The human epidermal growth factor receptors (HER) play an important role in cellular growth. Tumors with HER2 receptor overexpression exhibit greater metastatagenicy and virulence compared with non-overexpressing tumors. Recently, the development of the anti-HER2 monoclonal antibody, trastuzumab (Herceptin; Genentic, Inc., South San Francisco, CA, U.S.A.), has been put on the market because of its activity in the treatment of patients with HER2-overexpressing metastatic breast cancer.1,2) Trastuzumab is the first biologic modifier with significant activity in advanced breast cancer patients to amplify the HER2 gene.3) A new generation of clinical trials will evaluate the role of this drug in improving the curability of patients with early stage disease. This effort has been complicated by the unexpected observation of cardiac toxicity in patients treated with trastuzumab. Trastuzumab has some toxicities, including cardiotoxicity.4—6) The pathogenesis of cardiotoxicity associated with trastuzumab is unknown.2) Trastuzumab has not been shown to localize to the heart in animal models. Furthermore, monkeys treated with trastuzumab at doses more than 10-fold higher than in humans for up to 6 months exhibited no cardiotoxicity.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 have been widely used in pharmacological and toxicological experiments for evaluating drug action on the fetus.7—9)

Chick embryonic heart develops through a similar process to that in mice, rats and humans, and also has a similar atrioventricular system.10)

In order to develop alternative methods, we have studied the biological effects of drugs on the cardiovascular system of chick embryos using physiological techniques.11—14) In this system, we recorded good ECG tracings of chick embryos from the 8th to 18th day of incubation. The present study evaluated the effect of trastuzumab on the heart 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%. They were turned automatically every hour, and candled daily for viability.

Trastuzumab preparation (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) was used for the treatment. Trastuzumab at 5 mg/egg (low dosing) or 15 mg/egg (high dosing) 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 low dosing of trastuzumab, the heart rate was not different compared with the control. However, the heart rate was significantly decreased by high dosing of trastuzumab. In addition, arrhythmia was produced by high dosing of trastuzumab. These findings indicate that trastuzumab has a marked dose- and time-dependent influence on the heart rate in chick embryos.

**Key words** cardiotoxicity; trastuzumab; chick embryo; electrocardiogram

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The cardiac toxicity of trastuzumab was studied in chick embryos. Fertilized eggs of White Leghorns were incubated and investigated. Trastuzumab 5 mg/egg (low dose) or 15 mg/egg (high dose) 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 low dosing of trastuzumab, the heart rate was not different compared with the control. However, the heart rate was significantly decreased by high dosing of trastuzumab. In addition, arrhythmia was produced by high dosing of trastuzumab. These findings indicate that trastuzumab has a marked dose- and time-dependent influence on the heart rate in chick embryos.

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**Fig. 1. Schema of ECG-Recording System for Chick Embryo in Egg Shell**
Tokyo, Japan) (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 for significance.

RESULTS

The body weight of chick embryos gradually increased with the day of incubation. After low dosing of trastuzumab, the heart rate was not different compared with the control. However, the heart rate was significantly decreased by high dosing of trastuzumab (Fig. 2). In addition, arrhythmia was produced by high dosing of trastuzumab (Fig. 3).

DISCUSSION

The discovery of HER2 and its role in the pathogenesis of breast cancer tumors, as well as the development of the anti-HER2 monoclonal antibody, trastuzumab (Herceptin), directed against the HER2 receptor, represent major milestones in research development in breast cancer, making trastuzumab the first monoclonal antibody available for treatment of this disease.1—6) For HER2-positive patients, the use of trastuzumab is associated with improved time-to-disease progression and overall survival.4—6,15,16) Unfortunately, findings also demonstrate an increased risk for cardiotoxicity using trastuzumab.4,17) It is important that we must consider the carefully monitored use of trastuzumab.

Cardiotoxicity of trastuzumab (15 mg/egg) was demonstrated in chick embryos. And trastuzumab led to an arrhythmia.

We have reported that the toxicological effects of several drugs in chick embryos are stronger in the air sac injection route than other injection routes, i.e., the yolk sac and the albumen of fertile eggs.18) In this study, 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 in chick embryos should be investigated further.

Fig. 2. Changes in Heart Rate of Chick Embryo after Administration of Trastuzumab (Herceptin)
Saline (○), trastuzumab low dose 5 mg/egg (●) or high dose 15 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; saline: 238±8 beats/min, trastuzumab 5 mg/egg: 230±9 beats/min, trastuzumab 15 mg/egg: 246±15 beats/min. Data are the mean±S.D. (bar) of six eggs.

Fig. 3. ECG Tracing in Chick Embryos Treated with Trastuzumab
(A) Before injection of trastuzumab. (B) Arrhythmia was shown at 60 min after injection of 15 mg/egg of trastuzumab

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 cardiotoxicity induced by trastuzumab could be clearly analyzed in chick embryos, as in mammals, especially the decrease in heart rate, accompanied by prolongation of the QTc interval in the chick embryos, as was observed in humans. The clinical use of trastuzumab led to cardiotoxicity.4—6) Cardiotoxicity has been noted with single-agent trastuzumab (4 mg/kg loading dose, followed by 2 mg/kg/week intravenously). This indicates that developing chick embryos are appropriate as an alternative experimental animal, rather than traditional mammals.

There is substantial evidence that the HER receptor family plays an important role in the development of the heart. However, there is limited information regarding the HER receptor family in response to cardiotoxicity.2) Although the exact mechanism of this cardiotoxicity remains to be clarified, the drug seems to induce the cardiotoxicity in chick embryos.

In conclusion, our in ovo recording system for ECG of chick embryos may be useful for investigating the cardiotoxicity of trastuzumab.

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REFERENCES