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Letter

Predicting clinical cardiotoxicity caused by trastuzumab and E-8010 using human cardiomyocytes

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ABSTRACT — In addition to low molecular weight drugs, many antibody drugs have been developed in recent years. The safety of these drugs is mainly evaluated in animal toxicity experiments and it is difficult to detect all the toxicities that may occur in humans. Although trastuzumab (Herceptin®), an antibody drug that is used for the treatment of breast cancer, as well as E-8010, a low molecular inhibitor of phosphodiesterase-5, did not cause cardiotoxicity (*e.g.* dysfunction, QT prolongation, and arrhythmias) in monkeys, they caused cardiotoxicity in humans. The present study examined, whether or not the human cardiotoxicity of these drugs could be predicted using cardiomyocytes derived from human-induced pluripotent stem cells (hiPS-CMs) using an MED64-multielectrode array and assessed the usefulness of hiPS-CMs in antibody drug testing. At 1 and 3 mg/mL, trastuzumab prolonged the field potential duration (QT interval on electrocardiography) by $\geq$ 10% and induced arrest, respectively. At 0.1 and 1 µmol/L, E-8010 prolonged the field potential duration by $\geq$ 10% and induced early after-depolarization (proarrhythmia), respectively. The human cardiotoxicity induced by trastuzumab and E-8010 could be predicted using hiPS-CMs. It was thought that a multielectrode array using hiPS-CMs would be a useful tool for predicting the clinical cardiotoxicity of antibody drug candidates in addition to small molecular drug candidates in the preclinical setting.

Key words: Trastuzumab, E-8010, Cardiotoxicity, Human cardiomyocytes, Herceptin

INTRODUCTION

More than 80% of candidate drugs have been withdrawn in clinical trials in the past (Hay *et al*., 2014), usually due to a lack of efficacy or safety concerns. With regard to safety, in the preclinical setting, information about the toxicity of candidate drugs is mainly obtained from animal experiments, especially non-rodent animals; however, the results do not always reflect toxicity in humans (Olson *et al*., 2000; O'hara and Ruddy, 2012; Vargas *et al*., 2015; Dubois *et al*., 2016). Human cell experiments, which can potentially overcome this issue, are being investigated. Cardiotoxicity was most common type of adverse event in clinical studies; such events have sometimes led to death (Martin, 2015). Recently, many studies using human cardiomyocytes derived from human-induced pluripotent stem cells (hiPS-CMs) have been reported (Guo *et al*., 2013; Harris *et al*., 2013; Nakamura *et al*., 2014; Nozaki *et al*., 2014, 2016; Qu

and Vargas, 2015; Kitaguchi *et al*., 2016; Takasuna *et al*., 2017). hiPS-CMs are expected to be a useful tool for predicting human cardiotoxicity. Author investigated whether or not human-specific drug-induced cardiotoxicity could be predicted using hiPS-CMs and assessed the usefulness of hiPS-CMs in antibody drug testing.

The test drugs were chosen from the drug labels and studies that revealed they had no effect on the cardiovascular system of monkeys in preclinical testing but had cardiotoxic effects in clinical studies. Trastuzumab (Herceptin®), an antibody-drug for breast cancer, had no effect on the cardiovascular system of monkeys at 25 mg/kg (i.v.) weekly for 6 months (Martin, 2015), but was associated with the development of serious cardiac disorders (*i.e.,* dysfunction, arrhythmias, and bradycardia) in the clinical setting (Cardinale *et al*., 2010). E-8010 (MR-118585), a low-molecular inhibitor of phosphodiesterase type 5, showed no effect on the cardiovascular system of monkeys at 1000 mg/kg (p.o.) once daily

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for 4 weeks, but it induced a prolongation of the electrocardiogram QT-interval (QT) in clinical trials, resulting in the termination of its clinical development (Adachi *et al*., 2013). In those days, the International Committee for Harmonization (ICH) guideline for the conduct of safety pharmacology studies for human pharmaceuticals had not been issued. The clinical cardiotoxicity of drug candidates might not have been predicted in repeated dose toxicity studies in monkeys or due to differences in the pharmacokinetics of humans and monkeys. However, if the clinical cardiotoxicity of drug candidates could be predicted using hiPS-CMs, this finding would represent an important advance in drug development because it is a preclinical *in vitro* assay.

MATERIALS AND METHODS

Drugs

Trastuzumab was made in-house, dissolved in sterile distilled water at 100-fold of the tested concentration applied. E-8010 was also made in-house, dissolved in dimethylsulfoxide at 1000-fold of the tested concentration applied.

Cell culture and field potential assay

The hiPS-CMs used were iCell® cardiomyocytes[2] (the donor of human-induced pluripotent stem cells was Caucasian female, Cellar Dynamics International Japan, Tokyo, Japan). The frozen hiPS-CMs were thawed according to the manufacturer's instructions, seeded at 3.0×10^4 cells/probe onto the MED probe (MED-RG515A or -PG515A, Alpha Med Scientific, Osaka, Japan) precoated with 50 µg/mL human fibronectin (Sigma-Aldrich, St. Louis, MO, USA). The cells were then cultured in a 5% CO_2 incubator at 37°C under humidification. Seven days later, the cell sheets were used in the assay.

An MED probe with cell sheets was placed in the MED64-multielectrode array device (Alpha Med Scientific), and then stabilized for 30 min at 37°C in 5% CO_2. The field potential from the spontaneously beating cell sheets was recorded through a 0.1 Hz high-pass filter and a 5 kHz low-pass filter for 10 min before (control) and after applying of the drugs. The drugs were cumulatively applied to obtain the test concentration. The final concentrations of water and dimethylsulfoxide were limited to within 5 and 0.5 vol%, respectively.

Data analysis

The criteria used for their assay were those reported by Asakura *et al*. (2015). The data were presented the mean ± standard error of 4-6 individual preparations. The field

potential duration (FPD) was calculated from the last 30 waveforms of field potential during each 10 min-recording period. The FPD was corrected using the correction formulae of *Bazett* and *Fridericia* (FPDcB and FPDcF, respectively). The concentrations of FPDcB and FPDcF that prolonged the FDP by 10% in comparison to the control value were calculated (FPD10, FPDcB10 and FPDcF10, respectively). These parameters were considered as criteria for the occurrence of arrhythmia. The beats per minute (BPM) and first peak amplitude of field potential waveform (AMP) were calculated based on the first peak of the field potential waveform. Early after-depolarization (EAD) and arrest were observed during the field potential recording.

RESULTS

Trastuzumab (0.1, 0.3, 1, 3 mg/mL) prolonged the FPD at ≥ 1 mg/mL; the FPD10, FPDcB10 and FPDcF10 were all 1 mg/mL (Table 1, Fig. 1). The BPM only slightly decreased until 1 mg/mL (Table 1, Fig. 1). A 3 mg/mL caused arrest (Table 1, Fig. 1). The beating recovered after a few minutes of arrest in a few preparations, and after washout in all preparations. AMP was decreased at ≥ 0.3 mg/mL (Table 1, Fig. 1).

E-8010 (0.01, 0.03, 0.1, 0.3, 1 µmol/L) prolonged the FPD by ≥ 10% at 0.1 µmol/L, and the FPDcB10 and FPDcF10 were both 0.3 µmol/L (Table 1, Fig. 2). At 1 µmol/L, E-8010 caused EAD (Table 1, Fig. 2). The BPM was hardly affected until 1 µmol/L (Table 1, Fig. 2). The AMP decreased gradually with increasing drug concentrations at ≥ 0.03 µmol/L (Table 1, Fig. 2).

DISCUSSION

The major toxicity associated with trastuzumab is cardiac dysfunction and cognitive heart failure (Oldham and Dillman, 2009). In the present study, this drug induced arrest after prolonging the FPD (a surrogate of the QT interval on electrocardiography) (Luerman *et al*., 2014). Arrest likely represents cardiac dysfunction or hypofunction, due to the abnormality of contraction signaling rather than sudden death. The mechanisms underlying this drug's effects have not been sufficiently clarified; however, because trastuzumab-induced arrest was reversible, it was not thought to be associated with cardiac injury. The C_{max} in clinical therapy is 0.11-0.21 mg/mL (attached document drafted by Genentech, revised Jan 2000 and others). It was suggested that the FPD prolongation (≥ 10%) would occur at several-fold of the clinical therapeutic plasma concentration and that arrest would occur

Clinical prediction from *in vitro* testing
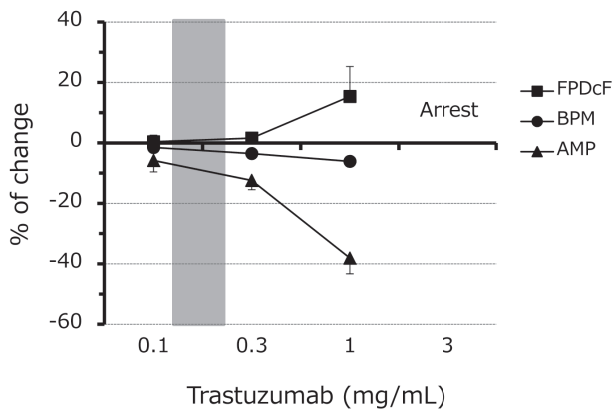
Fig. 1. Effect of trastuzumab on the field potential of beating cardiomyocytes. The field potential at each concentration was recorded for 10 min and the occurrence of early after-depolarization (EAD) and arrest were observed. Each value represents the mean ± standard error to the control value of 6 individual preparations. The closed vertical column represents the C_{max} in clinical therapy Closed square: field potential duration corrected by Fridericia's correction formula (FPDcF), closed circle: beats per minute (BPM), closed triangle: amplitude of 1st peak (AMP). FPDcF prolongation by ≥ 10% was 1 mg/mL.
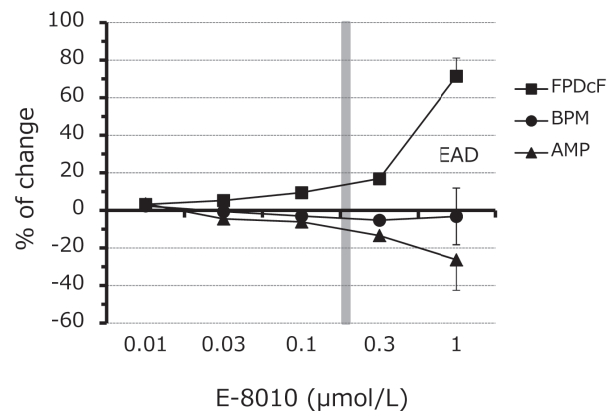
Fig. 2. Effect of E-8010 on the field potential of beating cardiomyocytes. The field potential at each concentration was recorded for 10 min and the occurrence of early after-depolarization (EAD) and arrest were observed. Each value represents the mean ± standard error to the control value of 6 individual preparations except 1 μmol/L (4 preparations). The closed vertical column represents the C_{max} in clinical therapy Closed square: field potential duration corrected by Fridericia's correction formula (FPDcF), closed circle: beats per minute (BPM), closed triangle: amplitude of 1st peak (AMP). FPDcF prolongation by ≥ 10% was 0.3 μmol/L.

Table 1. Effects of trastuzumab and E-8010 on field potential duration (FPD), corrected FPD with Buzett's (FPDcB) and Fridericia's (FPDcF) formula, first peak amplitude (AMP) and beats per minute (BPM).

Drug	Concentration	N	FPD (msec)	FPDcB (msec)	FPDcF (msec)	BPM (beats/min)	AMP (μV)	EAD/Arrest
	(mg/mL)							
Trastuzumab	0	6	486.9 ± 11.6	415.0 ± 9.5	437.7 ± 10.2	43.6 ± 0.5	2078 ± 303	
	0.1	6	491.2 ± 14.7	415.3 ± 11.6	439.2 ± 12.5	42.9 ± 0.7	1943 ± 277	
	0.3	6	500.7 ± 14.7	419.1 ± 11.4	444.7 ± 12.3	42.1 ± 0.6	1831 ± 287	
	1	6	571.6 ± 44.5	473.2 ± 40.0	503.9 ± 41.5	41.0 ± 0.5	1318 ± 260	
	3	6	NC	NC	NC	NC	NC	Arrest (N=6)
	(μmol/L)							
E-8010	0	6	505.2 ± 30.7	415.7 ± 13.9	443.4 ± 18.9	41.6 ± 2.6	2817 ± 389	
	0.01	6	517.3 ± 30.9	431.1 ± 16.0	458.0 ± 20.5	42.4 ± 2.2	2895 ± 379	
	0.03	6	533.4 ± 33.3	437.6 ± 15.6	467.3 ± 20.9	41.3 ± 2.5	2707 ± 402	
	0.1	6	560.4 ± 38.7	453.5 ± 18.5	486.5 ± 24.6	40.4 ± 2.7	2688 ± 444	
	0.3	6	601.9 ± 39.2	481.5 ± 16.5	518.4 ± 23.2	39.5 ± 2.8	2461 ± 383	
	1	6	890.4 ± 154.9 (N=4)	698.7 ± 106.6 (N=4)	749.7 ± 114.8 (N=4)	41.7 ± 1.3 (N=4)	1182 ± 264 (N=4)	EAD (N=6)

Each value represents the mean ± standard error. NC: not calculable because of arrest, EAD: early after-depolarization, N: number of preparations, Two among 6 preparations of E-8010 (1 μmol/L) assay were not calculable because of EAD was continued during recording period.

at $\geq$ 10-fold the therapeutic concentration. So long as this drug is used at the indicated dose in the clinical setting, it is not likely to cause cardiotoxicity. However, for patients with preexisting heart risk factors (high blood pressure, high cholesterol and obesity), the administration of other chemotherapies, comorbid disease and others conditions, the susceptibility to trastuzumab is increased, and cardiac dysfunction or hypofunction can easily develop. The findings of the present study suggest that the clinical cardiotoxicity caused by trastuzumab was able to be predicted using hiPS-CMs.

E-8010 prolongs the QT interval in humans, but not monkeys at a dose of 1000 mg/kg (oral) (Adachi *et al.*, 2013). E-8010 prolonged the FPD; while the FPD10, FPDcB10 and FPDcF10 were 0.1-0.3 μmol/L. Because the experimental medium contains serum protein and there are differences in the types and amounts of proteins and lipids present in plasma (*in vitro*) compared with tissues (*in vivo*), Author cannot discuss the protein-bindings ratio of E-8010 in the present study. At a therapeutic plasma concentration (0.183 μmol/L), E-8010 may inhibit the hERG channel (IC_{50}: 0.04 μmol/L) (Adachi *et al.*, 2013). At 1 μmol/L, E-8010 caused EAD (a surrogate of arrhythmia) (Charpentier *et al.*, 1993). EAD is an abnormality of the repolarization process of the action potential, which induces ventricular tachycardia *torsades de pointes* (Charpentier *et al.*, 1993). These findings suggested that E-8010 would prolong the QT in clinical therapy by inhibiting the hERG current, and that either patients receiving an overdose or those with preexisting heart disease have the potential to develop arrhythmia. The findings of the present study suggest that the clinical cardiotoxicity caused by E-8010 was able to be predicted using hiPS-CMs

The results of the present study show that the hiPS-CM-based assay can be used to predict the risk of clinical cardiotoxicities of drugs directly affecting cardiomyocytes.

Conflict of interest---- The authors declare that there is no conflict of interest.

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