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Effect of Digoxin on Chick Embryos with Hypothyroidism Induced by Thiamazole

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Abstract

Heart failure patients with hypothyroidism show unexpected reactions to cardiovascular drugs. In the present study, we proposed a chick embryonic model of hypothyroidism by injection of thiamazole (TMZ) and investigated whether this model can be used to examine the pharmacological and toxicological effects of cardiovascular drugs. When 1.2 mg/egg or more of TMZ was injected into the albumen of fertile eggs on the 9th day of incubation, the thyroid gland in the 16th day-chick embryos showed the same pathological characteristics as those in mammals. Although the effect of TMZ on the chick embryonic heart was morphologically mild, the heart rates decreased with the increase of dose of TMZ. An injection of digoxin into the TMZ-treated eggs increased the sensitivity of the heart to TMZ compared with that in the embryos treated with digoxin alone at the same dose. These results suggest that the TMZ-treated chick embryonic models can be used to investigate the effects of cardiovascular drugs.

Keywords: *Chick embryo, hypothyroidism, thiamazole, digoxin, electrocardiogram*

Introduction

The toxicological and pharmacological effects of cardiovascular drugs are usually studied in mammals and the results obtained are extrapolated to humans. In an attempt to reduce the number of mammals used in drug research, we have been examining the use of

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electrocardiograms (ECGs) in chick embryos and found that they may be superiorly used for predicting the effect of cardiovascular drugs (Sugiyama et al., 1997, 1999, Yoshiyama et al., 1997, Miyazaki et al., 1998).

Antithyroid drugs, thiamazole (TMZ) and propylthiouracil, have been widely used as therapeutic drugs in patients with hyperthyroidism. Toxicological studies have shown that the use of antithyroid drugs at an overdose causes functional and morphological changes in the thyroids in rats (Searle et al., 1950, Clark et al., 1992) and dogs (Wakamatsu and Ogawa, 1982, Shigemasa et al., 1990). 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 (Marrow et al., 1963). Therefore, to predict the effects of cardiovascular drugs, experimental animals with a very sensitive heart condition such as hypothyroidism or hyperthyroidism are required to be used. A convenient thyrotoxic model would be of great benefit for evaluating the side effects and toxicity of cardiovascular drugs.

In the present study, we produced a cardiac abnormality model in chick embryo, by TMZ treatment, and evaluated the effects of cardiotonic using this model.

Materials and Methods

Eggs and incubation

Fertile eggs of White Leghorn chicks (Ohmiya Poultry Science, Ohmiya, Japan) were incubated at 37.5 ± 0.2℃ at a relative humidity of about 65%, tuned automatically every hour (P-1 type, Showa Incubator Laboratory).

Drugs used

Thiamazole (TMZ, Mercazole, Chugai Pharmaceutical Co., Ltd., Tokyo), digoxin (Chugai Pharmaceutical Co., Ltd., Tokyo), urethane (Sigma Chemical Inc., St.Louis, USA) and α-chloralose (Sigma) were obtained from commercial sources. These drugs were dissolved at the concentration desired with physiological saline.

Injection of TMZ into fertile eggs

1.2, 2.4, 4.8 or 9.6 mg/0.2mL/egg of TMZ was injected into the albumen of fertile eggs on the 9th day of incubation as previously described (Sugiyama et al., 1985). The control group was given 0.2 mL/egg of physiological saline in the same manner.

ECG recordings for chick embryos

Twenty min after the injection (0.1 mL/egg) of anesthetic (urethane 450 mg/mL + α-chloralose 45mg/mL), ECG waves of the 16th day-chick embryos treated with and without TMZ were recorded using the methods previously reported (Sugiyama et al., 1996, Miyazaki et al., 1998) and heart rates (HR) were calculated from RR intervals.

Determination of the LD_{50} value of TMZ, and gross and histopathological observations

After injection of TMZ at the indicated doses, all eggs were candled daily for viability until the 16th day of incubation. The LD_{50} value of TMZ in the chick embryos was calculated from the number of dead embryos using the Probit method. After the ECG waves in the surviving embryos had been recorded, the embryos were removed and weighed, and the thyroid glands, heart and liver were examined. These organs were fixed in 10% buffered formalin and stained with hematoxyline and eosin (HE) for histopathological examination.

Effect of digoxin on HR in the TMZ-treated embryos

A single injection of digoxin (25, 37.5 or 50 μg/egg) was made into the air sac of saline-treated eggs on the 16th day of incubation. An injection of digoxin (25 μg/egg) was also made into the air sac of the TMZ (1.2 or 2.4 mg/egg)-treated eggs on the 16th day of incu-

Table 1 Survival on the 16th day of incubation and hatching rate in chick embryos treated with thiamazole

	A	B	C	Incubation priod (Days) and hatching rate (%)
Control	28	28 (100)	10	21-22 (100)
Thiamazole (mg/egg)				
1.2	28	28 (100)	10	23-27 (40)
2.4	28	26 (89)	10	25-28 (25)
4.8	28	22 (67)	10	28-31 (0)
9.6	20	0 (0)	0	-

Thiamazole was injected into the albumen on the 9th day of incubation and the survival of chick embryos were observed on the 16th day. A: the number of eggs used. B:the number of of surviving embryos and rate (%) on the 16th day of incubation. C: ten embryos surviving at the 16th day were incubated again until hatching and the hatching rate was examined.

Table 2 Organ weights in chick embryos treated with thiamazole

	No. of observed embryos	Wet weight of organs (relative weight to body weight, %)			
		Body (g)	Thyroid (mg) ($\%\times10^{-2}$)	Heart (mg) (%)	Liver (mg) (%)
Control	18	16.5 ± 1.67	1.47±0.45 (0.9±0.25)	165± 24 (1.0 ±0.16)	435 ±69 (2.7± 0.47)
Thiamazole (mg/egg)					
1.2	18	14.9 ±1.25	5.31±1.13* (3.6 ±0.76)*	141 ±21 (0.95± 0.13)	431± 64 (2.9 ± 0.47)
2.4	16	14.7±1.01	3.7 ±1.23* (2.5 ±0.92)*	144 ±18 (0.98± 0.12)	379± 49 (2.6± 0.26)
4.8	12	15.4±0.80	5.51± 1.76* (3.6 ±1.11)*	156 ±18 (1.01± 0.11)	375± 33 (2.4± 0.16)
9.6	0	-	-	-	-

Thiamazole was injected into the albumen of eggs on the 9th day of incubation and the surviving embryos were examined on the 16th day of incubation. Data are the mean±SE.
*; $p<0.01$, significant difference to the control.

bation. ECG waves were recorded from 0 to 30 min every 2-10 min after the injection and HR was calculated, as described above.

Statistical analysis

Statistical analysis of data was carried out using Student's *t*-test or Dunnett's multiple comparison tests. A value of $p < 0.05$ was considered significant.

Results

Survival rate in developing embryos

Both the survival rate on the 16th day of incubation and the hatching rate in TMZ-treated chick embryos decreased with the increase of the dose of TMZ, as shown in Table 1. A prolongation of embryonic development of three to 5 days beyond the normal 21 days was

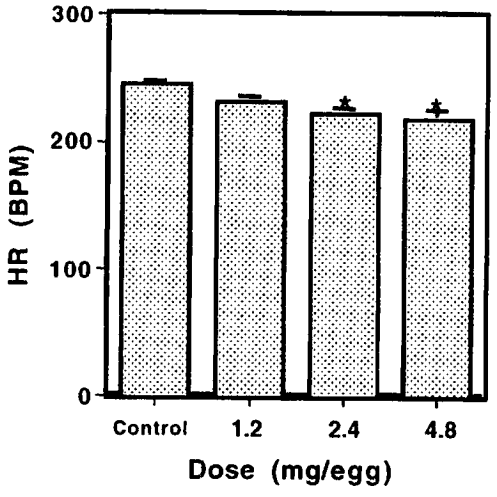

Fig.1 Changes of HR in chick embryos treated with thiamazole

Thiamazole was injected into the albumen of eggs on the 9th day of incubation and ECG waves were recorded 20 min after injection of anesthetic on the 16th day of incubation. *; $p<0.05$ to the control (saline).

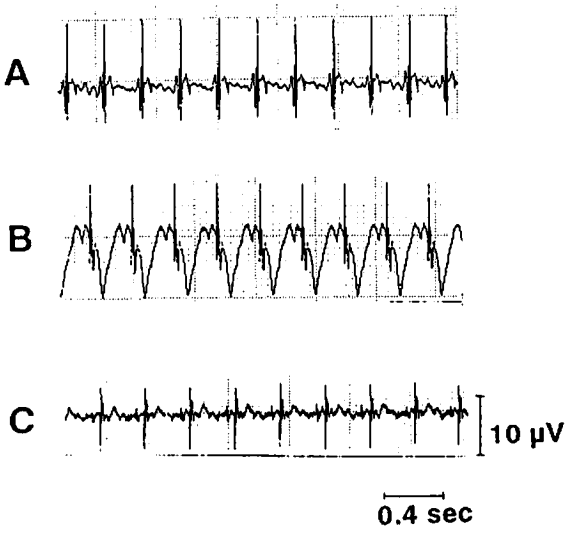

Fig.2 ECG waves in chick embryos treated with thiamazole

TMZ was injected into the albumen of eggs on the 9th day of incubation and ECG waves were recorded on the 16th day. A: Control (saline, 238 BPM), B: Bradycardia (215 BPM) in embryos with injection of 1.2. mg/egg of TMZ. C: Bradycardia (203 BPM) in embryos with 2.4 mg/egg of TMZ.

demonstrated in the chick embryos treated with TMZ. The LD_{50} value of TMZ in chick embryos given on the 16th day of incubation was 4.4 mg/egg (95% confidence limit, 3.40-5.43 mg/egg).

Gross observations

The weights of body, heart, thyroid glands and liver of the 16th day-chick embryos, and their relative body weight are shown in Table 2. The body weight tended to decrease dose-dependently and was significantly low in embryos treated with 1.2 mg/egg of TMZ. The wet and relative weights to body weight in thyroid of the embryos treated with TMZ were almost twice those of the control (saline) group. The heart and liver weights were not significantly different from those in the control embryos, and slightly decreased.

Histopathological observations

The thyroid follicles were larger, the epithelial cells were tall columnar and the colloid was reduced in size and amount in embryos treated with 1.2 or 2.4 mg/egg of TMZ, when compared with those in the control embryos. Degenerative changes in epithelial cells of follicles were observed with a vacuolated cytoplasm, and colloid droplets in follicles were scarce in embryos treated with 4.8 mg of TMZ. Slight atrophy of myocardial cells was seen in embryos treated with 2.4 mg TMZ. In addition, slight lymphocytic infiltration was observed in heart muscle fibers in embryos treated with 4.8 mg TMZ. However, no changes in liver were observed in embryos treated with 4.8 mg/egg.

Changes of HR and ECG waves in TMZ-treated embryos

As shown in Figs. 1 and 2, the HR in chick embryos gradually decreased, and bradycardia on injection of 1.2 mg /egg of TMZ was significantly reduced following injection of 2.4 mg/egg compared to the control.

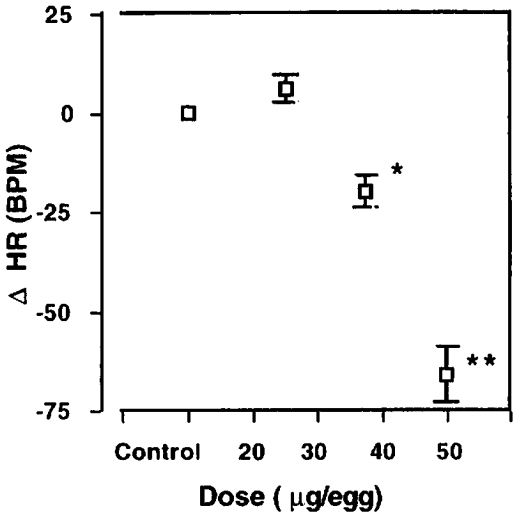

Fig.3 Changes of HR in chick embryos treated with digoxin

Data represent the changes in the HR 30 min after an injection of the indicated doses of digoxin into the air sac in 16th day-fertile eggs. Data are the mean ± SE. *, **; Significantly different from the control at $p<0.05$ and 0.01, respectively.

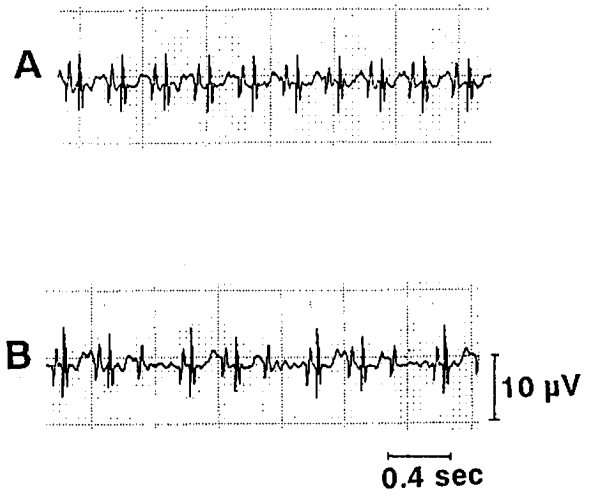

Fig.4 Arrhythmia in chick embryos treated with digoxin

Digoxin was injected into the air sac of saline-treated eggs on the 16th day of incubation. ECG waves were recorded 20 min after injection of anesthetic. A: Control (saline) B: 2 ° A-V block 30 min after single injection of digoxin 50 μg/egg.

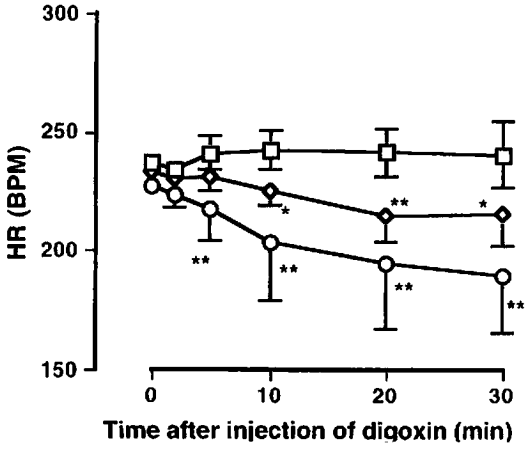

Fig.5 Effect of digoxin on HR in chick embryos treated with thiamazole

After the injection of indicated doses of TMZ on the 9th day of incubation, 25 μg/egg of digoxin was injected into the air sac of eggs on the 16th day of incubation. □ ; 25 μg/egg of digoxin, ◇ ; TMZ 1.2 mg/egg + 25 μg digoxin, ○ ; TMZ 2.4 mg/egg + 25 μg digoxin. Data are the mean ± SE. *, **; $p<0.05$, $p<0.01$ to the control (saline), respectively.

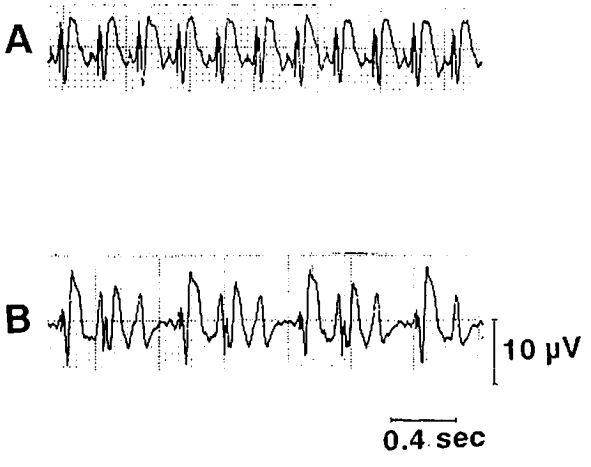

Fig.6 Arrhythmia in TMZ-treated embryos by additional injection of digoxin

TMZ (1.2 mg/egg) was injected into the eggs on the 9th day of incubation, and digoxin (25 μg/egg) was injected into the air sac of TMZ-treated eggs on the 16th day. A: ECG waves of TMZ-treated embryos before injection of digoxin. B: 2 ° A-V block 30 min after injection of digoxin

Effects of digoxin on HR in TMZ-treated embryos

The HR was constant until 30 min after injection of 25 μg/egg of digoxin into saline-treated fertile eggs. However, the HR was decreased markedly in embryos injected with 37.5 or 50 μg/egg of digoxin 5 min after the injection (Fig.3) and arrhythmia A-V block occurred 30 min after the injection (Fig.4). On the other hand, an injection of digoxin at 25 μg/egg to the TMZ-treated eggs reduced the HR remarkably (Fig.5). Regardless of the changes in ECG waves caused by the injection of 25 μg/egg of digoxin, an injection of the same dose of digoxin into the TMZ-treated eggs induced A-V block arrhythmias (Fig.6).

Discussion

In the present study, we proposed an experimental animal model with heart disease originated from abnormalities of the thyroid gland in chick embryos produced by the treatment with thiamazole (TMZ) and the pharmacological and toxicological effects of cardiotonic were examined using this model.

It is now clear that the growth, development and differentiation of cells in various organs of the chick embryos and in mammals are regulated by thyroid hormones. TMZ is structurally related to thiourea and interferes with the biosynthesis of thyroid hormone (Cooper and Ridgway, 1985). Therefore, the prolongation of the hatched period produced by TMZ is probably due to the inhibition of the biosynthesis of thyroid hormone by TMZ. As the lethal toxicity of TMZ in chick embryos was dose-dependent, this effect seems to be directly targeted for the whole body.

The pharmacological and toxicological activities of TMZ has characteristics in common with that of thiourea. Romanoff and Romanoff (1972) reported that when thiourea derivatives were injected into the albumen of eggs, the time of the injection strongly affected in the body weight and the thyroid gland weight from the 9th to 12th day of incubation. In addition, they showed that a state of hypothyroidism could be produced in chick embryos by injection of these drugs. It is important to determine the suitable period and site for the injection of drugs into fertile eggs. We already found that the same agent can exhibit significantly different effects in chick embryos when given at different sites or on different days of incubation (Sugiyama et al., 1982). According to the results obtained, in the present study, we injected TMZ into the albumen of fertile eggs on the 9th day of incubation.

Gaworski et al.(1994) observed histopathological changes, follicular cell hyperplasia and small amount of colloid in the enlarged thyroid gland in rats, on administration of the thiourea derivative, 2-mercaptobenzimidazole. When 1.2 mg/egg or more of TMZ was injected into the albumen on the 9th day of incubation, the enlarged thyroid glands in the 16th day-embryos showed pathologically marked changes involving tall epithelial follicular cells and small amounts of colloid in follicles. The colloid droplets were reduced by 4.8 mg/egg of TMZ. Namely, the pathological changes of thyroid gland in the chick embryos treated with TMZ were similar to those in rats already reported.

Although it was not reported whether the chick embryos with hypothyroidism had heart diseases caused by thiourea derivatives, it has been reported that human patients (Wartofsky, 1991) and rats (Dowell, 1992) with hypothyroidism may suffer bradycardia. In the present study, the HR in the TMZ-treated chick embryos showed bradycardia as that in mammals.

In addition, although no information was available on the liver of mammals treated with TMZ, the liver of the TMZ-treated chick embryos did not show any significant morphological changes.

The results obtained suggest that TMZ-treated chick embryos may be a model of the

heart diseases associated with hypothyroidism.

It has been reported that an overdose of antihyperthyroid drugs often results in heart disease accompanied by hypothyroidism in humans (Marrow et al., 1963) and in dogs (Wakamatsu and Ogawa, 1982, Shigemasa et al., 1990).

Digoxin has been widely used as a therapeutic drug in patients with congestive heart failure. However, digoxin at a lower dose is usually given to patients with hypothyroidism because of their enhanced sensitivity to this drug (Smith, 1989).

It was not known whether digoxin brings about any changes in the HR of chick embryos at a late stage of incubation. We showed in the present report that the HR of chick embryos decreased by injection of digoxin in dose-dependent manner and arrhythmias were produced by digoxin at high dosages. In addition, when a small amount of digoxin, which did not change the HR in the control, was injected into chick embryos with hypothyroidism, the HR was significantly decreased compared with that in control. It is suggested that digoxin increased the sensitivity to TMZ in the heart of chick embryos with hypothyroidism as same as in dog and man.

The increase in the sensitivity to digoxin in TMZ-treated embryos is considered to be due to one or more of the following factors: 1) An increase in digoxin concentration in plasma (Shenfield et al., 1977). 2) A decrease in Na^+, K^+-ATPase activity in the myocardium (Matthews and Jim, 1990). 3) The prolongation of digoxin half time due to the decrease in atrial natriuretic peptide (ANP) (Croxson and Ibbertson, 1975, Morise et al., 1991). 4) A decrease in the sensitivity of cardiac beta-adrenergic receptors to the activation by catecholamine (Akera and Brown, 1982). 5) An increase in the distribution of digoxin in heart (Morrow et al., 1963).

As thyroid hormone has multiple functions in chick embryos as well as in mammals, further investigation is necessary to clarify the mechanism underlying cardiac function induced by digoxin in chick embryonic hypothyroidism. However, TMZ-treated chick embryos may prove to be an alternative animal model with which to examine unexpected effects of cardiovascular drugs.

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