Heart Toxicities: Cultured Myocardial Cell Injury Induced by Adriamycin and Its Regulation

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Cultured myocardial cells show various interesting functions, resembling those of heart muscle tissue *in vivo*. Cultured myocardial cells are anatomically and functionally separated from nerves, connective tissue and blood vessels, and moreover, they are in indirect contact with the extracellular medium. Therefore, cultured myocardial cell systems are very simple and useful model for assaying the direct effects of cardioactive agents and for studying on cell-cell communications. In many instances it has been generally accepted that the force and rate of rhythmic contractions observed in cultured myocardial cells are good indicators of functional integrity of the cells. We can monitor easily the spontaneous beating modes and rates of myocardial cells by using the optical video dimension analysis system. Furthermore, at the present time, the changes in cytosolic free calcium concentration ($[Ca^{2+}]_i$) of cultured myocardial cells can also be measured by using a fluorescent probe such as fluo-3.

In this workshop, we describe first the basic properties of cultured myocardial cells. Additionally, we also discuss the mechanisms of genesis of asynchronous beating of cultured myocardial cells induced by adriamycin.

1) The basic properties of cultured myocardial cells

When heart tissues obtained from mouse and quail are dissociated to their component cells with the aid of trypsin and these cells are cultivated in Petri dishes, all the cells are attached and spread on the surface of the dishes within 10 hrs. Single isolated myocardial cells are completely separated from neighboring cells and no impulses are conducted between cells, so each cell beats independently. Their beating rates vary widely from 10 to

250 beats/min in standard medium at 37 ± 1 °C. On the other hand, it is well known that when cultured myocardial cells come into contact each other, gap junctions having low electrical resistance are formed in the regions of cell to cell contact. Therefore, the myocardial cell sheet having gap junctions beats synchronously, and its beating rates are stable in a range of 100 to 180 beats/min.

Cultured myocardial cells also show the same electrophysiological properties as adult heart. Even if myocardial cells were obtained from ventricular myocytes, they exhibit transmembrane potentials similar to those of pacemaker cells.

These basic and characteristic properties of cultured myocardial cells as described above are often applied to study the toxic effect of compounds such as drugs, food additives, industrial chemicals, and so on.

2) Cultured myocardial cell injury induced by adriamycin and its regulation

Adriamycin (doxorubicin, ADM), an anthracycline antibiotics, is well known as one of the most widely used antineoplastic agents for the treatment of various tumors. However, the therapeutic use of this agent is limited by its undesirable cardiotoxicity. ADM has been reported to induce many biological and physiological changes in the heart. However, there is as yet no direct evidence that some of these changes result in cardiac lesions produced by ADM. In this study the toxic effect of ADM on the heart has been developed in cultured fetal mouse ventricular myocytes.

ADM added to the culture induced a decrease in beating rates and an increase in the number of arrhythmic beating cells in cultured myocardial cell sheets. When incubated with 35 μ M ADM over 10 hrs, many of the cell sheets began to beat not only irregularly but also asynchronously. Fig.-(A) shows an example of asynchronous beating 12 hrs after incubation with 35 μ M ADM. As shown with arrows in the figure, the myocardial cells (a) and (b) in the same cell sheet occasionally showed asynchronous beatings. At these points, any morphological abnormality of cultured myocardial cells was not observed under a transmission electron microscope. Moreover, with exposure to ADM, there was a significant increase in the diastolic $[Ca^{2+}]_i$

without affecting the systolic $[Ca^{2+}]_i$. ADM-induced asynchronous beating disappeared dramatically after loading of 0.5 μ M quin-2-AM but not 0.5 μ M fluo-3-AM as an intracellular Ca^{2+} chelating agent (Fig.-(B)). Therefore, the asynchronous beating by ADM was suspected to be due to an increase in $[Ca^{2+}]_i$, which induces the closing of the channels of gap junctions between myocardial cells.

The present results suggest that the asynchronous beating induced by ADM may be a trigger reaction toward lethal cardiotoxicity of ADM.

Fig.