Cardiovascular and Adenylate Cyclase Stimulating Effects of Colforsin Daropate, a Water-Soluble Forskolin Derivative, Compared With Those of Isoproterenol, Dopamine and Dobutamine

Masahiko Yoneyama, MD; Atsushi Sugiyama, MD; Yoshioki Satoh, MD; Akira Takahara, PhD; Yuji Nakamura, MS; Keitaro Hashimoto, MD

Colforsin daropate is a recently developed water-soluble derivative of forskolin that directly stimulates adenylate cyclase, unlike the catecholamines. The chronotropic, inotropic and coronary vasodilator actions of colforsin daropate were compared with those of isoproterenol, dopamine and dobutamine, using canine isolated, blood-perfused heart preparations. The stimulating effect of each drug on adenylate cyclase activity was also assessed. Colforsin daropate, as well as each of the catecholamines, exerted positive chronotropic, inotropic and coronary vasodilator actions. The order of selectivity for the cardiovascular variables of colforsin daropate was coronary vasodilation >> positive inotropy >> positive chronotropy; whereas that of isoproterenol, dopamine and dobutamine was positive inotropy >> coronary vasodilation >> positive chronotropy. Thus, a marked characteristic of colforsin daropate is its potent coronary vasodilator action. On the other hand, each drug significantly increased the adenylate cyclase activity in a dose-related manner: colforsin daropate >> isoproterenol > dopamine = dobutamine. These results suggest that colforsin daropate may be preferable in the treatment of severe heart failure where the coronary blood flow is reduced and \( \beta \)-adrenoceptor-dependent signal transduction pathway is downregulated. (Circ J 2002; 66: 1150–1154)

Key Words: Adenylate cyclase; Colforsin daropate; Contraction; Inodilator

METHODS

All experimentation was performed in accordance with the rules and regulations of the Committee for Research at Yamanashi Medical University, which are equivalent to those of the Japanese Pharmacological Society. Animals were obtained from the Animal Laboratory for Research of Yamanashi Medical University.

Assessment of the Effects of the Drugs on the Canine Isolated, Blood-Perfused Heart Preparations

Isolated Heart Preparations  The preparation was obtained from a beagle dog of either sex, weighing approximately 10 kg. The dog was anesthetized with pentobarbital sodium (30 mg/kg, iv), given heparin calcium (500 U/kg, iv) and exsanguinated. The heart was excised and plunged into cold Tyrode’s solution kept at approximately 4°C.

The sinoatrial node preparation consists of the entire right atrium. The sinus node artery was cannulated. Bipolar recording electrodes were attached on the atrial epicardium close to the sinus nodal region.

The papillary muscle preparation consists of the anterior papillary muscle of the right ventricle attached to the interventricular septum. The anterior septal artery, which was the sole nutrient artery of the preparation, was directly cannulated. Bipolar stimulating electrodes were attached onto the His-bundle region.

Blood-Donor Dog  HBD dogs (Kitayama Labes, Yoshiaki Farm, Gifu, Japan) of either sex, weighing 17–20 kg, were used as a blood donors. The dog was anesthetized
with pentobarbital sodium (30 mg/kg, iv), and supplemented with 4–5 mg·kg⁻¹·h⁻¹. After intubation, the dog was artificially ventilated with room air (SN-480-3, Shina-no, Tokyo, Japan). The systemic blood pressure and surface lead II ECG were monitored using a polygraph system (RM-6000, Nihon Kohden, Tokyo, Japan). At the start of cross-circulation, heparin calcium (500 U/kg, iv) was given followed by an additional dose of 200 U·kg⁻¹·h⁻¹.

**Cross-Circulation** The preparations were placed in a double-wall glass jacket maintained at 38°C by circulating warm water and were perfused with arterial blood from the carotid artery of the blood-donor dog. Perfusion pressure was kept at 120 mmHg using a peristaltic pump (7553-00, Cole-Parmer, Chicago, IL, USA) with Starling’s pneumatic resistance placed parallel to the perfusion circuit. Venous blood from the preparations and excess blood passing through the pneumatic resistance were collected in a blood reservoir and returned to the jugular vein of the blood-donor dog.

**Parameters** The spontaneously beating rate of the sinoatrial node preparation (ie, sinoatrial rate) was measured with a heart rate counter (AT-601G, Nihon Kohden) triggered by the atrial electrogram. The papillary muscle preparation was electrically driven through the stimulating electrodes at a cycle length of 500 ms using a stimulator (SEN-7203, Nihon Kohden) and an isolation unit (SS-2011, Nihon Kohden). The stimulation pulses were rectangular in shape, 1–2 V amplitude (approx. 20% above the threshold voltage) and 5 ms in duration. The developed tension of the papillary muscle under a resting tension of 2 g was measured isometrically using a force displacement transducer (DRM-T20, Dia Medical, Tokyo). The coronary blood flow through the nutrient arteries of each preparation was continuously monitored with an electromagnetic flowmeter (MVF3200, Dia Medical). The coronary blood flow through the nutrient arteries together with the coronary blood flow through the anterior septal artery were recorded on a rectilinear recorder (RJG-4124, Nihon Kohden) at a paper speed of 25 mm/min.

**Experimental Protocol** After confirming that the preparations were stabilized, colforsin daropate (10 pmol–10 nmol), isoproterenol (0.1–30 pmol), dopamine (10 pmol–10 nmol) or dobutamine (10 pmol–1 nmol) was injected into each nutrient artery using a small microsyringe (Ito, Tokyo) in volumes of 10–30 µl over 4 s. Because a relatively small amount of drug was administered to the preparations compared with that needed in a whole animal model, multiple doses of each drug were studied in the same preparation, which has been shown not to interfere with the result. The effluent blood through each preparation immediately after the drug injection was discarded to eliminate the drug effects on the blood-donor dog. The vehicle saline did not affect any of the parameters assessed.

**Assessment of the Effects of the Drugs on Adenylyl Cyclase Activity**

**Plasma Membrane Preparation** When the experiment with the blood-perfused heart preparations was finished, the heart of the blood-donor dog was excised and immediately placed in ice-cold SET buffer (0.25 mol/L sucrose; 0.1 mmol/L EDTA; 5.0 mmol/L tris-acetate, pH 7.4). The left ventricular tissue was trimmed and homogenized in 5 volumes of SET buffer. The homogenate was filtered (Nitex filter, Tetko, CA, USA) and centrifuged at 10,000 G for 5 min at 4°C. The pellet was resuspended in SET buffer and the mixture was centrifuged 3 more times. Protein analysis was performed using a commercially available protein assay reagent (Pierce, Rockford, IL, USA). The membrane suspension was diluted with SET buffer to a concentration of 3–5 mg protein/ml and was stored at −80°C until its enzyme activity was measured.

**Adenylyl Cyclase Activity** The adenylyl cyclase activity of the membrane preparation was measured using an enzymatic fluorometric assay technique. We took 50 µL of reaction mix (100 mmol/L tris-acetate, pH 7.4; 20 mmol/L KCl; 10 mmol/L MgCl₂; 20 mmol/L phosphoenolpyruvate; 2 mmol/L ATP; 20 µmol/L GTP; 2 mmol/L diithiothreitol; 0.4 mg/L bovine serum albumin; 100 µmol/L 3-isobutyl-1-methylxanthine (IBMX); 100 µg/ml pyruvate kinase) and added it to each microcentrifugation tube in duplicate with or without either of colforsin daropate (2 × 10⁻⁸–2 × 10⁻³ mol/L), isoproterenol (2 × 10⁻⁷–2 × 10⁻⁴ mol/L), dopamine (2 × 10⁻⁶–2 × 10⁻³ mol/L) or dobutamine (2 × 10⁻⁶–2 × 10⁻² mol/L). Next, the membrane suspension in a volume of 50 µL was added to each tube on ice. The reaction was initiated by placing the tubes in a water bath maintained at 37°C. After 30 min, the reaction was terminated by heating at 95°C for 5 min. The mixture was vortexed 3 times and centrifuged at 10,000 G for 5 min. A volume of 5 µL of the supernatant was transferred to a 10 × 75 mm disposable assay tube (Iwaki Lab Ware, Tokyo) in triplicate. The cyclic AMP concentration was assayed using the enzymatic fluorometric method.

**Drugs**

The following drugs were purchased; colforsin daropate hydrochloride, 6-(3-dimethyl-aminopropionyl) forskolin hydrochloride (Nippon Kayaku, Tokyo, Japan); isoproterenol hydrochloride (Nikkun Kagaku, Tokyo, Japan); dopamine hydrochloride (Nacalai tesque, Kyoto, Japan); dobutamine hydrochloride (Shionogi, Osaka, Japan); pentobarbital sodium (Tokyo-Kasei, Tokyo, Japan); and heparin calcium (Mitsui, Tokyo, Japan). All other substrates and enzymes were bought from Sigma Chemical Company (St Louis, MO, USA).

**Data Analysis And Statistics**

The data are presented as the mean ± SE. Peak responses in each parameter of the blood-perfused heart preparations are expressed as percentages of the respective basal values. The doses that produced a 50% increase in the developed tension of the papillary muscle were determined for each drug from the dose–response curves fitted by linear regressions. Percentage changes produced by these particular doses in sinoatrial rate and coronary blood flow were also determined from the dose–response curves fitted by linear regressions. The statistical comparisons of mean values were carried out using a paired t-test or one-way repeated-measures analysis of variance (ANOVA) followed by Contrast. A p value <0.05 was considered significant.

**Results**

**Chronotropic, Inotropic and Coronary Vasodilator Effects of the Drugs**

One hour after the start of cross-circulation, the sinoatrial node preparation showed spontaneous regular automaticity of 99±9 beats/min (n=6). Meanwhile, the papillary muscle preparation showed a developed tension of...
5.6±0.5 g and coronary blood flow of 5.1±0.7 ml/min (n=6). Administration of each drug increased the sinoatrial rate, developed tension and coronary blood flow in a dose-related manner, but the potency of the effects on each variable was different, and the duration of each effect of colforsin daropate was longer than those of the other drugs. In addition, colforsin daropate increased the sinoatrial rate and developed tension more gradually than the receptor agonists. The peak effects of colforsin daropate on the sinoatrial rate and developed tension were observed at 2.5±0.4 min and 2.1±0.1 min, respectively, after the injection of the highest dose, whereas for the other drugs those effects were all attained at less than 0.6 min. Typical tracings are depicted in Fig 1, and the dose–response curves for the peak increase are summarized in Fig 2. Ventricular tachycardia at more than 120 beats/min was induced in 3 of 6 preparations after the administration of colforsin daropate (10 nmol) and isoproterenol (30 pmol), SAR, sinoatrial rate of the sinoatrial node preparation; DT, developed tension of the papillary muscle; CBF, coronary blood flow through the anterior septal artery of the papillary muscle preparation.

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The doses that produced a 50% increase in developed tension were obtained from the dose–response curves and were 8 nmol for colforsin daropate, 8.2 pmol for isoproterenol, 4 nmol for dopamine and 0.3 nmol for dobutamine. Colforsin daropate was the least potent of the drugs in producing positive inotropy. The selectivity of each drug for developed tension vs other cardiac variables is schematically summarized in Fig 3. In addition, the selectivity of toborinone, a phosphodiesterase inhibitor, was also calculated from our previous result for comparison! The order of selectivity for the cardiovascular variables of colforsin daropate was coronary vasodilation >> positive inotropy >> positive chronotropy; and that of isoproterenol, dopamine and dobutamine was positive inotropy >> coronary vasodilation >> positive chronotropy; whereas that of toborinone was coronary vasodilation ≥ positive inotropy, but positive chronotropy was hardly detected.

Effects of the Drugs on Adenylate Cyclase Activity

The effects of colforsin daropate, isoproterenol, dopa-
mine and dobutamine on the adenylate cyclase activity of the ventricular membrane preparation are summarized in Fig 4. Each drug significantly increased the adenylate cyclase activity in a concentration-related manner, but their potency varied significantly. The maximal adenylate cyclase activity (pmol·min⁻¹·mg protein⁻¹) was 923±225 for the colforsin daropate group (n=6), 108±18 for the isoproterenol group (n=6), 66±6 for the dopamine group (n=6) and 59±5 for the dobutamine group (n=6). The order of potency in stimulating adenylate cyclase activity was colforsin daropate >> isoproterenol > dopamine = dobutamine. When compared at a concentration of 10⁻⁵ mol/L, colforsin daropate was approximately 9-fold more potent than isoproterenol in increasing adenylate cyclase activity.

**Discussion**

Because comparative information regarding the cardiovascular effects and subcellular responses of colforsin daropate with those of clinically well-established cardiotonic agents is still limited,2,3 we assessed its chronotropic, inotropic, coronary vasodilator and adenylate cyclase stimulating effects with those agents that have effects on β-adrenoceptors. As clearly shown in the results, colforsin daropate, as well as each catecholamine, exerted positive chronotropic, inotropic and coronary vasodilator actions, and stimulated adenylate cyclase activity, which accords directionally with previous reports;1,3,7,9,10,11 however, the potency of the selectivity for each cardiac variable has not previously been fully compared.

As schematically depicted in Fig 3, the potency of the selectivity for each cardiac variable in the blood-perfused heart preparations was quite different among the drugs; namely, colforsin daropate was selectively effective for coronary vasodilation, and each catecholamine was more specific for positive inotropy, whereas the phosphodiesterase inhibitor toborinone was almost equipotent for coronary vasodilation and positive inotropy. A similar cardiovascular profile to that of toborinone has been reported for classical phosphodiesterase inhibitors, including IBMX and theophylline.15 Thus, the most marked difference between colforsin daropate and the catecholamines or phosphodiesterase inhibitors is its potent coronary vasodilator action, although all these drugs should increase intracellular cyclic AMP. It can be speculated that there might be a difference in the distribution of the adrenoreceptor-coupled adenylate cyclase activation system, the density of adenylate cyclase that colforsin daropate would directly or indirectly stimulate and the concentration of cyclic AMP-hydrolyzing phosphodiesterases.12,13 Experiments are now going to explain the different cardiovascular profiles of these drugs by assessing the regional production rate of cyclic AMP of the sinus node, ventricular tissue and coronary artery. Such an assessment may also explain why the effect of colforsin daropate on the coronary blood flow peaked soon after the injection, whereas that on the sinoatrial tissue and developed tension was slow in onset.

It is generally considered that positive inotropic drugs with less arrhythmogenic potential and less tendency to increase heart rate are desirable.1 Colforsin daropate has a stronger positive chronotropic effect than the catecholamines or phosphodiesterase inhibitors at doses that produced similar positive inotropic effects. Similar results have been observed previously in vivo in a study of colforsin daropate, as well as isoproterenol, caused ventricular tachycardia in some preparations, which may be a premonition of the former in certain clinical situations. On the other hand, as the most common cause of heart failure is coronary artery disease, the potent coronary vasodilator effects of colforsin daropate may be desirable in such cases.

In the present study, the effects of colforsin daropate on adenylate cyclase activity were precisely compared with those of isoproterenol, dopamine and dobutamine under the same experimental conditions, which has not been reported elsewhere. As we described, the stimulatory effect of colforsin daropate was the most prominent among the four drugs assessed; for example, the adenylate cyclase activity in the presence of 10⁻⁵ mol/L of colforsin daropate was approximately 9-fold greater than that with the same concentration of isoproterenol, yet the effect of colforsin daropate on inotropism or chronotropism was not necessarily the highest in this functional study. These results suggest that colforsin daropate could effectively promote cyclic AMP production in the heart under various conditions because the physiological signal transduction cascade between the β-adrenoceptor and adenylate cyclase might have been degraded in the membrane preparation we used in this study.8

In conclusion, colforsin daropate, isoproterenol, dobutamine and dopamine exerted positive chronotropic, inotropic and coronary vasodilator actions in addition to stimulating adenylate cyclase activity. The most marked characteristic of colforsin daropate is its potent coronary vasodilator and adenylate cyclase stimulating actions, which may be desirable in the treatment of acute heart failure where coronary blood flow is reduced and β-adrenoceptors are down-regulated. In addition, data from this study may provide guidelines for comparing the inotropic action as well as potential adverse effects of new cardiotonic agents, such as colforsin daropate. We propose that assessment of the drug-induced functional, as well as biochemical, responses may be better for the characterization of the cardiovascular profile of these drugs.

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**References**

7. Sugiyama A, Zhu BM, Takahara A, Sato Y, Hashimoto K. Cardiac effects of Salvia miltiorrhiza/Dalbergia odorifera mixture, an intra-

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