangle) or fluconazole 1.2 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 fluconazole 0.4 mg/egg group, P < 0.05. #Significantly different from fluconazole 0.8 mg/egg group, P < 0.05.

Fig. 3. Heart rate after administration of fluconazole in chick embryos with hypothyroidism induced by thiamazole. Fluconazole 0.4 mg/egg in untreated eggs (c) or thiamazole-treated eggs (●) was injected into the air sac of fertile eggs on the 16th day of incubation. Heart rates are 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 the untreated eggs, p < 0.05.

In conclusion, our in ovo ECG recording system in chick embryos may be useful for investigating the toxicity of fluconazole. In addition, thiamazole-treated chick embryos may prove to be an alternative animal model with which to examine the cardiotoxicity of some drugs, including fluconazole, under certain experimental situations.

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