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CHEMILUMINESCENCE ASSOCIATED WITH DOXORUBICIN-INDUCED LIPID PEROXIDATION IN RAT HEART MITOCHONDRIA

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Abstract—Chemiluminescence was observed in a rat heart mitochondrial suspension containing NADH, $FeCl_3$ and doxorubicin (Adriamycin®) (DXR). There was good correlation between the total intensity of chemiluminescence and the total amount of thiobarbituric acid reactive substances (TBARS) produced during DXR redox cycling. Thus, the chemiluminescence was shown to be associated with lipid peroxidation. The chemiluminescence was quenched by superoxide dismutase (SOD), suggesting that superoxide anion radicals contributed to its production. Upon addition of 1,4-diazabicyclo[2,2,2]-octane (DABCO), a singlet oxygen emission enhancer, to the mitochondrial suspension emitting the chemiluminescence, the chemiluminescence intensity increased transiently, indicating the involvement of singlet oxygen. Furthermore, spectral analysis of the chemiluminescence showed it to be due to singlet oxygen and excited carbonyls.

Key words: chemiluminescence; doxorubicin; heart mitochondria; oxidative stress; lipid peroxidation; reactive oxygen species; singlet oxygens; excited carbonyls

DXR†, a widely used antineoplastic agent, is well known to induce cardiotoxicity. This toxicity is considered to be mediated by reactive free radicals produced by DXR, and is characterized by changes in the morphology and function of mitochondria [1, 2]. DXR undergoes NADH dehydrogenase-catalyzed one-electron reduction to semiquinone free radicals in mitochondria [3]. Semiquinone radicals are reoxidized by reaction with molecular oxygen, resulting in the formation of superoxide anions and other reactive oxygen species, such as hydrogen peroxide and hydroxyl radicals [4–6]. These reactive free radicals are considered to participate in DXR-induced lipid peroxidation of mitochondrial membranes.

DXR-induced lipid peroxidation is generally evaluated by TBARS formation. Fluorescent substances and high molecular weight protein aggregates, both nonspecific indicators of lipid peroxidation, are also employed [7]. ESR measurements have been used to specify the molecular nature of reactive oxygen species generated during DXR redox cycling [4, 8]. Hydroxyl radicals have been shown to be involved in DXR-induced lipid peroxidation in phosphate buffer [4], while the perferryl–ADP–DXR complex has been indicated to be important in DXR-induced lipid peroxidation in Tris–HCl buffer [8].

Chemiluminescence is a useful and sensitive tool for monitoring oxidative stress [9]. However, chemiluminescence produced during DXR-induced lipid peroxidation has not been studied. In the present report, we investigated the chemiluminescence in a rat heart mitochondrial suspension during DXR redox cycling and identified its origins by chemiluminescence spectroscopy. Further, the effects of scavengers on chemiluminescence and TBARS production were studied. Based on these results, a molecular mechanism for the production of chemiluminescence is presented.

MATERIALS AND METHODS

Materials. Alkaline protease (Nagase) was purchased from Nagase Biochemicals Ltd. (Osaka, Japan). DEM, DABCO, mannitol and thiourea were from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Dimethylfuran was from the Aldrich Chemical Co., Inc. (Milwaukee, WI, U.S.A.). SOD (from bovine erythrocytes) and catalase (from bovine liver) were from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). NADH was from the Oriental Yeast Co., Ltd. (Tokyo, Japan), and benzoic acid sodium salt from Nacalai Tesque, Inc. (Kyoto, Japan). DXR was a gift from the Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). All other reagents were of the highest grade available.

Preparation of heart mitochondria. Heart mitochondria were isolated from male Wistar rats (Japan SLC Inc., Shizuoka, Japan), weighing 160–250 g, according to Mela and Seitz [10] and Fuller *et al.* [11] with a slight modification. Isolated hearts were treated with alkaline protease (2 mg/g heart) and homogenized in medium containing 0.25 M sucrose, 1 mM EDTA and 25 mM Tris–HCl at pH 7.4. The

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† Abbreviations: DXR, doxorubicin; TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase; DABCO, 1,4-diazabicyclo[2,2,2]octane; DEM diethyl maleate; MDA, malondialdehyde; and CL, chemiluminescence.

homogenates were centrifuged for 7 min at 60 *g* and the supernatant was recentrifuged for 7 min at 600 *g*. This supernatant was then centrifuged for 10 min at 7740 *g*, and both the resultant supernatant and fluffy layers were removed. The precipitate was resuspended in medium containing 0.25 M sucrose, 1 mM EDTA, 25 mM Tris–HCl and 1% (w/v) bovine serum albumin, centrifuged for 7 min at 7740 *g* and the supernatant removed. The resultant precipitate was resuspended in medium containing 150 mM KCl and 50 mM Tris–HCl (pH 7.4) and centrifuged for 7 min at 7740 *g*. The precipitate (mitochondria fraction) was resuspended in medium containing 150 mM KCl and 50 mM Tris–HCl (pH 7.4). Mitochondria were stored in liquid nitrogen and used within 1 week. The protein concentration of the mitochondria was determined by the method of Lowry *et al.* [12] with bovine serum albumin as the standard.

Preliminary treatment of mitochondria. Since Griffin–Green *et al.* [13] reported that TBARS were not produced in rat heart mitochondria, in the present study GSH-depleted heart mitochondria were prepared as follows. Heart mitochondria (5 mg protein/mL) were preliminarily incubated at 37° for 60 min with 40 mM DEM, a glutathione-depleting agent. KCl (150 mM)–Tris (50 mM)–HCl buffer (pH 7.4) through which 95% O_2–5% CO_2 gas had been bubbled for more than 15 min was used throughout.

Chemiluminescence. CL was measured using a single photoelectron counting system, CLD-100 and CLC-10 (Tohoku Electronic Industries Co., Ltd., Sendai, Japan), connected to a personal computer PC-9801 NS (NEC Corp., Tokyo, Japan) for integration. Two milliliters of the mitochondrial suspension (0.5 mg protein/mL) containing 25 μM $FeCl_3$ with or without DXR was placed in a stainless steel dish (diameter: 50 mm, height: 10 mm) for 5 min where the temperature was maintained at 37°, after which the reaction was started by adding 2.5 mM NADH solution to the mixture. The chemiluminescence emanating from the mitochondrial suspension was measured continuously as counts per minute or counts per 10 seconds. The reaction was terminated by the addition of 1 mM EDTA at the designated times, and amounts of TBARS formed in the reaction mixture during the reaction period were determined. Similarly, the chemiluminescence from the mitochondrial suspension containing 0–100 μM DXR, 2.5 mM NADH and 25 μM $FeCl_3$ with radical scavengers such as SOD (3500 U), catalase (3500 U), 50 mM thiourea, 100 mM mannitol, 50 mM DABCO or 100 mM dimethylfuran was measured in the same way as above. The chemiluminescence intensity was shown by subtracting the background counts from the observed counts of the reaction mixtures according to Nakano *et al.* [14]. The background counts were very low against the observed values. The TBARS contents of the reaction mixtures were also subtracted from that of the mitochondrial suspension before the reaction was started.

Spectral analysis of chemiluminescence. The spectral distribution of the chemiluminescence was investigated according to the method of Inaba *et al.* [15]. In this method, chemiluminescence emissions with certain wavelengths were sorted using colored glass filters. A set of colored glass filters with different sharp short-wavelength cutoffs (390–650 nm; L-39, L-42, Y-43, Y-44, Y-45, Y-46, Y-47, Y-48, Y-49, Y-50, Y-51, Y-52, O-53, O-54, O-55, O-56, O-57, O-58, O-59, R-60, R-61, R-62, R-63, R-64, R-65; Toshiba Glass Co., Ltd., Shizuoka, Japan) were inserted successively into the optical path between the sample cuvette holder and the photomultiplier in the chemiluminescence analyzer. Chemiluminescence intensity was measured for every filter, and was also measured with no filter each time to correct for time-dependent changes. Chemiluminescence intensity was expressed as the ratio of photon counts with filter to those without filter, and the chemiluminescence spectrum was plotted relatively by subtraction of the chemiluminescence intensities observed with two successive filters.

TBARS assay. TBARS formed in the heart mitochondrial suspension incubated with or without DXR were assayed according to Buege and Aust [16] and expressed as nanomoles of MDA equivalents per milligram of protein. Briefly, 0.5 mL of KCl–Tris–HCl buffer and 2 mL of TBA stock solution consisting of 15% (w/v) trichloroacetic acid, 0.375% (w/v) 2-thiobarbituric acid and 0.25 N HCl were added to 0.5 mL of mitochondrial suspension. The reaction was terminated by the addition of 1 mM EDTA as described above. The mixture was then boiled for 15 min, cooled, and centrifuged at 1000 *g* for 15 min. The absorbance of the supernatant at 535 nm was determined with 1,1,3,3-tetraethoxypropane as the standard.

RESULTS

Chemiluminescence and TBARS induced by DXR. Rat heart mitochondria were preliminarily treated with DEM to deplete intra-mitochondrial glutathione, so as to increase the DXR-induced lipid peroxidation observed in native heart mitochondria. The mitochondrial suspension containing NADH, $FeCl_3$ and DXR produced chemiluminescence which was measured directly at 37° and expressed as total chemiluminescence counts (Fig. 1). Total chemiluminescence increased slowly at the early stage of the reaction, and after about 15 min increased time dependently. The total amount of TBARS formed in the mitochondrial suspension was determined at designated times when the chemiluminescence measurements were terminated. The total amount of TBARS increased as the reaction proceeded. The production of both chemiluminescence and TBARS in mitochondrial suspensions incubated with NADH-$FeCl_3$, NADH-DXR or $FeCl_3$-DXR was negligible, and it was the same in the buffer containing NADH, $FeCl_3$ and DXR without mitochondria.

Relationship between chemiluminescence and TBARS. The relationship between chemiluminescence and TBARS was investigated by incubating the mitochondrial suspension containing NADH and $FeCl_3$ with 0, 5, 10, 25, 50 and 100 μM DXR for 1 hr. Total intensity of chemiluminescence

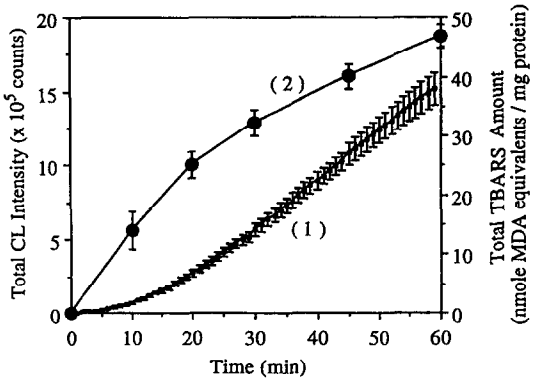


Fig. 1. Time courses of chemiluminescence and TBARS production in the rat heart mitochondrial suspension with DXR. Heart mitochondrial suspension (0.5 mg protein/mL) containing 2.5 mM NADH and 25 μM $FeCl_3$ was incubated at 37° with or without 50 μM DXR. Chemiluminescence (CL) and TBARS production were measured for the same samples, as described in Materials and Methods, and were expressed as total CL intensity (1) and total amount of TBARS (2) produced during the reaction time, t. Chemiluminescence data represent the means ± SEM of 3–24 experiments. TBARS data represent the means ± SEM of 3–12 experiments.

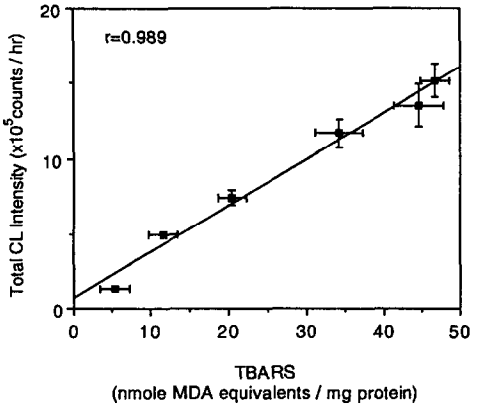


Fig. 2. Relationship between chemiluminescence and TBARS production in the heart mitochondrial suspension. The total chemiluminescence (CL) intensities produced for 1 hr in the presence of 0, 5, 10, 25, 50 or 100 μM DXR were plotted against the total amounts of TBARS formed in the same mitochondrial suspension. The data represent the means ± SEM of 3–12 experiments. Correlation coefficient: $r = 0.989$.

was plotted against total amount of TBARS (Fig. 2), and good correlation was observed between them (correlation coefficient: $r = 0.989$).

Effects of radical scavengers on chemiluminescence.

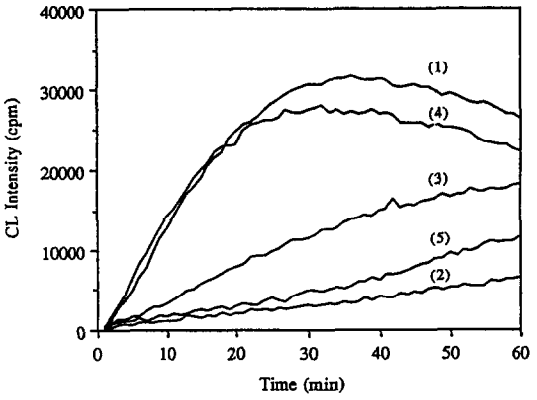


Fig. 3. Time courses of chemiluminescence in the presence of DXR and radical scavengers. The time course of chemiluminescence from the heart mitochondrial suspension (0.5 mg protein/mL) containing 2.5 mM NADH and 25 μM $FeCl_3$ with 50 μM DXR was detected at 37°: (1) without scavenger, (2) with SOD (3500 U), (3) with catalase (3500 U), (4) with 100 mM mannitol, and (5) with 50 mM DABCO. Chemiluminescence (CL) intensity is expressed as the production rate (cpm). The data shown here are typical ones.

Various types of radical scavengers were added to the mitochondrial suspension before the start of the reaction, and the chemiluminescence with DXR was measured. The time course of chemiluminescence production is shown in Fig. 3. Chemiluminescence was inhibited markedly in the presence of SOD (superoxide anion scavenger) [4, 17, 18], catalase (hydrogen peroxide scavenger) [4, 17, 18], and DABCO (singlet oxygen quencher) [18, 19]. Mannitol, as a hydroxyl radical scavenger [4, 17, 18], showed no significant inhibition of chemiluminescence. The total intensities of chemiluminescence produced during the 60 min following the start of the reaction are shown in Table 1. The presence of SOD (3500 U), catalase (3500 U), or thiourea (50 mM) as a hydroxyl radical scavenger [20] or DABCO (50 mM) caused 89.3, 57.8, 62.8 and 68.4% reductions in the total chemiluminescence intensity, compared with that without scavengers, respectively. Mannitol and dimethylfuran, a singlet oxygen quencher [21], both induced slight reductions in total chemiluminescence intensity (6.2 and 16.4%, respectively). Changes in TBARS production in the mitochondrial suspension with these scavengers were similar to the changes observed in chemiluminescence (Table 1). SOD, catalase, thiourea and DABCO all significantly reduced the total amount of TBARS, by 94.6, 56.6, 47.7, and 79.1%, respectively, compared with that in the absence of scavengers. Both mannitol and dimethylfuran induced only slight reductions in TBARS content i.e. 15.4 and 24.1%, respectively. Heat-denatured SOD showed no inhibition of either chemiluminescence or TBARS, whereas heat-denatured catalase effectively inhibited both to the same extent as native catalase (data not shown).

Effects of DABCO, a singlet oxygen emission enhancer, on chemiluminescence. The time course of

Table 1. Effects of various radical scavengers on chemiluminescence and TBARS production

	Total CL intensity (x 10^5 counts/hr)	Relative intensity (%)	Total TBARS amount (nmol MDA equivalents/ mg protein/hr)	Relative amount (%)
None	13.62 ± 1.02	100	38.90 ± 1.84	100
SOD (3500 U)	1.45 ± 0.11*	10.7	2.10 ± 1.68*	5.4
Catalase (3500 U)	5.75 ± 0.36†	42.2	16.90 ± 0.65*	43.4
100 mM Mannitol	12.78 ± 0.96	93.8	32.92 ± 3.18	84.6
50 mM Thiourea	5.06 ± 1.59†	37.2	20.34 ± 7.46†	52.3
50 mM DABCO	4.31 ± 0.84*	31.6	8.13 ± 2.18*	20.9
100 mM Dimethylfuran	11.38 ± 1.55	83.6	29.51 ± 3.67	75.9

The heart mitochondrial suspension containing 2.5 mM NADH, 25 μM $FeCl_3$ and 50 μM DXR was incubated at 37° for 1 hr with or without scavengers. The total chemiluminescence (CL) intensity and the amount of TBARS produced in 1 hr were determined. Values are means ± SEM of 3 experiments.
*,† Significantly different from control (without scavenger): *$P < 0.001$ and †$P < 0.01$.

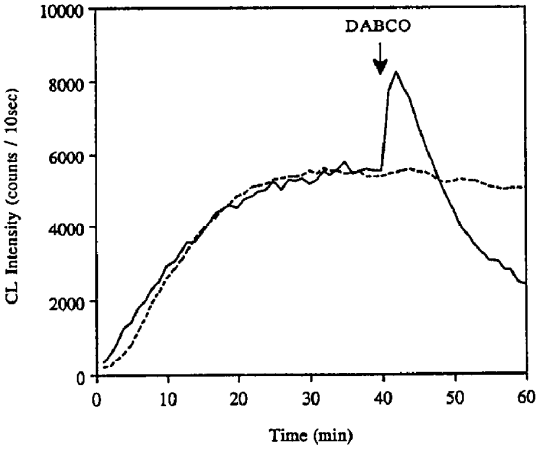


Fig. 4. Effect of DABCO as a singlet oxygen emission enhancer on chemiluminescence. The time course of chemiluminescence from the heart mitochondrial suspension (0.5 mg protein/mL) containing 2.5 mM NADH and 25 μM $FeCl_3$ with 50 μM DXR was recorded, and at 40 min (indicated by an arrow) DABCO (250 mM) was added (——). The time course of chemiluminescence without addition of DABCO is indicated by the dotted line (. . . .). Chemiluminescence (CL) intensity was recorded as counts/10 sec.

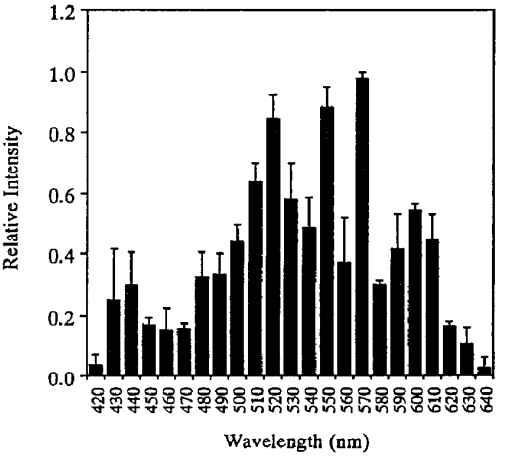


Fig. 5. Spectral distribution of DXR-induced chemiluminescence. Spectral analysis of the chemiluminescence from the heart mitochondrial suspension (0.5 mg protein/mL) containing 2.5 mM NADH and 25 μM $FeCl_3$ with 50 μM DXR was carried out as described in Materials and Methods. The data represent the means ± SEM of 3 experiments.

chemiluminescence in the mitochondrial suspension containing NADH and $FeCl_3$ with DXR was recorded, and at 40 min when it reached a plateau, DABCO (250 mM) (a singlet oxygen emission enhancer) [22] was added (Fig. 4). Chemiluminescence was initially enhanced by DABCO, followed by a rapid and marked decrease.

Chemiluminescence spectrum. Spectral analysis of chemiluminescence produced in the mitochondrial suspension with DXR began 20 min after initiation of the reaction. The spectral distribution of the chemiluminescence presented in Fig. 5 shows peaks at around 440 and 520 nm, which were considered to be due to excited carbonyls [23, 24]. The spectral distribution also showed two peaks at around 570 and 600 nm and a shoulder around 480 nm, probably due to singlet oxygen [25]. The shoulder at around 480 nm may be ascribed to rapid quenching in aqueous solution [14].

DISCUSSION

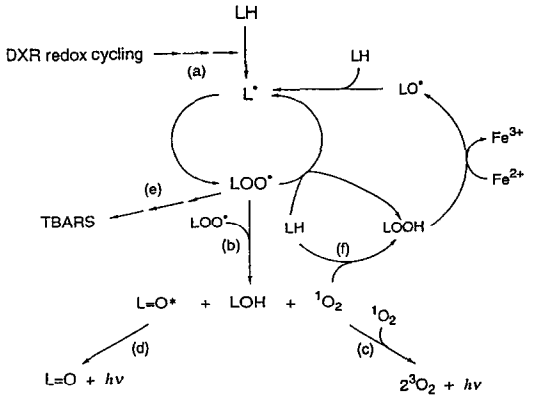
The present study describes the chemiluminescence emitted from a rat heart mitochondrial suspension containing NADH, $FeCl_3$ and DXR (Fig. 1). The chemiluminescence shown here was detected directly in the absence of reagents, such as luminol, for amplification. Luminol-mediated chemiluminescence is not specific for a particular activated oxygen species. Hence, direct detection facilitates the identification of the molecular species responsible for the chemiluminescence. The total chemilumi-

nescence intensity correlated with the total amount of TBARS formed (Fig. 2), indicating that the DXR-induced chemiluminescence in the heart mitochondrial suspension was associated with lipid peroxidation.

The effects of various radical scavengers on DXR-induced chemiluminescence and TBARS production in the heart mitochondrial suspension were investigated (Fig. 3 and Table 1). The inhibition of chemiluminescence and TBARS production by SOD indicated the involvement of superoxide anions. This was consistent with the findings of Doroshow and Davies [4], Mimnaugh *et al.* [26] and other groups who used spectrophotometric and biochemical techniques. Although inhibition by both native and heat-denatured catalase gave no substantial support for the participation of hydrogen peroxide, this does not exclude its involvement as suggested by Mimnaugh *et al.* [18]. The hydroxyl radical identified by ESR spectroscopy was suggested as a potent reactive oxygen species in DXR-induced lipid peroxidation [4]. However, mannitol (a hydroxyl radical scavenger) showed no significant inhibition of either chemiluminescence or TBARS production in the present study. This discrepancy is likely due to the Tris–HCl buffer used in this experiment, since Tris effectively traps hydroxyl radicals [27]. In contrast, thiourea (a hydroxyl radical scavenger) markedly inhibited both chemiluminescence and TBARS production (Table 1). This was probably due to the low specificity of thiourea, which is able to react not only with hydroxyl radicals, but also with hydrogen peroxide [28]. Dimethylfuran (a singlet oxygen quencher) inhibited both chemiluminescence and TBARS production, but these reductions were not statistically significant. On the other hand, DABCO (a singlet oxygen chemiluminescence enhancer [22]) significantly inhibited both chemiluminescence and TBARS production. The differences in inhibition may reflect solubility differences, as dimethylfuran is water insoluble whereas DABCO is water soluble. Furthermore, the addition of DABCO, which is known to enhance singlet oxygen dimol emission in aqueous media [22], to the mitochondrial suspension emitting the chemiluminescence markedly enhanced its emission, transiently, based on an increased singlet oxygen life time (Fig. 4). These results suggest the generation and involvement of singlet oxygen in chemiluminescence and TBARS production.

The generation of electronically excited species, predominantly singlet oxygen and/or excited carbonyls, is probably the origin of the chemiluminescence shown here, as both emit light [9, 14, 29]. The spectral analysis presented in Fig. 5 shows that the DXR-induced chemiluminescence in the mitochondrial suspension originated from both singlet oxygen and excited carbonyls.

Based on these results, the molecular mechanism of chemiluminescence production in heart mitochondria with DXR-induced lipid peroxidation can be represented as in Scheme I. The superoxide anion generated during the DXR redox cycling (Scheme I, a) eventually results in the production of lipid peroxyl radicals (LOO·) by reacting with mitochondrial lipids (LH). The collision of two lipid



Scheme I. Molecular mechanism of the chemiluminescence production from heart mitochondria on DXR-induced lipid peroxidation. In the presence of NADH and oxygen, superoxide anions are produced during DXR redox cycling (a). Superoxide anions eventually result in lipid peroxyl radicals (LOO·) by reacting with mitochondrial lipids (LH). The peroxyl radicals produce singlet oxygen ($^1O_2$), excited carbonyls (L = O*) and hydroxyl compounds (LOH) (b). The singlet oxygen ($^1O_2$) and the excited carbonyls (L = O*) emit chemiluminescence upon relaxation to the ground state, triplet oxygen ($^3O_2$) and (L=O), respectively (c,d). The reaction processes (a–f) in the scheme were described in the following reports: (a) [8, 25], (b) [29, 33, 37, 38], (c)[24, 37], (d) [22, 23, 37], (e) [39], and (f) [40]. Reaction processes (b–f) were explained based on observations from different anthracycline reaction systems.

peroxyl radicals (LOO·) is assumed to produce singlet oxygen ($^1O_2$) and excited carbonyls (L = O*) according to Russell's mechanism [30–33] (Reactions 1 and 2; Scheme I, b).

$$LOO\cdot + LOO\cdot \rightarrow LOH + {}^3O_2 + L = O^* \quad (1)$$

(Scheme I, b)

$$LOO\cdot + LOO\cdot \rightarrow LOH + L = O + {}^1O_2 \quad (2)$$

(Scheme I, b)

Singlet oxygen ($^1O_2$), emitting chemiluminescence, decomposes to the triplet ground state ($^3O_2$) (Reaction 3; Scheme I, c). Excited carbonyls (L = O*) also emit chemiluminescence upon relaxation to the ground state (L = O) (Reaction 4; Scheme I, d). These two electronically excited species are considered to be responsible for the chemiluminescence induced here by DXR.

$$^1O_2 + {}^1O_2 \rightarrow 2\,{}^3O_2 + h\nu\ (480, 580, 630\ nm) \quad (3)$$

(Scheme I, c)

$$L{=}O^* \rightarrow L{=}O + h\nu\ (440, 520\text{–}530\ nm) \quad (4)$$

(Scheme I, d)

Some of the present results can be interpreted based on Scheme I. As in Fig. 4, the addition of DABCO to the mitochondrial suspension emitting the chemiluminescence resulted in a transient increase in chemiluminescence. This is possibly due to an increased lifetime of singlet oxygen [34]. On the other hand, as in Fig. 3 and Table 1, addition of DABCO to the mitochondrial suspension at the

start of the reaction markedly inhibited both chemiluminescence and TBARS production. This contrary result may be attributed to the action of DABCO as a free radical scavenger, since DABCO can react with not only singlet oxygen but also lipid peroxyl radicals (LOO·) [35]. That is, inhibition of lipid peroxyl radicals results in a reduction in both chemiluminescence (Scheme I, b–d) and TBARS production (Scheme I, e). Furthermore, the rapid and marked decrease in chemiluminescence following temporal enhancement by the addition of DABCO, as shown in Fig. 4, is also considered to be due to the reaction of DABCO with lipid peroxyl radicals (LOO·), inhibiting the formation of singlet oxygen and/or excited carbonyls (Scheme I, b–d).

As shown in Fig. 1, the time course of chemiluminescence differed from that of TBARS production, although there was a good correlation between total chemiluminescence and the total amount of TBARS over a period of 60 min (Fig. 2). As shown in Fig. 1, TBARS formation occurred immediately, while chemiluminescence emission commenced in a nonlinear fashion after an initial lag period. A similar relationship between both chemiluminescence and TBARS production was reported in liver microsomes during NADPH/ADP-iron-promoted lipid peroxidation [36]. TBARS formation occurs via the early and continuous chain-reaction process of reactive oxygen production, which can stimulate lipid peroxidation. On the other hand, the generation of singlet oxygen and/or excited carbonyls required for chemiluminescence is induced by the collisional self-reaction of lipid peroxyl radicals (LOO·). This requires a sufficiently high concentration of lipid peroxyl radicals (LOO·) to induce collision of two lipid peroxyl radicals (LOO·), hence, the observed lag period. The same can be said of singlet oxygen ($^1O_2$). This requirement for lipid peroxyl radical (LOO·) and singlet oxygen ($^1O_2$) concentrations appears to produce nonlinearity in the production of chemiluminescence. Furthermore, the temporal differences in chemiluminescence and TBARS production indicate that singlet oxygen is regarded as a consequence of DXR-induced lipid peroxidation rather than as an initiator of the process.

In conclusion, it was shown that chemiluminescence was produced from a heart mitochondrial suspension during DXR redox cycling. This chemiluminescence was found to be closely associated with lipid peroxidation and was indicated to be due to singlet oxygen and excited carbonyls. Singlet oxygen and excited carbonyls in the heart mitochondria during DXR redox cycling were considered as a consequence of lipid peroxidation rather than as an initiator of the process. Thus, this highly sensitive chemiluminescence could be of great use in evaluating DXR-induced cardiotoxicity.

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