Benzo-(a)-Pyrene-induced Chemiluminescence Emission in a Primary Culture of Rat Parenchymal Hepatocytes

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Benzo-(a)-Pyrene-induced Chemiluminescence Emission in a Primary Culture of Rat Parenchymal Hepatocytes

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Summary

Benzo-(a)-pyrene (BP) is known to emit chemiluminescence (CL) in the postmitochondrial supernatant of liver homogenates supplemented with NADPH. In this study, we tried to detect CL emission in a primary culture of rat parenchymal hepatocytes. BP was dissolved in dimethylsulfoxide (DMSO) and dropped directly into the petri dishes cultivating the liver cells. We discovered that the resulting CL had an intensity that changed as a function of the incubation time.

PCB-pretreated hepatocytes emitted a significantly stronger CL than non-PCB-pretreated ones. This suggests that the CL resulted from the metabolic reaction of BP with PCB-induced enzymes. The CL intensity from 6-day-old cultured cells was about 10 times stronger than that from 3-day-old cultured cells. This result suggests that the emissive ability of the cultured hepatocytes changes dramatically over that period.

It is well known that a significant CL emission is observed during the metabolic reaction between BP and arylhydrocarbon hydroxylase when this reaction is induced in microsomes 1) or in a post-mitochondrial supernatant supplemented with NADPH 2). When BP is metabolized in such cell-free systems, many types of intermediate metabolites are produced from BP 3), 4). Of these typical intermediate metabolites, one that is able to emit CL is 7, 8-diol-9, 10-dioxetane 5), 6). Recently, we detected a red-colored CL emission from BP, and we suggested that this CL originates from singlet oxygen 7).

On the other hand, there have been but few reports that CL emission can be detected from isolated cells of from cell culture systems. One example is the report by Cadenas *et al.* that oxygenation of isolated hepatocytes leads to an increase in low-level chemiluminescence and to an accumulation of malondiablde-hyde 8) in a manner similar to that described for liver homogenates 9). Moreover, 4-hydroxynonenal enhances the O_2-induced CL of isolated hepatocytes in a

concentration-dependent manner, but this enhancement is not associated with further accumulation of malondialdehyde 10).

Recently, our co-workers reported that N-methyl-N′-nitro-N-nitrosoguanidine can induce CL in a cell culture system derived from Chinese hamster fibroblasts 11). However, nothing is known about CL emission when BP is added to an isolated cell system or to a cell culture system.

In this report, we tried first to determine whether CL is induced by adding BP to a primary liver cell culture system and then to determine the nature of that CL.

Materials and Methods

In all experiments, male Sprague-Dawley rats (B.W. 200 g) were used. Induction of enzymes in rat liver that would metabolize BP was achieved using PCB (KC500) according to the method of Yahagi *et al.* 12).

The techniques used in this study for isolating hepatocytes and for producing a primary cell culture were described in detail in our previous report 13). Briefly, a cannula was inserted as quickly as possible through an incision in the portal vein of an anesthetized and abdominally operated rat, and the blood was washed from the vein by perfusion with phosphate-buffered saline solution (pH 7.2). Immediately, the subhepatic inferior vena cava was tied off and cut open, and the washed-out blood and pre-perfusion buffer were discarded. A peristaltic pump operating at a flow rate of 30 ml/min was used to perfuse the liver at a constant rate for 4–5 min.

Then, the phosphate buffer was replaced by 0.05% collagenase solution (pH 7.5) maintained at 37°C. The perfusion was replaced by 0.05% collagenase solution (pH 7.5) maintained at 37°C. The perfusion was halted when 30 ml had been perfused out of the 50 ml collagenase solution that have been prepared. The live was then carefully removed from the body and placed in a dish.

After the remaining 20 ml of the 0.05% collagenase solution had been added to the dish, the liver was cut into pieces using a razor blade. The solution containing the dispersed hepatic cells was then poured into a conical flask and shaken with the same concentration of collagenase solution for 15 min at 37°C. After that, the cell suspension was filtered through a gauze and a nylon mesh.

The cells collected by this method were cooled with ice, and centrifuged at 50 g for one minute. After the supernatant had been removed, the precipitated cells were resuspended in CMF-PBS solution. This process was repeated until a virtually clear supernatant was obtained. After that, precipitated cells were resuspended in William's Medium mixed with insulin (10^{-6} M) and 5% of newborn calf serum. The cell suspension ($3\times10^{5\#}$ cells/ml) was poured into 35 mm dishes and cultured at 37°C in an atmosphere of 5% CO_2-95% air. The medium

was changed on alternate days. Three or six days later, these cell cultures were used as the material in which CL was induced.

CL was measured at 37°C using a single photoelectron counting apparatus, namely a Chemiluminescence Analyzer OX-71 manufactured by Tohoku Electronic Industries Co., Sendai, equipped with a Hamamatsu R878 photomultiplier with a special response range of 300-650 nm (sensitivity maximum at 400 nm). After the medium had been removed, 2 μmol of BP dissolved in dimethyl sulfoxide was dropped into each cell culture dish, and it was then incubated in the OX-71. All experiments were carried out in a dark room.

Data was analyzed by an analysis of variance (ANOVA) followed by a paired t-test to assess the difference between groups.

Results and Discussion

Typical patterns of chemiluminescence induced by BP in primary liver cell culture systems are shown in Fig. 1. The CL intensity changed with incubation time in a sigmoid fashion whether or not the cells in which the induction drug was metabolized by enzymes were treated with PCB. With or without PCB pretreatment, CL emission reached a maximum 7-8 min after the start of the incubation period, then plateaued, and finally began a gentle decline. The hepatocyte cell culture group in which the drug-metabolizing enzymes were induced by PCB emitted a significantly stronger CL than those not treated with PCB. This result suggests that the process by which CL is emitted during the metabolic reaction

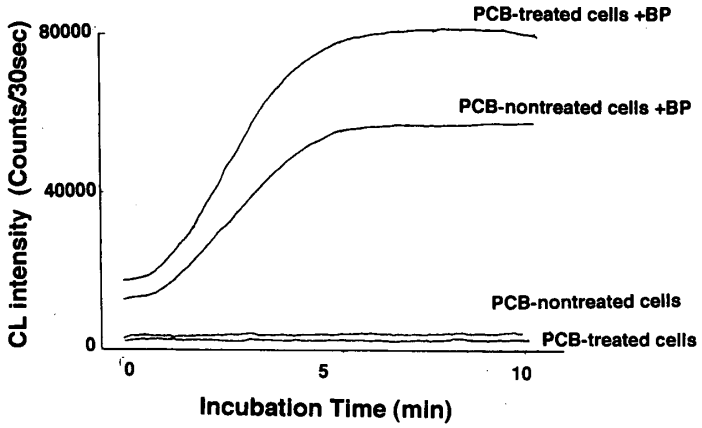

FIG. 1. Dependence on Incubation Time of BP-induced CL Intensity in 6-Day-old Primary Cell Culture Systems Derived from PCB-treated or PCB-nontreated Hepatocytes.
Activated groups were treated with PCB (KC500) 3 days before the separation of the hepatocytes. The methods used for CL measurement and primary liver cell culture are described in Materials and Methods. Results shown are from a single experiment that was representative of the five experiments conducted.

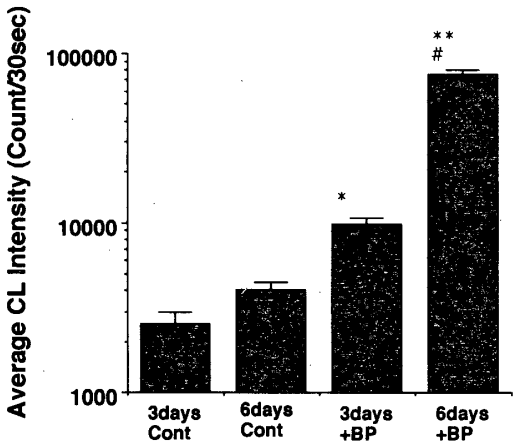

FIG. 2. The Average CL Emission over a 2 min Period (i.e. Between 3 and 5 min from the Initiation of the Metabolic Reaction Involving BP) in 3-Day-old and 6-Day-old Primary Cultured Hepatocytes.
The height of each column represents the mean of triplicate measurements of average CL intensity, with the bar representing the SD. Significance levels from paired *t*-tests : **, $P<0.001$, for the difference from control. *, $P<0.01$, for the difference from control. #, $P<0.001$, for the difference from 3 days + BP.

between BP and drug-metabolizing enzymes is much like that involving cytochrome P-450, epoxidehydrase, and so on. The CL intensity induced by BP was averaged over the 2 min period from 3 to 5 min after the start of the incubation phase in 3-day-old and 6-day-old cultured hepatocytes. These results are shown in Fig. 2. In both cases, addition of BP led to a significantly stronger CL intensity than that seen in a control group (in which only DMSO was added). In the 6-day-old cultured cells, CL intensity was about 10 times stronger than in the 3-day-old cultured cells.

In the 3-day-old culture, the hepatocytes formed a monolayer, and the number of cells was at a maximum. On the other hand, there was fewer hepatocytes in the 6-day-old culture, and the cells formed a bilayer or aggregation (data not shown). These formational changes between day 3 and day 6 in culture may have been a causal relation to the dramatic change in CL intensity.

From these results, it is suggested (i) that the chemiluminescence induced by BP in primary liver cell culture is emitted as a consequence of the reaction between the drug and the metabolizing enzymes induced by PCB and (ii) that the CL intensity depends to a large extent on the condition of the cultured hepatocytes.

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