Cell Injury by Antineoplastic Agents and Influence of Coenzyme Q₁₀ on Cellular Potassium Activity and Potential Difference across the Membrane in Rat Liver Cells

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ABSTRACT

Potential difference across the cell membrane and intracellular activity of the potassium ion in rat liver cells were measured simultaneously using double-barreled potassium ion-selective microelectrodes.

Both potential difference across the membrane and K⁺ activity in liver cells were depressed after treatment with the antineoplastic agents mitomycin C and 5-Fluorouracil Dry Syrup, suggesting that these drugs would induce disturbances of cellular energy metabolism in liver cells.

When the antineoplastic agents were used in combination with coenzyme Q₁₀, the depression of potential difference across the membrane and K⁺ activity and the hypofunction of liver cells in energy metabolism were significantly prevented.

INTRODUCTION

In the treatment of neoplasms with antineoplastic agents, the relationship among the neoplasm, host, and antineoplastic agent must always be taken into consideration. In particular, a potent antineoplastic agent affects not only the neoplasm but also normal cells (the host cells), so that in a number of cases its use must be discontinued because of untoward side effects. It is not rare that the host cells, which have undergone some damage as a side effect, take time to recover. This would, in turn, facilitate the growth of the neoplasm, so that the cancer cells are allowed to proliferate when the drug is withdrawn. The degree of damage done to normal host cells by an antineoplastic agent must always be taken into consideration. In particular, a potent antineoplastic agent affects not only the neoplasm but also normal cells (the host cells), so that in a number of cases its use must be discontinued because of untoward side effects. It is not rare that the host cells, which have undergone some damage as a side effect, take time to recover. This would, in turn, facilitate the growth of the neoplasm, so that the cancer cells are allowed to proliferate when the drug is withdrawn. The degree of damage done to normal host cells by an antineoplastic agent has thus far been determined by histopathological studies and blood analyses.

Often, however, the cell damage has already been aggravated to an irreversible degree by the time it can be detected histopathologically or chemically. The authors have already provided experimental evidence from a functional point of view that a cytopathic change usually precedes such cell damage (21). If treatment were given to prevent further damage to the host cells in this stage, the cell damage would be cured or would remain mild and reversible.

The present investigation has been undertaken to clarify the degree of damage in the functional cell produced by the antineoplastic agents MMC² and 5-FUraDS, which inhibit the synthesis of nucleic acids. This study has also been carried out to determine the effect of CoQ₁₀ used in combination with those antineoplastic agents on such cell damage. CoQ₁₀ has been attracting attention for its facilitation of electron transport as a mobile carrier of electron flow across the inner mitochondrial membrane, thus activating the energy metabolism in the cell. Cell function has been studied by the use of the double-barreled potassium ion-selective microelectrodes developed by Fujimoto and Kubota (5). These newly developed microelectrodes enable us to measure the intracellular ionic activity of K⁺, which is essential to the maintenance of cell function and the generation of the PD, as a measure of cellular metabolic function. This paper deals with the preventive effect of CoQ₁₀ on the damage done by antineoplastic agents with respect to cellular potassium metabolism and membrane potential.

MATERIALS AND METHODS

Animals. Male Wistar rats (body weight, 150 to 200 g) were anesthetized with ether and laparotomized. A portion of the liver was isolated and sliced into sections 1 mm thick or less. The liver section was immediately placed in the chamber of the closed perfusion system shown in Chart 1. The liver sections were perfused at 31°C with Krebs-Ringer-bicarbonate solution (NaCl, 103 mM; KCl, 4.7 mM; CaCl₂, 2.57 mM; MgCl₂·6H₂O, 1.13 mM; NaHCO₃, 25 mM; NaH₂PO₄·2H₂O, 1.15 mM; d-glucose, 2.8 mM; sodium pyruvate, 4.9 mM; sodium glutamate, 4.9 mM; and sodium fumarate, 2.7 mM, adjusted to 7.4 with 0.1 N HCl or NaOH). The perfusing medium was continuously bubbled with 95% O₂–5% CO₂. All measurements were taken under conditions that were as uniform as possible.

Antineoplastic Agents. 5-FUraDS and MMC were obtained from Kyowa-Hakko Kogyo Co., Ltd. (Chiyoda-ku, Tokyo, Japan).

Double-Barreled K⁺-selective Microelectrode. The double-barreled K⁺-selective microelectrode was fabricated according to the method described by Fujimoto and Kubota (5). Only its main features will be described here. Two Pyrex glass capillaries (0.9 mm, outside diameter) were mounted in parallel on an electrode puller (PD-5 Narishige), twisted 180°, and pulled to a tip less than 1 μm in diameter. Only the tip of one side of the 2 capillaries was siliconized with 0.3% silicone oil diluted with trichloroethylene, while the other was kept hydrophilic by filling it with acetone. The hydrophilic barrel was filled with 1.0 M NaCl, while a small quantity of a liquid ion exchanger (No. 477317; Corning Glass Works) was placed into the tip of the siliconized limb of the double-barreled micropipet, and the rest (stem) of the siliconized side was filled with 0.5 M KCl. The double-barreled microelectrode thus made is shown schematically in Chart 2. The capillary filled with 1.0 M NaCl serves as a reference electrode which is used to measure the PD. The potential obtained from the siliconized barrel of the double-barreled microelectrode equals the sum of PD and Eₓ. Accordingly, the...
Chart 1. Schematic diagram of the perfusion system in rat liver slices. The liver section was immediately placed in the chamber, and the section was perfused with Krebs-Ringer-bicarbonate solution at 31°C.

Chart 2. Double-barreled K⁺ microelectrode. The capillary filled with 1.0 M NaCl serves as a reference electrode which is used to measure the membrane PD. The potential obtained from the ionic barrel of the double-barreled microelectrode equals the sum of membrane potential and chemical potential for K⁺ ($E_K$).

difference in potential between the 2 electrodes directly represents the intracellular K⁺ concentration ($a_K$) at the site where the tip of the double-barreled microelectrode is lodged. For the operational principle of the double-barreled K⁺-selective microelectrode, see the report of Fujimoto et al. (6).

To estimate the liquid junction potential between the internal reference solution of the PD barrel and the test solution, we compared the EMF of a 0.1 M KCl-filled PD barrel dipped into 2 test solutions: an artificial solution mimicking the cell fluid, consisting of 15 mM NaHCO₃, 30 mM KCl, 40 mM K₂HPO₄, 40 mM KH₂PO₄, and 10 mM glucose (cell Ringer); and a solution mimicking the EMF, a normal Ringer solution, the composition of which was described previously. The difference in EMF of the 2 solutions (i.e., the EMF in cell Ringer minus that in normal Ringer) averaged 2.73 ± 2.81 (S.D.; n = 5). We chose the double-barreled microelectrodes with a tip potential of less than 5 mV for routine use.

The tip potential for the individual electrodes was estimated at the calibration stage, and the membrane potential obtained by the PD barrel was corrected for the tip potential; i.e., the tip potential was subtracted from the recorded membrane PD and was added to the differential output of the double-barreled electrode as the net ionic potential. Further, the electrode used in this study complied with the following standards (13): (a) reactivity to K⁺ [slope of the curve of EMF (mV) arising from a 10-fold change in K⁺ activity], 50 mV or more; (b) K⁺ selectivity (reactivity to K⁺ as compared to reactivity to the same molality of Na⁺ ions), 80/1 or more; (c) response time (rise time of potential record when the microelectrode is immersed in the standard solution, i.e., the time required for the maximum reaction amplitude to change from 5 to 95%), 2 sec or less; outer diameter of the microelectrode, less than 1 μm.

The method of electrode calibration and estimation of selectivity coefficient, slope constant, etc., have been described in detail by Fujimoto and Kubota (5).

**Micropuncture Procedure and Equipment.** A liver slice 1 mm thick or less is placed in a chamber of a perfusion system. With a micromanipulator under a stereomicroscope (Leitz TS with magnifications of ×10 to ×320), the double-barreled K⁺-selective microelectrode is inserted into a cell of the liver slice perfused with Ringer solution.

The measurement and recording were performed by the method described in the paper of Kubota and Fujimoto (11). The membrane potential was measured and recorded in both digital and analog presentations. The cables from the double-barreled microelectrode were directly connected to solid-state electrometers (Keithley Instruments, Models 610C, 160, and 616). The K⁺ concentration ($a_K$) in the cell was determined by the ion-selective microelectrode from the differential output of the PD andionic barrels. Data recording was carried out by a 2-channel EPR3T polygraph (Toa Electronics). An MEI-2 optical fiber-light source (Narishige, Tokyo, Japan) was used for illumination, and the microelectrode was advanced to the specimen using hydraulic stepping micromanipulators (MO-81 combined with MM3; Narishige).

When the microelectrode is advanced into the liver slice, a cell membrane potential of about −30 mV is recorded from the output of the PD barrel, and a sudden change appears in the
differential output of the ionic barrel and in association with the change of membrane potential (15, 16).

**Calculation.** The ionic activity was calculated and the ion selectivity constant was chosen according to the methods of Fujimoto and Kubota (5) and Walker (20), using the following equation.

$$a_{ICF} = (a_{ECF} + K_ya_{ECF}) 10^{\Delta F - E_{mem}/S} - K_ya_{ECF}$$

where $a_i$ and $a_j$ are activities of $i$th and $j$th ions, $K_y$ is the $y$th ion selectivity constant as compared to the $i$th ion, $\Delta F$ is the change in potential of ionic barrel on cellular impalement (difference between values in ECF and ICF), $E_{mem}$ is the cell membrane potential, and $S$ is the slope of the curve of EMF arising from a 10-fold change in $i$th ion activity.

In practice, the $i$th ion corresponds to $K^+$ and the $j$th ion corresponds to $Na^+$ as an interfering ion with $K^+$. As stated previously, the value of $K_{Na}^{K+}$ is less than 1/80, or 0.0125 in our microelectrodes. Since the intracellular $Na^+$ activity is usually less than 30 mM, the product $K_{Na}^{Na^{K+}}$ would be less than 0.4 mM. Thus, $K_{Na}^{Na^{K+}}$ (the last term of the equation) is negligibly small, as compared with the size of the intracellular $K^+$ activity ($a_{K}^{ICF}$ on the left side of the above equation), which is usually more than 50 mM. For this reason, we neglected the last term from the calculation of intracellular activity of $K^+$. In conversion of the chemical concentration (c) to the activity (a), c was multiplied by activity coefficient, $\gamma$; i.e., $a = c \cdot \gamma$. The activity coefficient was derived from the Debye–Hückel equation [see Robinson and Stokes (18)]. The numerical analysis depended on the work of Croxton (2), in which the values were expressed as mean ± S.D., and the significance of difference was tested by Student’s t test on ungrouped data with unequal variances according to the method of Kendall (9).

**RESULTS**

**Effect of CoQ10 on Sarcoma Solid-type Tumor.** In determining the effect of CoQ10 on tumors, such as Sarcoma 180 treated with MMC, the following criterion was used. Although CoQ10 can exhibit a preventive effect on the liver cell damage which is otherwise produced by an antineoplastic agent, it must be considered to be ineffective if it eventually allows the tumor to continue to grow.

A total of 41 male ddN mice, 4 weeks old (body weight, 20 g or less), were used in this experiment, in which Sarcoma 180 (solid-type tumor 3 mm in diameter) was transplanted in the right axillary region in each animal. About 24 hr after transplantation, the animals were divided into the following 3 groups: control group (n = 14), 1 ml of 0.85% NaCl solution administered i.p. for 10 consecutive days; MMC + CoQ10 group (n = 13), MMC and CoQ10 administered in doses of 1 and 3 mg/kg, respectively, for 10 consecutive days; MMC + placebo group (n = 14), MMC and placebo administered in doses of 1 and 3 mg/kg, respectively, for 10 consecutive days.

After 10 days of treatment, the animals were sacrificed, and the tumors were removed and weighed. The weight of Sarcoma 180 in the control group was 0.28 ± 0.19 g (n = 14), that of the MMC + CoQ10 group was 0.15 ± 0.14 g (n = 13), and that of the MMC + placebo group was 0.10 ± 0.17 (n = 14). The difference in sarcoma weight was significant between the control and MMC + CoQ10 groups at $p < 0.05$. The difference between the control and MMC + placebo groups was also significant at $p < 0.05$. These results indicated that CoQ10 did not affect the antineoplastic action of MMC. On the other hand, there was no significant difference in sarcoma weight between the MMC + CoQ10 and the MMC + placebo groups. This also indicated that CoQ10 did not adversely affect the antineoplastic action of MMC.

The experiment with 5-FUraDS and Sarcoma 180 produced much the same results as those obtained with MMC.

**Effects of MMC and CoQ10 on the Liver Cell.** Chart 3 shows the PD and $a_K$ which were measured after 4 days of treatment with MMC (1 mg/kg; n = 44).

In the control group, the mean PD was $-27.4 ± 2.4$ mV (n = 53), and the $a_K$ was $95.5 ± 6.9$ mM (n = 53); whereas, after 4 days of treatment with MMC (1 mg/kg), the PD was $-23.6 ± 2.5$ mV (n = 44) and the $a_K$ was $93.6 ± 8.6$ mM (n = 44). The differences in PD and $a_K$ between the 2 groups were significant at $p < 0.005$. These data indicate that treatment with MMC produced a fall of the intracellular $a_K^+$, on the average, by $-11.9$ mV and of the membrane PD by 3.8 mV. This suggests that $K^+$ loss in the liver cell was produced by the administration of MMC in association with a concomitant fall of the membrane PD, a finding which is in agreement with an impairment of liver cell activity.

The PD and $a_K$ were measured in the group given MMC (1 mg/kg; n = 32) and CoQ10 (3 mg/kg; n = 32) for 4 days and in the group given MMC (1 mg/kg; n = 32) and placebo for 4 days.

The results are shown in Chart 4. The PD of the MMC + CoQ10 group was $-25.9 ± 2.0$ mV, and the $a_K$ was 99.2 ± 10.4 mM (n = 32); while the PD of the MMC + placebo group was $-23.4 ± 2.2$ mV, and the $a_K$ was 83.5 ± 8.3 mM (n = 32). The differences in PD and $a_K$ between the 2 groups were 2.5 mV and 14.2 mM, respectively, both of which were statistically significant at the 0.1% level, suggesting that the admin-

**Antineoplastic Effect of CoQ10**

**Chart 3.** Effect of MMC on membrane potential and potassium activity in rat liver cells. The membrane potentials and potassium activities were measured after 4 days of treatment with MMC (1 mg/kg; n = 44). The membrane potentials on the ordinate are plotted against the potassium activities on the abscissa. Cross-bars: intersection, mean value for each group; length, S.D. mM/L, mmol/liter.

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istration of CoQ₁₀ and MMC was effective in preventing the cell damage which would be induced by MMC alone.

Effects of 5-FUraDS and CoQ₁₀ on the Liver Cell. Chart 5 shows the effects of 5-FUraDS (20 mg/kg; n = 31) on the PD and aK after 7 days of treatment. In the control group, the PD was -27.4 ± 2.4 mV and the aK was 95.5 ± 6.9 mm; while in the 5-FUraDS group, the PD was -22.6 ± 1.5 mV, and the aK was 83.2 ± 9.1 mm. Both PD and aK became significantly depressed in the 5-FUraDS-treated group as compared to the values obtained in the control group (p < 0.005). This fact would suggest that liver cell function was impaired by the administration of 5-FUraDS, because the membrane PD was depressed by 4.8 mm and the aK was lowered by 12.1 mm.

In order to know the preventive effect of CoQ₁₀ on the impairment of liver cell function, the PD and aK were compared for rats treated with CoQ₁₀ and nontreated rats, both of which were affected commonly by administration of the antineoplastic agent (5-FUraDS). The animals were given 5-FUraDS (20 mg/kg) with CoQ₁₀ (3 mg/kg; n = 40) and without CoQ₁₀ or placebo (n = 27). The values obtained in these groups are plotted in Chart 6. The PD in the 5-FUraDS + CoQ₁₀ group was -25.1 ± 2.0 mV, and the aK was 97.6 ± 7.9 mm, while the corresponding values for the 5-FUraDS + placebo group were -21.5 ± 1.5 mV and 87.5 ± 8.8 mm, respectively. The differences in PD and aK between the 2 groups were significant at p < 0.001. These values suggested that CoQ₁₀ inhibited the depression of PD and aK.

DISCUSSION

Physiological function of the liver cell still remains unclear in many respects, as compared to the information on the morphological and biochemical aspects of the liver. The majority of investigations which have been carried out thus far on the liver cell damage induced by various antineoplastic agents were done by morphological or biochemical means, and little work has been done on the functional aspect or by physiological means. This is mainly due to the fact that the pathophysiological method at the cell level has not yet been fully established.

Recently, it has become possible to measure intracellular K⁺, Cl⁻, Na⁺, and pH in situ with the aid of microelectrodes, as well as PD. Application of this method to the liver would provide new information on the ion transport mechanism across the membrane and intracellular electrolytes in the interior of the liver cell.

Walker (20) was the first investigator who developed the K⁺-selective microelectrode using a K⁺ ion exchanger and described its operational principle. He was soon followed by Lux and Neher (12) and Khuri et al. (10). Fujimoto et al. (7), on the other hand, developed the double-barreled ion-selective micro-electrode for use in the measurement of K⁺ and Cl⁻ and recently developed one for use in the measurement of Na⁺ and pH. They widened the range of biological application of their microelectrodes.
The use of the double-barreled $K^+$-selective microelectrode enables us to measure both the $PD$ and $a_K$ of the liver cell simultaneously and continuously. It has been conceived that the membrane $PD$ is generated by the outward movement of $Na^+$ across the membrane (3, 8, 14, 19). The intracellular and extracellular compartments are generally polarized, the former being negatively charged. The active process of the cell membrane acts such that $Na^+$ ions are pumped out of the intracellular compartment, while $K^+$ ions enter it. This action of the cell membrane has been found universally in almost all cells. The liver cell, which is one of the gland cells, is by no means an exception. The active transport of $Na^+$ and $K^+$ ions across the cell membrane is often referred to as the "Na$^+$-K$^+$ exchange pump," and it would serve for the maintenance of the ICF and ECF environment of $Na^+$ and $K^+$, and hence the membrane $PD$. In the present study, we examine the effect of MMC and 5-FUraDS, which are in wide clinical use as inhibitors of nucleic acid synthesis, on cellular $K^+$ and $PD$. After 4 days of treatment with MMC at a dose of 1 mg/kg, both the $PD$ and $a_K$ became significantly depressed as compared to the levels in the control group ($p < 0.001$). In other words, the energy metabolism in the liver cell was suppressed in the animals given MMC. With 5-FUraDS, on the other hand, both the $PD$ and $a_K$ became significantly depressed after 7 days of treatment as compared to the levels in the control group ($p < 0.005$). Similarly, it was postulated that, as a result of cell damage done by 5-FUraDS, the ion transport as well as the energy metabolism was inhibited.

CoQ$_{10}$, a quinone derivative with 10 isoprenoid chains, has been recognized as an activator of metabolism in animal tissues. This compound was initially isolated from the mitochondria of beef heart muscle by Crane et al. (1) in 1957, and it is now known to be distributed ubiquitously in all the cells in which mitochondria are present. As well as cytochrome $b$, CoQ$_{10}$ is located in the lipid layer of the inner mitochondrial membrane, playing an important role in transferring electrons from NADH or reduced flavin-adenine dinucleotide to cytochrome $b$ in the respiratory chain; therefore, it can necessarily be involved in the oxidative phosphorylation or the synthesis of ATP (4). Therefore, under conditions in which electron transport has been disturbed, the supply of CoQ$_{10}$ would benefit the damaged cell which lacks metabolic energy. In the present study, the effect of CoQ$_{10}$ on ion transport in the liver cell was investigated after combined use with antineoplastic agents, and evidence was provided to support the above-mentioned activities of CoQ$_{10}$. As already shown in Chart 4, the $PD$ and $a_K$ of the MMC + CoQ$_{10}$ and MMC + placebo groups were significantly depressed, as compared to the values obtained in the control group at $p < 0.001$; with 5-FUraDS, a similar tendency was observed, as shown in Chart 6.

The results obtained in this study may suggest that CoQ$_{10}$ would probably activate the Na$^+$-K$^+$ exchange pump by increasing the production of ATP. Accordingly, it seems that CoQ$_{10}$ is a useful drug in the prevention of liver damage by antineoplastic agents.

In conclusion, $PD$ and $a_K$ were measured by the use of double-barreled $K^+$-selective microelectrodes. Both the $PD$ and $a_K$ became depressed after treatment with MMC or 5-FUraDS, suggesting that these antineoplastic agents suppressed energy metabolism in the liver cell. When CoQ$_{10}$ was combined with these antineoplastic agents, the depression of $PD$ and $a_K$ was significantly suppressed. In other words, CoQ$_{10}$ seemed to prevent the depression of energy metabolism in the liver cell by the antineoplastic agents. Thus, the combined therapy of antineoplastic agents with CoQ$_{10}$ would help to prevent the side effects of the antineoplastic agents.

Acknowledgments

The authors thank Eisai Co. (4-8-10, Koishigawa, Bunkyo-ku, Tokyo, Japan) for a gift of CoQ$_{10}$ injections and CoQ$_{10}$ solvent used as a placebo in this investigation.

References

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