ORIGINAL ARTICLE

EFFECTS OF CADMIUM CHLORIDE ON ISOLATED RAT HEPATOCYTES

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SUMMARY

The cytotoxic effects of cadmium chloride was studied in isolated rat hepatocytes. Cadmium chloride appeared to have the direct toxic effects on plasma membrane integrity as shown by the leakage of cytoplasmic enzymes and intracellular potassium. The metabolic capability of isolated cells was also effected by cadmium chloride. There was the sign of inhibition on carbohydrate metabolism especially the gluconeogenesis. These toxic effects of cadmium chloride were dose-related and could be detected as early as 30 minutes of incubation.

It has been known for many years that cadmium is toxic to man and other animals. After its administration, cadmium is accumulated initially in the liver (Friberg et al,1974). Cadmium uptake by hepatic parenchymal cells is approximately half of the administered dose (Perry et al, 1970). Acute liver damage will result if the dose is high enough, thus, the liver is a major target organ for acute cadmium toxicity(Dudley et al, 1982).

Isolated hepatocytes have been employed recently as a very effective and expeditious means of in vitro screening for various toxic agents (Klaassen and Stacey 1982 ; Cantilena et al, 1982 and 1983). Many hepatotoxins and cytotoxic agents can be elucidated their toxic mechanisms and effects by using this in vitro model.

The purpose of the present investigation was to study the utility of isolated rat hepatocytes as a model system for testing the

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cytotoxic effect of cadmium chloride. Cadmium chloride was selected on the basis of its known toxicity in vivo. The indices used for measure early cadmium cytotoxicity were the membrane and metabolic integrity of the isolated cells. These criterion were more sensitive than routine nonspecific measures, such as cellular protein content which detected only the later stage of cell injury (Santone and Acosta 1982).

MATERIALS AND METHODS

Male Wistar rats (200-250 g) from Mahidol University Central Animal House were used as liver donors. They were allowed free access to food (charlick's M and V mouse cubes) and water. Some experiments involved food deprivation(24 hours fasting). Surgery was performed between 8.30 - 9.30 a.m. for each experiment. Under ether anesthesia hepatocytes were isolated by the method of Berry and Friend(1969) with some modifications by stacey and Priestly(1978). Liver was perfused with Ca^{+2}-free physiological solution(96 mM NaCl, 1.4 mM KCl, 0.7 mM $MgSo_4$, 2.5 mM KH_2PO_4, 30 mM $NaHCO_3$ and 21.7 mM sodium gluconate, equilibrated with 95% O_2/5% CO_2 at pH 7.4) via the portal vein. When the perfusion of all hepatic lobes was rapid and complete, the liver was perfused with 100 ml of 0.05%(w/v) collagenase (sigma Type IV) in the same calcium free buffer equilibrated with carbogen (95% O_2/5% CO_2), under the recirculating condition. Flow maintained at 30-35 ml/min with a pressure head of 20 cm of water, and temperature was maintained at 37° C throughout the procedure.

After perfusion with collagenase(10-15 minutes), the liver was dispersed with a blunt spatula in 50 ml of fresh collagenase buffer(0.05%)

and incubated(37°C, 80 oscillations/min) for 10 minutes. Bovine serum albumin(BSA) was added to give a final concentration of 12 mg/ml and cells were harvested through nylon mesh(250 μm followed by 61 μm). Hepatocytes were separated from other cells and cellular debris by differential centrifugation(50 g, 1 minute). The resulting cell pellet was washed twice with this fresh physiological medium containing 12 mg/ml BSA and once with the incubation medium (Krebs-Henseleit physiological solution with 12 mg/ml BSA). The final cell pellet was resuspended in the incubation medium at the concentration of approximately $4\text{-}5\times10^{6}$ cells/ml. Viability of freshly isolated hepatocytes was determined routinely by the trypan blue exclusion test (Seglen, 1976). Cell preparations with a trypan blue exclusion index of less than 90% were never used in any studies.

Hepatocyte suspensions(3 ml) were incubated with various concentrations of cadmium chloride(1,5,10,25,50 and 100 μM, respectively) for 30 minutes at 37°C. Aliquots(0.5 ml) of cell suspension were centrifuged at 50 g for 1 minute. ALT activity in the supernatant was determined by the method of Reitman and Frankel(1957). The cell pellet was extracted with 1 ml 3%(w/v) perchloric acid(PCA), then centrifuged at 3500-5000 r.p.m. for 5-10 minutes. The resulting supernatant was diluted with distilled water and the K^{+} concentration determined by flame photometer.

The gluconeogenesis was used as the index for metabolic capability of isolated hepatocytes. Using the method of Cornell and Filkins(1972), rats were fasted 24 hours prior to isolation. Cell suspensions(3 ml) were preincubated with cadmium chloride(1,5,10,50 and

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100 μM, respectively) for 30 minutes (at 37°C) before the incubation with 10 mM lactate at 37°C for 60 minutes. Samples were deproteinized with 1 ml each of 1.8% barium hydroxide and 2.0% zinc sulfate, then centrifuged at 1000 g for 2-3 minutes. Aliguots 1 ml of supernatant were analyzed for glucose using the o-toluidine method.

Hepatocytes were isolated from individual rat livers and each data point represents the mean value of 3-5 separate preparations, within each experiment, replicates were used to generate the mean values. The statistical significance was determined by student t-test (probability, $p = 0.05$) using either a paired or unpaired test according to the experimental design.

RESULTS

Incubation of isolated rat hepatocytes with varying concentration of cadmium chloride (1-100 μM) resulted in a cytotoxic response as measured by the parameters of membrane integrity, namely intracellular K^+, release of cytoplasmic enzyme (ALT) and the trypan blue exclusion index. Cadmium chloride at low concentration (1 μM) did not affect any of these parameters (Figs 1).

A statistically significant release of intracellular K^+ was seen with cadmium concentrations of 5-100 μM as compared to control (Fig. 1A). Similar result was obtained with the release of ALT (Fig. 1B). Trypan blue exclusion index was the least sensitive test as the effect of cadmium was seen at the concentrations of 25-100 μM (Fig. 1C).

Effect of cadmium chloride on metabolic capability of isolated hepatocytes as measured by the gluconeogenesis (using lactate as the

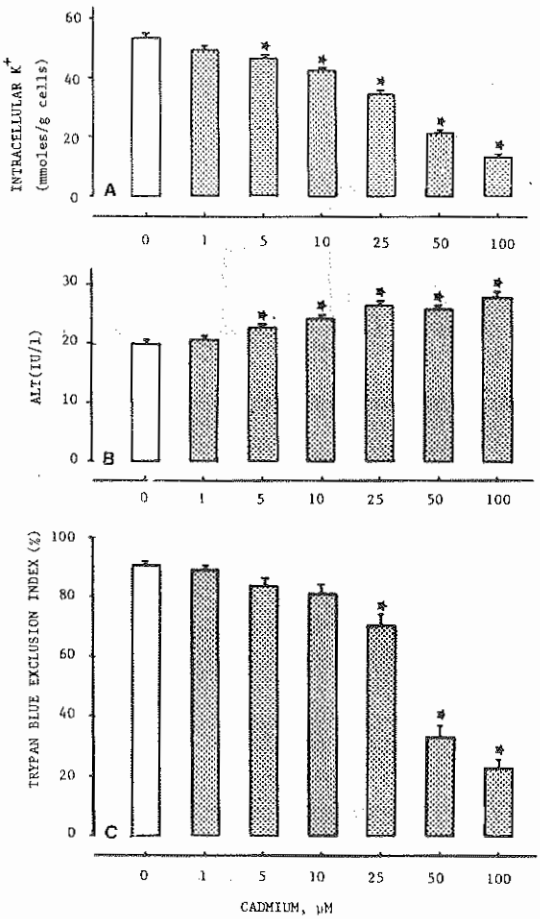

Fig.1. The effect of cadmium chloride on cell membrane integrity.
A. The release of intracellular K^+
B. The release of cytoplasmic enzyme (ALT)
C. Trypan blue exclusion index
Hepatocytes were incubated with cadmium chloride at the concentration of 1-100 μM for 30 minutes. Values represent mean ± S.E.(n = 5).
*Significantly different from control ($p<0.05$)

substrate for the generation of glucose) was shown in Fig 2. Gluconeogenesis was inhibited by cadmium at the concentrations of 1-100 μM. These effects of cadmium on both plasma membrane integrity and metabolic capability of isolated hepatocytes appeared to be a dose-related manner, as the cytotoxicity increased when increased dose of cadmium chloride (Figs 1,2).

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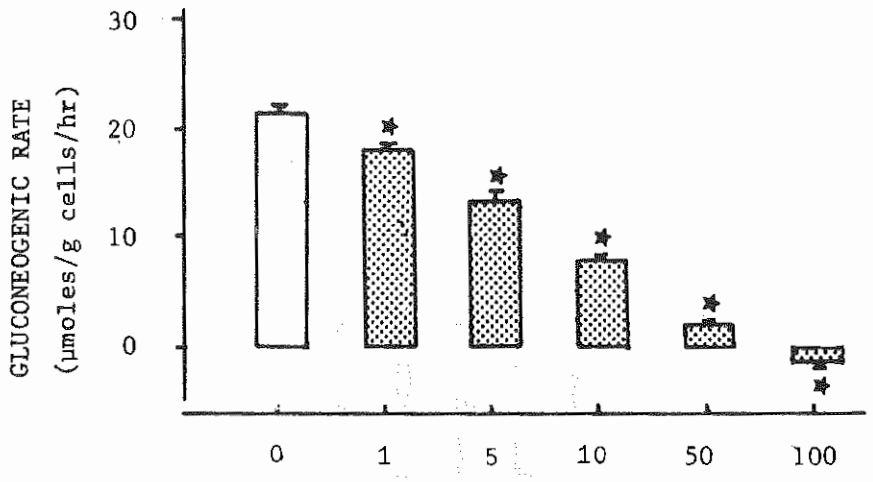

Fig 2. The effect of cadmium chloride on metabolic capability of isolated rat hepatocytes.
Hepatocytes from 24 hours fasting rats were incubated with cadmium concentrations of 1-100 µM, then the gluconeogenesis was determined. Values represent mean ± S.E.(n = 5).
* Significantly different from control ($p<0.05$)

DISCUSSION

Cadmium has been reported to have cytotoxic properties by other investigators(Olsen and Jonsen, 1979 ; Stacey et al, 1980). Most of these studies used higher concentrations of cadmium than the present experiments. It is proposed that such cytotoxic effect of cadmium may due to its effect on cell membrane(Fleisher et al, 1975 ; Kopp and Hawley, 1976) and this toxic response is not caused by the lipid peroxidation (Stacey et al, 1980).

Plasma membrane of isolated hepatocytes have the ability to retain intracellular K^+ and cytoplasmic enzymes(e.g. lactate dehydrogenase, ALT) and the ability to exclude substances which should normally be impermeable(e.g. trypan blue) (Seglen, 1976). In the present series of experiments, cadmium altered these properties of cell membrane by causing the leakage of intracellular K^+ and cytoplasmic enzyme(ALT). Trypan blue

exclusion index was also affected by the administered cadmium. This suggested that cadmium may have the direct toxic effect on cell membrane of isolated rat hepatocytes.

The inhibitory effect of cadmium on gluconeogenesis was correlated well with its effect on plasma membrane integrity. It should be noted that cadmium at the concentrations of 5-100 μM affected both plasma membrane integrity and metabolic capability of isolated hepatocytes. These effects of cadmium were aepended on administered dose in that toxicity was increased when increased dose.

In conclusion, cadmium may have the direct toxic effects on isolated rat hepatocytes and isolated rat hepatocytes prove to be a very useful and economical model in the studies of toxicity induced by chemicals in the environment.

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