Spontaneous Ultra-Weak Photon Emission and Delayed Luminescence during Carbon Tetrachloride-Induced Liver Injury and Repair in Mouse

Jungdae Kim,*[,a] Jaekwan Lim,[a] Byung-Cheon Lee,[a] Yong-ung Kim,[b] Seung Ki Lee,[b] Byeung Soo Cheun,[c] and Kwang-Sup Soh[a]

[a]*Biomedical Physics Laboratory 25–414, School of Physics, Seoul National University, San 56–1, Sillim-dong, Gwanak-gu, Seoul 151–747, Korea,* [b]*College of Pharmacy, Seoul National University, San 56–1, Sillim-dong, Gwanak-gu, Seoul 151–742, Korea, and* [c]*Department of Molecular Biology, College of Medicine, Kyung Hee University, 1, Hoegi-dong, Dongdaemun-gu, Seoul 130–701, Korea*

(Received October 13, 2004; Accepted November 30, 2004)

Spontaneous ultra-weak photon emission and delayed luminescence were measured from the mouse liver injured by carbon tetrachloride (CCl_4), a hepatotoxic chemical. After carbon tetrachloride in olive oil (4 ml/kg) was injected intraperitoneally into ICR mouse, spontaneous photon emission and delayed luminescence using metal halide lamp was measured from the excised liver. Twenty-four hours after injection, spontaneous photon emission from the livers was 69.3 ± 21.2 counts/min/cm^2, which was two times higher than that from controls of 29.5 ± 5.9 counts/min/cm^2. However, 72 hr after injection, spontaneous photon emission from the livers was lowered to 37.0 ± 14.9 counts/min/cm^2. These observations were closely correlated with those of the activities of aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT), which were released in the course of hepatocellular death. Delayed luminescence also showed clear distinction in its time course of relaxation between the carbon tetrachloride-treated and control groups. On the basis of these observations, we suggest that these photon emissions are involved in the process of death and/or proliferation of liver cells after acute exposure to carbon tetrachloride with sublethal doses. Further, these photon emissions might be originated from the process of lipid peroxidation and consequent radical scavenging by antioxidant enzymes in injured liver tissue. This model study might provide a basis for the analysis of hepatotoxicant induced liver injury and repair by means of measurements of the photon emissions.

Key words ——spontaneous photon emission, mouse liver, carbon tetrachloride, delayed luminescence

INTRODUCTION

For several decades, ultra-weak photon, often called bio-photon,[1)] emissions of various biological systems have been measured in studies of their physiological and pathological states. Biological systems for measurements of bio-photon emissions have been covered from the cellular levels to human bodies.[2–7)] Although there was still controversy over the origins of bio-photon emission, it was widely accepted that free radical reactions may play an important role for very weak photon emissions.[8)] Radical reactions can be produced by biological events such as lipid peroxidation. In the studies of microsomal lipid peroxidation,[9,10)] they showed that the biochemical data were related to the intensity of emitted light. It has been proposed that oxygen dependent light emission in rat liver nuclei resulted from lipid peroxidation in the nuclear membrane.[11)] Free radical decomposition of lipid hydroperoxides produced either singlet molecular oxygen or excited carbonyl groups which had chemiluminescent properties. Another interesting model for the mechanism of bio-photon emission[12)] was suggested that there was a negative feedback-loop in living cells which coupled together states of coherent ultra-weak photon field and the conformational state of the cellular DNA. Cells and organisms which were exposed to toxic substances showed different responses in photon emission dependent on the amount, type and expo-

*To whom correspondence should be addressed: Biomedical Physics Laboratory 25–414, School of Physics, Seoul National University, San 56–1, Sillim-dong, Gwanak-gu, Seoul 151–747, Korea. Tel.: +82-2-878-9172; Fax: +82-2-880-7697; E-mail: jade@phya.snu.ac.kr

sure time of toxin as well as on the organism investigated.[13–16] As a hepatotoxicant, carbon tetrachloride (CCl_4) has frequently been used in models for free radical damage in animal liver. This hepatotoxicant is known to be reductively bio-activated by cytochrome P450 into a trichloromethyl radical ($\cdot CCl_3$), which was subsequently converted into a peroxyl radical ($\cdot OOCCl_3$) in the presence of oxygen. The free radical initiates lipid peroxidation by abstracting a hydrogen atom from the polyunsaturated fatty acid of a phospholipid.[17]

In this paper we investigated properties of endogenous and exogenous ultra-weak photon emission from mouse liver injured by CCl_4 injections. The dose of drug injections was high enough to cause acute hepatic injury on mouse liver. The toxic effects were maximized within 24 hr after the injection and were slowly improved by self-regulation of antioxidant systems. We measured spontaneous photon emission from the mouse liver of 24 and 72-hr treated group and investigated spectral properties. The results are compared with the corresponding control groups along with transaminase activities in the plasma. We also investigated delayed luminescent property of liver tissue damaged by CCl_4.

MATERIALS AND METHODS

Animals and Drug Administration —— All mice were handled in accordance with National Institute of Health guidelines for the humane care of laboratory animals. Male ICR mice (Seoul National University Laboratory Animal Center, Seoul, Korea) in 4–5 weeks of age were maintained on a 12 : 12 hr light : dark cycle with diet and water available *ad libitum* and were adapted for 2 weeks to these conditions before the experiment. CCl_4 (4 ml/kg) was given intraperitoneally in the form of 5% (v/v) solution in olive oil. An equal amount of olive oil was given to the control group of mice. At 24 and 72 hr after the injection of CCl_4, the animals were killed by exsanguinations from the abdominal aorta under anesthesia with sodium barbital. Whole liver of the mouse was immediately extracted and placed in 37°C Ringer's solution for 30 min before the photon measuring. There were six mice in each group.

Photo-Detection System —— Number of emitted photons from the samples was measured by the photomultiplier tube (PMT, R331-05 selected, Hamamatsu, Japan) and associated data acquisition systems. The schematic block diagram of the experimental setup was presented in Fig. 1. The PMT operated at room temperature had spectral response in the range of 300 to 650 nm and the wavelength of maximum response was 420 nm. Proper housing and magnetic shield case for the PMT were used to ensure complete light-shielding and magnetic-shielding. The distance between the PMT and the sample was 7 cm. Actual photon detections were made within the dark chamber in a dark room. In the dark chamber the bio-sample was put in the sample holder. The sample holder was maintained at constant temperature (37°C) by the temperature controller with a thermocouple and a heater as shown in Fig. 1. The dark chamber and the sample holder were made of anodizing aluminum.

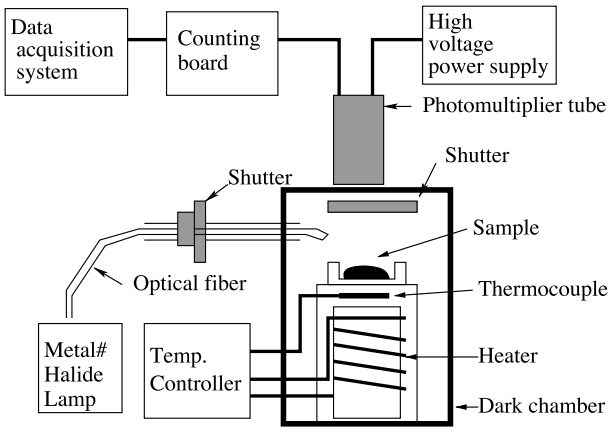


Fig. 1. Schematic Block Diagram of the Experimental Setup
This apparatus was designed to measure sequentially ultra-weak photon emission and delayed luminescence.

Measurements of Spontaneous Photon Emission —— The background was measured for 5 min in a situation that the shutter between the PMT and sample holder was open. Then, the shutter was closed and the liver sample was put inside the dark chamber on the sample holder within 1 min. After opening the shutter again, we measured spontaneous photon emission from the sample for 5 min. Data for spontaneous photon emission were presented by subtracting the background result. Dark adaptations of the samples were made in the dark room at least 30 min so that the intensity of emitted light was kept constant during spontaneous photon emission measurements. We also measured the liver area of which side was exposed to the detecting side of PMT.

Measurements of Activities of Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) in Serum —— Blood was collected during the sacrifice of mouse as de-

scribed in the above, and plasma was obtained by centrifugation at 3000 rpm (1500 × *g*) for 10 min. Each of AST and ALT activity was measured by the Reitman and Frankel's method[18] using AST or ALT test kit (Item No. BC101-O, BC101-P; YD Diagnostics, Korea) with the UV/Visible spectrophotometer (Beckman DU70, U.S.A).

Measurements of Delayed Luminescence —— We made the delayed luminescence experiments successively with each samples after the spontaneous photon emission measurements. For the measurements of delayed luminescence, we used 150 Watt metal halide lamp. Metal halide lamp is a lamp that uses an electric-discharge to produce light from a metal vapor such as sodium or mercury. The light produced by this lamp is in the broad white-blue spectrum. We illuminated the sample through the optical fibers. There were two shutters as shown in Fig. 1: one was for the PMT and the other for the light source. Their operations were connected in such a way that, when the shutter for the light source was open, the shutter for the PMT was closed, and vice versa. Each sample was illuminated for 10 sec, and the measurement of delayed luminescence was started at 117 ± 10 milli-sec just after cutting off the light. The time intervals between the cut-off from the light source and the beginning of delayed luminescence measurement were made all the same for every sample. Illumination area over the sample was 2 cm diameter within the range of whole area of mouse liver.

RESULTS AND DISCUSSION

The averages and standard deviations of spontaneous photon emission from the control and treated mice were plotted in Fig. 2. Quantum efficiency of the PMT was not counted on this presentation. The average value of spontaneous photon emission from the 24-hr control group was about two times higher than that from the 24-hr treated group. The intensities of spontaneous photon emission were decreased as the time elapsed from 24 to 72 hr. On the other hand, the spontaneous photon emission of the 24-hr control group was almost equal to that of the 72-hr control group. The data showed the time dependency of photon emission after the CCl_4 injections. We presented AST and ALT activities in Fig. 3. Two transaminase activities also had the time dependency after the CCl_4 administration and showed strong correlation with the spontaneous photon emission

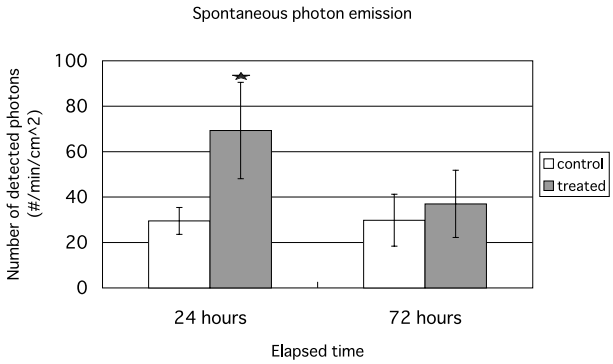
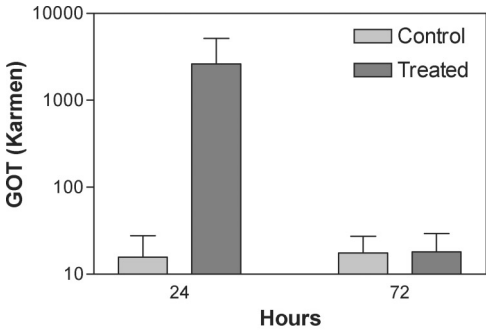


Fig. 2. Spontaneously Emitted Ultra-Weak Photons from Livers between the CCl_4-Treated and Control Mouse

Average area of the livers was measured in 3.8 ± 0.4 cm^2 and its average weight was 1.5 ± 0.2 g. Unpaired *t*-test between two groups showed $p = 0.001$ ($n = 6$).



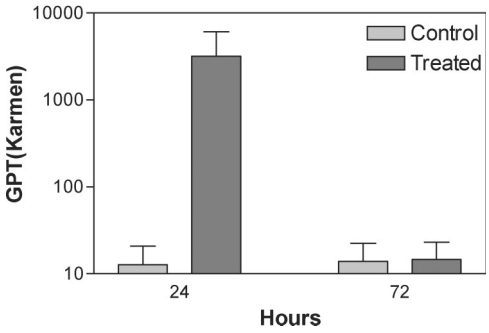


Fig. 3. AST and ALT Activities in Serum between the CCl_4-Treated and Control Mouse

The AST and ALT activities were calculated in the standard curves by extrapolation from the data of diluted plasma with distilled water.

data. Thus, the phenomena of intrinsic ultra-weak photon emissions appeared to be involved in the process of liver tissue repair as well as hepatocellular death after acute exposure to carbon tetrachloride.

Figure 4(A) and 4(B) showed the averaged delayed luminescence data for the control and treated groups after 10-sec irradiations over the livers. The

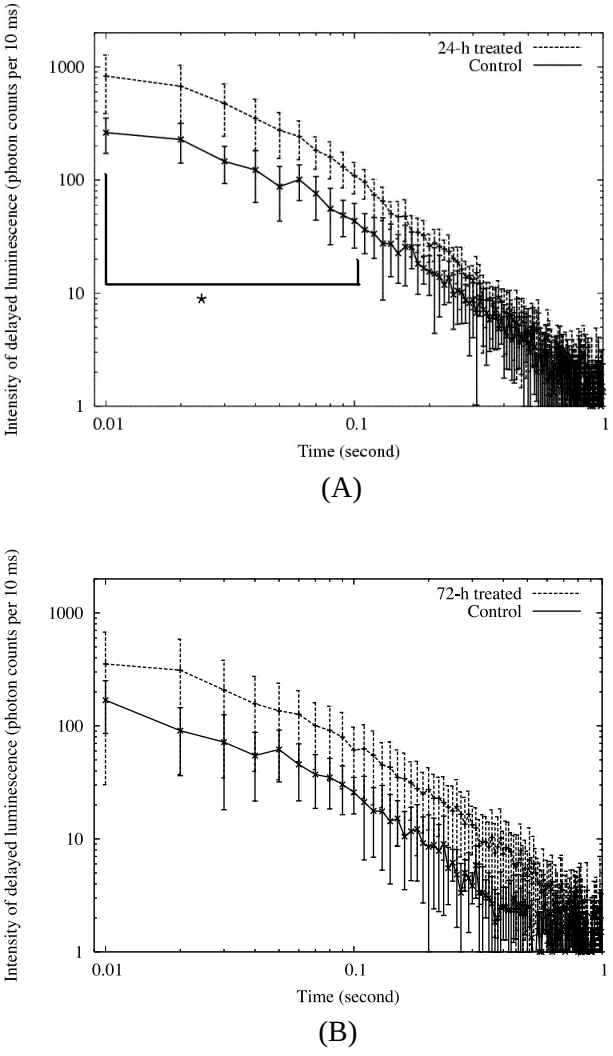


Fig. 4. Time Course of Delayed Luminescence from Liver between the CCl_4-Treated and Control Mouse after the 0.01-sec Illumination

(A) 24-hr treated (dashed line) and control (solid line) group, the asterisk means the region where significant differences ($p < 0.05$) were shown by unpaired *t*-test ($n = 6$ for each group); (B) 72-hr treated (dashed line) and control (solid line) group.

averages and standard deviations were obtained from the data of 6 mice in each group. Initial photon emissions of the treated group give more intensive peaks than those of the control group. The peak intensities in the delayed luminescence plots were 262 ± 90 counts per 10 ms and 830 ± 445 counts per 10 ms in the case of 24-hr control and treated group, respectively. In the 72-hr case, the peak intensities were 169 ± 83 counts per 10 ms and 353 ± 323 counts per 10 ms for the control and treated groups, respectively. As in the spontaneous photon emission case, the delayed luminescence data of 24-hr control and treated groups showed significantly different peak intensities at the start of delayed luminescence. The time behaviors of photon emission after the start of delayed luminescence also showed different patterns in the 24-hr group. Unpaired *t*-test showed that significant differences ($p < 0.05$) in the intensity of delayed luminescence were kept until the elapsed time, 0.1 sec. These differences were disappeared in the 72-hr group.

Carbon tetrachloride is a classic model of liver injury, and the accumulation of lipids within the hepatocyte and the appearance of hepatocellular death are primary pathological events of the hepatocellular lesion produced after exposure to this experimental hepatotoxicant.[19] Generally, active proliferation of liver cells occurs during the process of liver tissue repair after carbon tetrachloride intoxication with sublethal doses. Also, the temporal elevations in emission intensities of spontaneous photon emission and delayed luminescence have been reported during the cell proliferation of human carcinoma cell culture.[20,21] Thus, these photon emissions appear to be involved in the hepatocellular proliferation during the process of the hepatocellular death and/or liver tissue repair. The functions of both endoplasmic reticulum and mitochondria are markedly perturbed after carbon teterachloride intoxication, and these subcellular organelles have been proposed as the site of action.[19] Thus, the changes in these subcellular organelles during hepatocellular death and/or proliferation might be reflected in the changes of these photon emissions after acute exposure to carbon tetrachloride.

The transaminase was one of the thousands kinds of liver enzymes, that had the function of transferring amino group of amino acids from alpha-amino acids to alpha-keto acids. The transaminase activity levels were used as one of the ways to indicate the liver injury. There were various other biochemical assays of which results showed strong correlation with the transaminase activity levels. In particular, lipid peroxide level was changed and antioxidant enzymes such as superoxide dismutase and catalase were produced in a similar fashion to the transaminase activities after the CCl_4 injection.[22,23] One of the possible mechanisms for the initiation of destructive radical reactions *in vivo* was the decomposition of lipid hydroperoxides. Thus, these ultra-weak photon emissions might show correlation to free radical reactions and oxidative damages induced by CCl_4. Another process for radical productions in organic material might be the photolysis of chemical bonds. Also, the results of delayed luminescent properties

of liver tissues showed that the intensities of delayed luminescence were also discriminated depending on the degree of toxicities. This discrimination could be seen in more amplified quantities through the initial peak values of delayed luminescence. This model study might provide a basis for the analysis of hepatotoxicant induced liver injury and repair by means of measurements of the photon emissions.

Acknowledgements This work was supported in part by Grant R01-2001-000-00023-0 from the Korea Science and Engineering Foundation, ITEP (A19-02-03) from the Ministry of Commerce, Industry and Energy, NRL (M1-0302-00-007) from the Ministry of Science & Technology, and the BK21 program of the Korea Research Foundation. Part of the measurements in this work (the transaminase assays) was done by the National Center for Inter-University Research Facilities, Seoul National University, Korea.

REFERENCES

1) Chwirot, W. B., Cilento, G., Gurwitsch, A. A., Inaba, H., Nagl, W., Popp, F. A., Li, K. H., Mei, M., Galle, R., Neurohr, J., Sławinski, J., Van Wijk, R. V. and Schamhart, D. H. J. (1988) Multi-author review on biophoton emission. *Experientia*, **44**, 543–600.
2) Niggli, H. J., Scaletta, C., Yu, Y., Popp, F. and Applegate, L. A. (2001) Ultra-weak photon emission in assessing bone growth factor efficiency using fibroblastic differentiation. *J. Photochem. Photobiol. B*, **64**, 62–68.
3) Cohen, S. and Popp, F. (1997) Biophoton emission of the human body. *J. Photochem. Photobiol. B*, **40**, 187–189.
4) Choi, C., Woo, W. M., Lee, M. B., Yang, J. S., Soh, K. S., Yoon, G., Kim, M., Zaslawsky, C. and Chang, J. J. (2002) Biophoton Emission from the Hands. *J. Korean Phys. Soc*., **41**, 275–278.
5) Kim, J. D., Lim, J., Sung, B. and Soh, K.-S. (2003) Biophoton Emission from Rat Liver. *J. Korean Phys. Soc*., **42**, 427–430.
6) Jung, H.-H., Woo, W.-M., Yang, J. M., Choi, C., Lee, J., Yoon, G., Yang, J. and Soh, K.-S. (2003) Photon counting statistics analysis of biophotons from hands. *Indian J. Exp. Biol*., **41**, 446–451.
7) Jung, H.-H., Woo, W.-M., Yang, J.-M., Choi, C., Lee, J., Yoon, G., Yang, J., Lee, S. and Soh, K.-S. (2003) Left-right asymmetry of biophoton emission from hemiparesis patients. *Indian J. Exp. Biol*., **41**, 452–456.
8) Tilbury, R. N., Sławinski, J., Ezzahir, A., Godlewski, M., Kwiecinska, T., Rajfur, Z., Sitko, D., Wienzuchowska, D., Kochel, B., Gu, Q., Popp, F. A., Lilius, E. M., Marnila, P., Van Wijk, R. and Van Aken, J. M. (1992) Multi-author review on biophoton emission, stress and disease. *Experientia*, **48**, 1029–1102.
9) Cadenas, E. (1984) Biological chemiluminescence. *Photochem. Photobiol*., **40**, 823–830.
10) Wright, J. R., Rumbaugh, R. C., Colby, H. D. and Miles, P. R. (1979) The relationship between chemiluminescence and lipid peroxidation in rat hepatic microsomers. *Arch. Biochem. Biophys*., **192**, 344–351.
11) Devaraj, B., Scott, R. Q., Roschger, P. and Inaba, H. (1991) Ultra-weak light emission from rat liver nuclei. *Photochem. Photobiol*., **54**, 289–293.
12) Nagl, W. and Popp, F. A. (1983) A physical (electromagnetic) model of differentiation: Basic considerations. *Cytobios*, **37**, 45–62.
13) Sławinski, J. (2003) Biophotons from stressed and dying organisms: Toxicological aspects. *Indian J. Exp. Biol*., **41**, 483–492.
14) De Zwart, L. L., Meerman, J. H., Commandeur, J. N. and Vermeulen, N. P. (1999) Biomarkers of free radical damage applications in experimental animals and in humans. *Free Radic. Biol. Med*., **26**, 202–226.
15) Basu, S. (2003) Carbon tetrachloride-induced lipid peroxidation: eicosanoid formation and their regulation by antioxidant nutrients. *Toxicology*, **189**, 113–127.
16) Fraga, C., Llesuy, S. and Boveris, A. (1984) Increased carbon tetrachloride-stimulated chemiluminescence in the *in situ* liver of barbital-treated mice. *Acta Physiol. Pharmacol. Latinoam*., **34**, 143–150.
17) Recknagel, R. O. (1967) Carbon tetrachloride hepatotoxicity. *Pharmacol. Rev*., **65**, 145–208.
18) Reitman, S. and Frankel, S. (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminase. *Am. J. Clin. Pathol*., **28**, 56–63.
19) Plaa, G. L. (2000) Chlorinated methanes and liver injury: Highlights of the past 50 years. *Annu. Rev. Pharmacol. Toxicol*., **40**, 43–65.
20) van Wijk, R. and van Aken, H. (1991) Light-induced photon emission by rat hepatocytes and hepatoma cells. *Cell Biophys*., **18**, 15–29.
21) Takeda, M., Tanno, Y., Kobayashi, M., Usa, M., Ohuchi, N., Satomi, S. and Inaba, H. (1998) A novel method of assessing carcinoma cell proliferation by biophoton emission. *Cancer Lett*., **127**, 155–160.
22) Boveris, A., Cadenas, E., Reiter, R., Filipkowski, M., Nakase, Y. and Chance, B. (1980) Organ chemi-

luminescence: Noninvasive assay for oxidative radical reactions. *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 347–351.
23) Nomura, T. and Yamaoka, K. (1999) Low-dose γ-ray irradiation reduces oxidative damage induced by CCl_4 in mouse liver. *Free Radic. Biol. Med.*, **27**, 1324–1333.