A Protective Action of Coenzyme Q₁₀ on Chlorpromazine-Induced Cell Damage in the Cultured Rat Myocardial Cells

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SUMMARY

The interactions between coenzyme Q₁₀ (CoQ₁₀) and chlorpromazine (CPZ) were studied after CPZ-induced injury of cultured myocardial cells of new-born Wistar rats. Administration of 7.5 × 10⁻⁶ M of CPZ caused decreases in adenosine triphosphate (ATP) contents and beating rates by 77.4% and 54.2%, respectively. These changes were dependent on the dosage of CPZ. Histologically, many lamellated granules appeared in the cytoplasm and seemed to originate from mitochondria. The decreased density of the mitochondrial matrix and an irregular arrangement of cristae were also observed. Coincubation of the cultures with CPZ and CoQ₁₀ led to a dose-dependent increase in ATP contents and beating rates. The appearance of increased number of granules in the cytoplasm was suppressed. Indomethacin, an inhibitor of prostaglandin synthesis, interfered with the effects of CoQ₁₀ on the CPZ-induced myocardial cell injury. These findings suggest that CoQ₁₀ may protect myocardial cells from CPZ-induced injury, and that prostaglandins may play an important role in the action of CoQ₁₀.

Additional Indexing Words:
Chlorpromazine    Cultured myocardial cells    Coenzyme Q₁₀    Prostaglandin

Since the 1950’s, chlorpromazine (CPZ), one of the phenothiazine derivatives, has been used in the treatment of psychiatric patients. Because of the high-dose and long-term administration of CPZ, various adverse action are often found in the cardiovascular system, the central nervous system, liver and other organs.¹ In the cardiovascular system, they include sudden death, arrhythmias, hypotension or electrocardiographic abnormalities such as conduction disturbances, prolonged QT-interval, depressed ST-segments and abnor-
malities of T-waves. The electrocardiographic abnormalities induced by CPZ may result from myocardial injury. Histologically, CPZ-induced myocardial injury appears separations of contracted myofibers by clusters of enlarged mitochondria, dilation of the sarcoplasmic reticulum and abundant glycogen storage. Biochemical changes include a decrease in intracellular cyclic AMP, the decreased Ca++-uptake in the mitochondria and the microsomes, and permeability changes of mitochondrial membranes.

Coenzyme Q₁₀ (CoQ) occurs in major organs of the human body and is a redox component of the respiratory chain in the mitochondria. It has been proposed that CoQ plays a role in oxidative phosphorylation on the metabolic pathway. In the ischemic myocardium, CoQ may have a protective action on disturbances in the oxidation and reduction system in the mitochondria.

The aim of the present study are to determine whether CoQ can protect myocardial cells from CPZ-induced injury.

**METHODS**

The preparations for the present experiments were made by the methods of Harary. Under aseptic conditions, ventricular muscles of new-born Wistar rats (2–3 days old) were removed and immediately immersed into a cold saline A solution (NaCl 8.00 Gm/L, KCl 0.40 Gm/L, glucose 1.00 Gm/L, NaHCO₃ 0.35 Gm/L). The tissue was minced into pieces about 0.5 mm in diameter, rinsed several times with 0.1% tripsin-saline A solution and stored at 37°C. After stirring for 7 min, the tissues were iced and maintained for 4 min. The stirring procedure was repeated 4 times. The supernatant fraction after the first digestion was discarded and filtered with a nylon mesh from the 2nd to 4th digestions. The filtered supernatant fraction was centrifuged to remove tripsin at 1,500rpm for 5 min at 4°C. The last sediment was resuspended in culture medium containing Basal MEM (GIBCO), 1% penicillin-streptomycin solution (Sigma), 0.01% L-glutamine and 10% fetal calf serum. One half million seeded cells were introduced into a 35×10 mm Petri-dish (Falcon) with 2 ml of a cultured medium and were cultured in an incubator under 95% room air and 5% CO₂ with a pH of 7.4 at 37°C. After 48 hours, the medium was changed to fresh medium which contained the experimental drugs. They were then cultured for 24 hours.

The beating rates were measured with a phase-contrast photomicroscope (Nicon) in a closed box, in which CO₂ was continuously introduced to maintain a buffer medium at a pH of 7.4 at 37°C. Photomicroscopic examination was also performed.

After the cultured cells were rinsed with saline A solution to remove the
medium, 2 ml of 5% trichloracetic acid was added into the dish in order to fix protein of the cells. The cultured cells were removed from the dish and homogenized in a glass homogenizer. The homogenate was centrifuged at 15,000 rpm for 20 min at 4°C. The supernatant was extracted with ether. Firefly Lantern Extract-50 (FLE-50, Sigma) was added to the supernatant. The protein content was measured by the methods of Lowry. The ATP amount was measured with a Luminescence Reader (Aloca). About 11 to 16 experiments were performed on each sample concentration. Each experiment was performed in 3 to 4 cultures. The results obtained were expressed as the ratio to the control. Statistical significance was determined with Student's t-test.

For electron microscopic examination, cultured myocardial cells were fixed with 2.5% glutaraldehyde buffered with 0.2 M cacodylate (pH 7.5) for 1 hour. The tissue was removed from the dish and post-fixed in 0.1 M phos-
Fig. 2. Phase contrast photomicrographs of cultured myocardial cells in the rat. A is the control. B is a $7.5 \times 10^{-6} \text{M}$ chlorpromazine-treated sample. Granules (arrow mark) in the cytoplasm are more numerous than in control cells. C shows cells treated with $5 \times 10^{-5} \text{M}$ coenzyme Q$_{10}$ (CoQ) and the same concentration of chlorpromazine (CPZ). The cytoplasmic granules, increased by CPZ, are suppressed by the addition of CoQ. D is a sample treated with the combination of CPZ, CoQ, and indomethacin. The granules are prominent. (Magnified by 200×)

phate buffered with 1% OsO$_4$ for 1 hour. After that they were dehydrate in a series of acetone solutions and embedded in Epon 812 using usual methods. Ultrathin sections were cut on a Porter-Blum MT-2B microtome and stained with uranyl acetate and lead citrate. Observations were performed with a JEM 100S electron microscope.

The drugs used were chlorpromazine (CPZ; the dose of $10^{-9}$, $10^{-8}$, $10^{-7}$, $10^{-6}$, and $7.5 \times 10^{-6} \text{M}$), coenzyme Q$_{10}$ (CoQ; the dose of $5 \times 10^{-6}$, $2.5 \times 10^{-5}$, $5 \times 10^{-8}$, and $10^{-4} \text{M}$), and indomethacin (the dose of $10^{-6}$, $10^{-5}$, and $2.5 \times 10^{-5} \text{M}$).

**RESULTS**

1. Changes of ATP contents and beating rates caused by chlorpromazine

The ATP contents and beating rates in the cultured myocardial cells were measured 24 hours after the addition of chlorpromazine (CPZ). Fig. 1 shows changes in ATP contents and beating rates caused by CPZ. The ATP content and beating rate in the controls were $16.0 \pm 3.77 \text{ (mean} \pm \text{SD)} \text{ nmol/}$
mg protein and 63.7±5.26 beats/min, respectively. The ATP contents and beating rates tended to increase after the addition of 10⁻⁹ and 10⁻⁸ M of CPZ. However, the ATP contents decreased to 77.4±4.1% and the beating rate was reduced markedly to 54.2±4.0% as compared to the control values, when the incubation medium contained 7.5 μM CPZ.

Histologically the number of cytoplasmic granules increased markedly as compared to the control. However, cell arrangements and nuclei did not show appreciable changes (Fig. 2-A, B). In thin sections, the mitochondrial cristae showed normal arrangement and the matrix had a normal density in the control cultures (Fig. 3-A). By contrast, 7.5×10⁻⁶ M CPZ markedly affected the mitochondria, resulting in a decreased density of the matrix of cristae (Fig. 3-B, C). In addition, lamellated bodies appeared in the cytoplasm (Fig. 3-B, C, D). Since these lamellated bodies are surrounded by
**Fig. 4.** Effects of coenzyme Q₁₀ (CoQ) and CPZ (7.5×10⁻⁶ M) on ATP contents and beating rates of cultured myocardial cells. CoQ dose-dependently suppresses the effects of CPZ on the ATP contents and beating rates. The recovery rates are statistically significant (p<0.01). Each value shows a mean±SD. (n<11 to 16)

a double membrane, they may be derived from the mitochondria (Fig. 3-D). These findings provide an evidence that CPZ dose-dependently injures cultured myocardial cells in the rat.

2. Effects of coenzyme Q₁₀ on chlorpromazine-induced changes of myocardial cells

Different concentrations of CoQ were placed in the medium containing 7.5×10⁻⁶ M CPZ and the myocardial cells were cultured for 24 hours. The control values of ATP contents and beating rates were 16.3±2.71 (mean±SD) nmol/mg protein and 62.4±5.64 beats/min, respectively. Several con-
Fig. 5. Effects of indomethacin, coenzyme Q₁₀ (CoQ, 5×10⁻⁶ M) and chlorpromazine (CPZ, 7.5×10⁻⁶ M) on ATP contents and beating rates of cultured myocardial cells. Indomethacin dose-dependently reduces ATP contents and beating rates. Each value indicates a mean ± SD. Asterisks show significant differences (p<0.05 to 0.01). (n=11 to 16)

Centrations (5×10⁻⁶, 2.5×10⁻⁵, 5×10⁻⁵, and 1×10⁻⁴ M) of CoQ with 7.5×10⁻⁶ M of CPZ were added to each dish, and cultures were incubated for 24 hours. The addition of CoQ produced a dose-dependent antagonism of the CPZ induced suppression of ATP contents and beating rates (Fig. 4). In a concentration greater than 2.5×10⁻⁵ M of CoQ, ATP contents and beating rates increased significantly (p<0.01). Administration of 5×10⁻⁵ M of CoQ and of solvent alone did not affect the ATP contents and beating rates.

Phase contrast photomicroscopy revealed a distinct change in the cytoplasm. While the CPZ treatment (7.5×10⁻⁶ M) led to an increase in the number of cytoplasmic granules (Fig. 2-B), the addition of 5×10⁻⁵ M of CoQ to the medium decreased the number of granules in the cytoplasm (Fig.
3-C). Thus, CoQ may protect the myocardial cell from CPZ-induced damage.

3. Mechanism of the action of coenzyme Q₁₀

During treatment with CPZ and CoQ, several concentrations of indomethacin (5×10⁻⁶, 1×10⁻⁵, and 2.5×10⁻⁵ M), a potent inhibitor of prostaglandin-forming cyclooxygenase, were added to the incubation medium. Fig. 5 shows changes in ATP contents and beating rates induced by the addition of indomethacin. The value of ATP contents and beating rates were significantly reduced by indomethacin, depending on the dosage (p<0.05–0.01). Namely, the CoQ induced protective effects on ATP contents and beating rates were suppressed by indomethacin. However, these concentrations of indomethacin alone did not appreciably affect ATP contents and beating rates.

DISCUSSION

In the present study, a high dose of CPZ distinctly caused myocardial cell damage in new-born Wistar rats. Exposure of cultured myocardial cells to CPZ resulted in a decrease in ATP contents and beating rates, histological changes which were especially severe in the mitochondria, a decrease in the density of the matrix and an irregular arrangement of cristae. It has been reported that CPZ causes clusters and swelling of the mitochondria. There were also prominent lamellated structures in the cytoplasm, which may originate from the mitochondria. CPZ treatment also decreases intracellular cyclic AMP and Ca⁺⁺-binding activity in the mitochondria. And it inhibits mitochondrial respiration and membrane enzyme activity, probably through the formation of free radicals. The effects of CPZ on energy production, a decrease in ATP contents and histological changes, may lead to a decrease in the beating rates in this study.

Coadministration of CoQ and CPZ to myocardial cells results in less damage than is induced by CPZ alone. CoQ occurs in major organs of the human body and plays an important role as a coenzyme in oxidative phosphorylation in the metabolic pathway. In the ischemic myocardium, CoQ protects the electron transport system in the mitochondria. A CoQ also is an antioxidant in vitro. These effects of CoQ may stabilize the permeability of the mitochondrial membrane, thus improving mitochondrial respiration and ATP production. These protective actions of CoQ have been applied to conditions which include ischemic heart muscle, isoproterenol-induced myocardial injury, Adriamycin cardiotoxicity, neuroleptica-induced cardiotoxic-
Indomethacin, a potent inhibitor of prostaglandin-forming cyclooxygenase, suppressed the effectiveness of CoQ on CPZ-induced myocardial cell injuries in the present experiments. These findings suggest that the protective actions of CoQ are partly related to prostaglandins. The identity of the prostaglandins that are related to the action is unknown. The site of the action of indomethacin is in the metabolic pathway of arachidonic acid to prostaglandin $G_2$. At this site, indomethacin inhibits cyclooxygenase activity, leading to the inhibition of the production of prostaglandins. The free radicals produced in the metabolic pathway of arachidonic acid in the injured cells could be scavenged by CoQ, which has a role of the free radical scavenger. Therefore, the action of prostaglandins may be superimposed under the influence of CoQ. Namely, the protective action of CoQ may be implicated in prostaglandins. E-type prostaglandins protect ischemic myocardial tissue by reducing cardiac oxygen demand and stabilizing cardiac lysosomal membranes. Thus, CoQ protects myocardium from CPZ induced injury. The mechanism of the protective action of CoQ may be due to both prostaglandins and the oxidative phosphorylation in the metabolic pathway.

Acknowledgments

The author wishes to express thanks to Prof. Hironori Toshima for his encouragement during the course of this research. I would also like to thank Assist. Prof. Tsunetaka Matoba and Dr. Katsu-toshi Ohta for their comments and discussion.

References
