POSITIVE CHRONOTROPIC AND INOTROPIC ACTIONS OF NEW
ANTITUMOR AGENT ADRIAMYCIN AND ITS CARDIOTOXICITY
—ITS SPECIAL REFERENCES TO MYOCARDIAL CONTRACTILE
FORCE AND THE CHANGE OF THE
TRANSMEMBRANE ACTION POTENTIAL—

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New antitumor antibiotic adriamycin, chemically glycoside linkage of the red pigmented aglycone and the water soluble aminosugar, has been clinically known to produce some electrocardiographic abnormalities such as sinus tachycardia, ST segment depression and the flattening of T wave, as its side effect. In order to clarify its genetic mechanism, isolated guinea pig heart perfused by Langendorff's technique was used and single shot of adriamycin into this preparation produced positive chronotropic and inotropic actions and the acceleration of the repolarization process manifested by the shortening of the duration of the membrane action potential, especially, the repolarization phase 2. This agent has lesser degree of acute accumulative effect in producing arrhythmia, as compared with the action of daunomycin. These effects were completely blocked by dl-propranolol.

ADRIAMYCIN, recently introduced in the field of cancer chemotherapy, especially for the treatment of child and adult solid tumors, various types of leukemias, malignant lymphoma so on, was isolated from culture of streptomyces peucetius caesius, and was rather new antibiotic with the nature of antimetabolite in inhibiting the action of DNA dependent RNA polymerase.

Key Words: Antitumor Antibiotic
Adriamycin
Glycoside-linkage
Red Pigmented Aglycone
Guinea Pig Heart
Langendorff's Perfusion
Positive Inotropism
Positive Chronotropism
Arrhythmias
Beta Blocking Agent

Recently, however, the cardiac effects of this agent such as sinus tachycardia, electrocardiographic ST segment depression and the flattening of T wave have been clinically reported as its side effect¹

These cardiotoxic effects may bring some fears to clinician to use this promising drug routinely, therefore, it may be urgent matter to investigate the genetic mechanism of this effect and to find some antidotes to overcome these problems.

This study was carried out to elucidate the genetic mechanism of the cardiotoxic effect of this drug by analysing the change of myocardial contractility, surface electrogram and the transmembrane action potential from the ventricular muscle of guinea pig.

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METHODS

Guinea pigs weighing 200 to 300 grams were used, and guinea pig was anesthetized with ether. Midline thoracotomy was performed and the entire heart was excised and the polyethylene cannula was inserted into the aorta for the *Langendorff's* perfusion. Schematic illustration for the experimental set-up was presented in Fig.2. Isolated heart was perfused through *Langendorff's* technique with 95% O₂-5% CO₂. Hydro-

static pressure of 400 mmH₂O was maintained for perfusion. Transmembrane action potential was recorded from the right ventricular epicardial surface by flexible mounted microelectrode (tip-diameter 0.5 micron with its resistance of 10 to 20 megohms) using a high gain amplifier (Nihon Kohden Model RDH-2) and a high in-put impedance amplifier (Nihon Kohden Model MZ-3B). Myocardial contractile force was recorded from the apex of the heart through the silk thread connected to a force transducer. All tracings were recorded on an eight channel direct writing polygraph (Nihon Kohden Model RM-150). Penetration of microelectrode was made on the right ventricular epicardial surface, and the transmembrane action potential was recorded between the microelectrode and an indifferent electrode placed in the vicinity of the microelectrode. For artificial driving by electrical stimulation, a bipolar electrode was placed in the vicinity of the SA node, and a square wave of 5 msec was given at the desired frequency through an isolating unit (Nihon Kohden Model MSE-JM) of electronic stimulator (Nihon Kohden Model MSE-3).

Following the regular basic sinus rhythm was resumed after the initiation of the perfusion, adriamycin 0.5-2.5 mg/0.1 ml was injected into the cannula by single shot, and the drained

Fig.1. Structural formulas of adriamycin, daunomycin and cardiac glycoside.

Fig.2. Schematic illustration of the experimental set-up for the Langendorff's perfusion used in the present study.

Fig.3. Positive chronotropic and inotropic effects appeared about 40 seconds after the administration of adriamycin 2.0 mg/0.1 ml, and returned to the control value about 5 minutes later. Upper tracing: Developed tension
Middle tracing: Surface electrogram
Lower tracing: Membrane action potential

Fig.4. Illustration of acute accumulative effect of adriamycin. This is the continued record of Fig.3. After approximately 5 minutes following the administration of 2.0 mg/0.1 ml and returned to the control value, second shot of adriamycin 0.1 mg/0.1 ml was given. The trigeminal rhythm appeared 3 minutes later, then returned to the control value about 4 minutes 30 seconds later.

solution which perfused through the heart was discarded and not returned into the perfusion reservoir.

In some experiments, the bipolar electrode was placed at the vicinity of the sinus node and artificial driving was done in order to eliminate the effect of the change of the heart rate upon the duration of the membrane action potential.

Experimental data were gathered from 10 guinea pig with approximately 150 penetrations by microelectrode.

RESULTS

1. Positive inotropic and chronotropic actions of adriamycin

After the administration of adriamycin 2 mg/0.1 ml, the heart rate increased from 106/min at the control to 125/min and reached to the max-

imum 150/min about one minute later and gradually returned to the control value about 5 minutes later. On the surface electrogram recorded from the right ventricular epicardial surface showed the decrease of the amplitude of T wave about 5 minutes later.

The developed tension at the upper tracing increased from 1.25 g to 2.5, 4.75, 2.8, 1.88 g at the 40 seconds, 1 minute, 3 minutes, 4 minutes respectively. Siegel's myocardial contractile index increased from 214 at the control to 314, 256, 236, and 214 sec^{-2} at 1 minute, 3 minutes, 4 minutes and 5 minutes respectively (Fig.2).

In the same animal, after approximately 5 minutes following the first administration of adriamycin 2 mg/0.1 ml, and returned to the control value, second shot of adriamycin 1 mg/0.1 ml was given. The positive chronotropic and inotropic effects were appeared 1 minute later, and the trigeminal rhythm was appeared 3 minutes later, then returned to the control value about 4 minutes 30 seconds later. This figure illustrated acute accumulative effect of adriamycin (Fig.3).

Second shot of adriamycin 1 mg/0.1 ml was given after the first shot of adriamycin 2 mg/0.1 ml. The bigeminal rhythm was appeared about 1 minute 40 seconds later as the acute accumulative effect. The duration of the membrane action potential measured at the one thirds of the depolarization upstroke was markedly shortened from 133 msec to 116 msec at the records of 1 minute and of 1 minute 40 seconds (Fig.4).

2. The change of the duration of the membrane action potential

The shortening of the duration of the membrane action potential was observed in Figs.2, 3, and 4. This change was not ascertained due to the drug effect itself or due to the change of the heart rate from the positive chronotropic effect. Therefore, in order to eliminate the effect of the change of the heart rate upon the duration of the membrane action potential, artificial electrical stimulation was given at the right atrial appendage in the frequency of 120/min. Then, adriamycin 0.6 mg/0.1 ml was administered, and the positive inotropic action was appeared about 1 minute later. The duration of the membrane action potential measured at the point "B" was markedly shortened from 175 msec at the control to 158 msec and 150 msec in the tracings recorded at 1 minute and 2 minutes 30 seconds respec-

Fig.5. The bigeminal rhythm was appeared as the acute accumulative effect of adriamycin. The duration of the membrane action potential was markedly shortened.

Fig.6. The effect of adriamycin upon the duration of the membrane action potential recorded from artificially driven heart with its frequency of 120/min.

tively, and returned to the control value approximately 5 minutes later (Fig.6).

3. Positive chronotropic and inotropic actions of daunomycin

The developed tension, surface electrograms recorded from the right atrial epicardial surface and from the right ventricular epicardial surface were simultaneously recorded, as may be seen in the upper, middle and lower tracings respectively. The tracings in both sides were illustrated in high speed for the better comparison. The positive inotropic and chronotropic effects were appeared about 40 seconds later, and arrhythmic rhythm was appeared about 1 minute 40 seconds later. At the control, the regular sinus rhythm was seen in 120/min as the normal atrio-ventricular conduction, but the atrio-ventricular dissociation was appeared in the atrial rate of 129/min and the ventricular rate of 85/min, as may be seen in the

Fig.7. Illustration of the positive chronotropic and inotropic effects, and arrhythmic property induced by administration of daunomycin 2 mg/0.1 ml. Atrio-ventricular dissociation was appeared, seen at the right side.

R. A. EG.: Surface electrogram recorded from the right atrial epicardial surface

R. V. EG.: Surface electrogram recorded from the right ventricular epicardial surface

Fig.8. Illustration of the bigeminal rhythm induced by administration of daunomycin 2.5 mg/1.25 ml.

high speed record at the right side (Fig.7).

The larger dose of daunomycin 2.5 mg/1.25 ml was administered, the bigeminal rhythm was appeared about 1 minute 30 seconds later, and returned to the control value about 4 minutes later (Fig.8).

4. The effect of dl-propranolol upon the actions of daunomycin and adriamycin

Daunomycin 1.0 mg/0.1 ml and dl-propranolol 0.01 mg/0.1 ml were simultaneously administered into the cannula by single shot. Approximately 30 seconds later, the developed tension was increased in the upper tracing, and the arrhythmia started to appear at the same time in the tracing of surface electrogram (middle), and the duration of membrane action potential, especially repolarization phase 2 was markedly shortened, as may be seen in the lower tracing. This amount of dl-propranolol was unable to eliminate or block the

Fig.9. Effect of dl-propranolol upon the action of daunomycin.

Fig.10. Effect of dl-propranolol upon the action of adriamycin. The upper figure illustrates the effect of adriamycin 2.0 mg/0.2 ml and dl-propranolol 0.01 mg/0.1 ml simultaneously administered. The positive chronotropic and inotropic actions, slight ST segment depression and the shortening of MAP duration appeared. However, when the half dose of adriamycin 1.0 mg/0.1 ml and dl-propranolol were simultaneously administered, all these effects were blocked, as may be seen in the lower figure.

positive chronotropic and inotropic actions, and arrhythmic property of daunomycin.

However, in the same animal, the half dose of daunomycin 0.5 mg/0.05 ml was given with the same amount of dl-propranolol 0.01 mg/0.1 ml,

Fig.11. Assumptive mechanism for the action of adriamycin.

the developed tension, the heart rate and the duration of the membrane action potential were not changed except slight prolongation of P-R interval. This amount of dl-propranolol was able to block the positive chronotropic and inotropic actions and arrhythmic property of daunomycin (Fig.9).

Similar experiment was carried out for the case of adriamycin. The amount of dl-propranolol was kept constant in 0.01 mg/0.1 ml. The equidose of adriamycin 1.0 mg/0.1 ml was blocked by the same amount of dl-propranolol 0.01

mg/0.1 ml which was used in the case of daunomycin in Fig.8. The double dose of adriamycin 2.0 mg/0.2 ml, as compared with the dose of daunomycin 1.0 mg/0.1 ml, was not blocked by dl-propranolol 0.01 mg/0.1 ml, namely the positive chronotropic and inotropic actions were appeared. Therefore, the equipotent dose was 1.0 mg and 0.5 mg for adriamycin and daunomycin respectively (Fig.10).

DISCUSSION

Adriamycin is a new anti-tumor antibiotic isolated in 1967 in the Farmitalia Research Laboratories from cultures of *streptomyces peucetius caesius*. The elucidation of this compound was carried out by *Arcamone* and associates² and its structure is very similar to that of daunomycin, namely 14-hydroxydaunomycin. The difference exists in the substitution of a hydrogen atom with a hydroxyl group on the acetyl radical, as may be seen in Fig.1.

Chemically this compound has consisted of red pigmented aglycone; adriamycinone and the water soluble aminosugar; daunosamine, and these two parts are connected by glycoside linkage. The basic structure of the red pigmented aglycone in either adriamycin or daunomycin is the anthracycline nucleus, which differs from that of steroid nucleus in cardiac glycosides. However, the experimental findings clearly demonstrated that these compounds have proven to possess the positive inotropic action to the ventricular myocardium of guinea pig heart, although species difference has to be considered for the appearance of this effect. It is speculated that the red pigmented aglycone may play the important role to produce this action.

The warning evidence of the side effect due to this agent has been reported clinically by *Bonadonna* and associates,¹ namely the sinus tachycardia, ST segment depression and the flattening of T wave electrocardiographically. These changes were observed experimentally in the present investigation.

The similar side effects were reported clinically in the cases treated by daunomycin, however, more severe effects were induced in some cases as the occurrence of the congestive heart failure.^{3,4}

The various experimental evidences have been already reported about the elucidation of the basic genetic mechanism of the cardiovascular effects to due daunomycin.⁵⁻⁷

Herman and associates⁷ investigated the influence of pharmacologic or physiologic pretreat-

ment on acute daunomycin cardiac toxicity in hamster, and observed that daunomycin induced sinus tachycardia and acute bidirectional arrhythmia, and an arrhythmic dose of daunomycin produced mild pressor response and an increased concentration of plasma epinephrine and norepinephrine. He suggested that daunomycin induced arrhythmia may be mediated by a central mechanism involving sympathetic nervous system.

The other possibility may exist that adriamycin or daunomycin induced cardiovascular effects such as positive chronotropic, inotropic effects, and arrhythmias may be mediated by the change of an intrinsic cardiac neurons or myocardial fiber itself, because the present study was carried out in the denervated excised heart, whereas *Herman's* experiment was done in the in situ hamster heart with the intact sympathetic nervous regulation, and furthermore it was found that the repolarization process was markedly accelerated as manifested by the shortening of the MAP duration in the present study.

Herman and associates⁶ also investigated that the influence of various cardiodepressant and beta adrenergic blocking drugs on the daunomycin induced bidirectional arrhythmia in the hamster, and concluded that diphenylhydantoin and racemic propranolol were the most effective to eliminate the side effects of daunomycin.

The experimental findings of the present study demonstrated that dl-propranolol produced the ability to eliminate the effects of adriamycin or daunomycin, such as the positive chronotropic and inotropic effects, and arrhythmic effect, and furthermore the shortening of the duration of the membrane action potential.

It is of interest to note that both agents adriamycin and daunomycin have the ability to produce the positive inotropism but its ability is stronger in daunomycin when the equidose was given comparatively.

As to the genetic mechanism in producing the positive inotropic and chronotropic actions by adriamycin, various mechanism can be speculated from the experimental findings in the present study, as may be seen in Fig.11. In the myocardial fiber, glucagon, prostaglandins and dibutyryl cyclic 3',5'-AMP may produce their actions through "Multi-responsive cyclase" to cyclic 3',5'-AMP, and this route of action was not blocked by dl-propranolol, and epinephrine, norepinephrine and isoproterenol may be mediated through "Epinephrine responsive cyclase" and this was blocked by dl-propranolol, whereas

cardiac glycoside may produce its action mediated by Ca-stimulated ATP-ase system? As to adriamycin or daunomycin, they may act upon the site of Ca-stimulated ATP-ase, because their chemical structure possess the red pigmented aglycone and glycoside linkage and resembled to that of cardiac glycoside. Second route speculated may be the site of epinephrine-responsive cyclase system, because these agents produce as increased plasma concentration of epinephrine and norepinephrine, and furthermore it was blocked by dl-propranolol. However, the exact mechanism involving in the pharmacological process and which route was predominant were not determined yet from the experimental findings in the present study.

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