First report on the persist time of the free radical produced by shock wave pulses employed in clinical ESWL

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ABSTRACT

The shock wave used in extracorporeal shock wave lithotripsy (ESWL) induces strong cavitation and generates a large amount of free radicals (FR). In order to evaluate the harmfulness of FR in the ESWL, information on the incidence and persist time of FR caused by shock waves is required. FR markers can estimate the amount of FR generated, but not how long the FRs will survive. The OH* FR generated by the ESWL shock wave reacts with luminol and emits blue light, which is called sonochemical luminescence (SCL) phenomenon. In this study, FR generation and persist time were measured by recording SCL phenomenon with a sensitive photomultiplier tube (PMT) that responds in nanoseconds. As a result of measurement with the PMT, when the electromagnetic shock wave used in clinical practice was irradiated to the luminol solution, the amount of light emitted per unit time reached its maximum value within a very short time (≈600us) and then exponentially decreased for a long time (~several hundred ms). The measured FR persist time reaches a maximum of 1000 ms. As the output setting of the shock wave generator increases, the minimum or average FR persist time increases, but the maximum value does not show a high correlation with the output setting. The amount of generated FR shows a very high correlation with the shock wave setting, and when the setting is changed from low to high, it increases very sensitively, rapidly and non-linearly. In order to reduce the risk of FR in patient treatment using lithotripsy, the output setting of the shock wave should be minimized, and the interval between the shock wave pulses should be sufficiently larger than the FR persist time. Therefore, it is recommended to avoid increasing the output setting and setting the shock wave irradiation frequency below 1 Hz to shorten the treatment time in clinical practice. For the purpose of formulating these recommendations, additional studies on the generation and persist time of FR depending on the shock wave generation method and set conditions in living tissue or similar environment are required in the future.

1. Introduction

Extracorporeal shock wave lithotripsy (ESWL) uses shock waves, which are high-amplitude pulsed ultrasound waves. This pulsed ultrasound always produces very strongly inertially collapsing cavitation bubbles that cause biological damage for therapeutic purposes [1,2]. The temperature and pressure in the imploding cavity can be thousands of degrees K and hundreds of atmospheres, respectively [3,4].

In water, this leads to the formation of hydroxyl radicals and hydrogen atoms due to thermal decomposition of water molecules [5]. In aqueous solution, it is more likely that the shock waves generates significant amount of free radicals (FR) [6-8]. Even in sonicated biological media, FR formation was detected by electron spin resonance (ESR) spin trapping [9] and by detection of sonoluminescence (SL) with a sensitive image intensification technique [8,10].

The role of FR and of mechanical effects induced by ultrasound in DNA degradation, inactivation of enzymes, lipid peroxidation, and cell killing is reviewed [5,10]. Although there is yet no conclusive evidence that ultrasound causes free radical formation in the interior of cells [9], the study of FR generated by ultrasound will continue to be a...
field of active research because of its significance to large areas of chemistry, biology, and medicine[10].

In order to evaluate the harmfulness of FRs generated by ESWL shock waves, information on the incidence and persist time of FRs is required. Using conventional FR markers, the amount of FR generated can be estimated [11], but not how long FR will survive. Several studies have been reported that the intensity of various acoustic or optical signals emitted as a result of cavitation has a high correlation with the amount of generated FR [5,7], but the measurement results for the duration of the FR has not been reported.

In this study, using the sonochemical luminescence (SCL) phenomenon in which OH* FR reacts with luminol and emits blue light [12-14], the generation and persist time of FR by ESWL shock wave were estimated. The luminol aqueous solution was irradiated with a shock wave and the emitted light (LE) pulse signal was recorded by using a very sensitive photomultiplier tube (PMT) that responds in nanoseconds. Based on the measurement results, the operating conditions of the shock wave generator to minimize the harmfulness of FR to the patient were discussed.

2. Materials and methods

2.1. FR from cavitation by shock pulse

The bubble generated by the shock wave collapses violently. When the bubble collapses, the inside of the bubble is in a state of high temperature and high pressure [3,4,12,14] and the surrounding medium, especially water, is easily decomposed to generate FRs such as O, O3, H2O2, and OH* [12,15]. Among the FRs, it is suggested that OH* FRs are possibly produced by the chemical reaction between H2O2 and O3 produced from the cavitation [16]. FRs generated as a result of cavitation are chemically unstable and are stabilized through various sonochemical reactions in a very short time (in the order of nano or sub-nano second) [12,14]. The energy difference before and after FR generation and chemical reaction processes can be emitted as light of a specific wavelength [17].

2.2. SCL of luminol reaction with OH*

As luminol (C8H7N3O2) reacts with OH* radicals produced by shock wave pulses, a divalent anion (dianion) is formed. When the divalent anion and oxygen react, nitrogen is separated and an unstable organic peroxide is formed, which is in an excited state. At this time, electrons are transferred from the excited state to the ground state, and energy is emitted as light (BLUE light) in this process [18,19]. Since the amount of LE is related to the amount of OH* FR, the amount of OH* FR can be estimated by measuring the LE [13,20].

2.3. Shock pulse production

An electromagnetic (EM) type ESWL device (Rifle, HnT Medical, Rep. Korea), one of clinical devices, was used as the shock wave generator. The EM type ESWL device is capable of generating high-output shock waves, and has excellent reproducibility of shock waves under a given operating [21,22]. The shock wave generator consists of a pulse power supply and a solenoid coil shock wave transducer. When electrical energy stored in a capacitor (1uF) charged to a high voltage (up to 20 kV) is discharged from the pulse power supply through the solenoid coil, the coil and the cylindrical metal film outside the solenoid interact electromagnetically to vibrate [21]. The fundamental frequency of the vibration pulse of the metal film is determined by the size of the capacitor and the inductance of the solenoid shock wave transducer [22,23]. By the parabolic reflector which has the co-central axis coincident with the solenoid shock wave transducer, the vibration pulses of the cylindrical metal film are concentrated at a focal point and become high pressure shock wave pulses. Pressure pulses generated at all locations on the cylindrical metal film are reflected by the parabolic reflector and propagate the same distance to the focal point. The collimator of the shock wave generator is placed vertically upward at the bottom of the cylindrical water tank (diameter 450, height 400 in mm) (Fig. 1). The time required for the shock wave generated immediately after turning on the device to reach focus was measured to be 169 μs.

Fig. 2 shows the typical waveform measured at the focal position (Fig. 2a) and the maximum pressure (Fig. 2b) measured as the output setting range (1 low – 9 high) of the shock wave generator is varied. A broadband (upto 150 MHz) optical hydrophone (FOPH 200 RP Acoustic, Germany) was used to clearly measure the shock front of the waveform. In Fig. 2a, the time axis is indicated based on the time the shock wave is generated (t = 0), and the maximum pressure of the shock wave is observed at t=~169us which is the time it takes for the shock wave to reach the focus.

The peak positive and peak negative pressure ranges of the shock wave at the focal point were measured as 33 – 95 MPa and –10 – –17 MPa, respectively, and increased nonlinearly as the output setting was changed from 1 to 9 (Fig. 2b).

2.4. Luminol solution

Luminol is a white-to-pale-yellow solid, soluble in ionized organic solvents, but insoluble in water. When luminol is added to an alkaline solution, it reacts with FR to release SCL. In this experiment, an alkaline solution was used in which sodium carbonate (Na2CO3) was dissolved and the pH was adjusted to 14 [18]. Based on Negishi [18], a luminol solution was prepared by dissolving 2.5 g of sodium carbonate in 500 mL of distilled water to dissolve luminol well, and then completely dissolving 0.1 g of luminol.

After filling an open-top acrylic resin container with luminol solution, the container was placed vertically on the upper part of the water tank. In order to place the focus of shock wave 50 mm above the bottom, the position of the container was adjusted. The internal size of the container is 50(W) x 50(D) 150(H) (in mm) and the bottom was made of 0.2 mm thick thin PMMA (poly methyl methacrylate to allow the shock wave to pass through. By using an absorber layer at the top of the tank, multiple reflections of shock waves in the tank were minimized. In addition, an optical shield (NPL Plain Tile, APTFLEX F28) was placed to shield the light generated from the shock wave generator pulse power supply or the light noise reflected from the stainless steel reflector at the bottom of the tank.

2.5. Light detection by PMT

The light emitted by the reaction of luminol and OH* FR produced as a result of strong cavitation at the focus of the shock wave was recorded using a PMT (H10721-110, HAMAMATSU, Hamamatsu City, Shizuoka, Japan) in a dark room. The PMT sensor used in the experiment is a current output type and can measure the rise time up to 0.57 ns. The PMT was placed close (~35 mm apart) to the outer surface of the luminol solution container, and the axial direction of the sensor was positioned perpendicularly to the shock wave beam axis at the shock wave focus point.

2.6. Measurements

The shock wave generator located in the darkroom was operated with a trigger signal from the function generator outside the darkroom. This trigger signal was simultaneously transmitted to a high definition oscilloscope (HD06104A, 10GS/s, 12-bit ADC resolution, TELEDYNE LECROY) to collect the PMT output signal. The water tank was filled with distilled water, and the temperature of the luminol solution and water was maintained at room temperature, 25 °C. In case of repeated measurements, the interval of shock wave generation was maintained for at least 30 s to minimize the change in the state of the medium due to
3. Results

Fig. 3 shows the typical LE pulses recorded by the PMT in water and the luminal solution for 1 s. The output of the shock wave generator was set to the maximum of 9 (the charging voltage of 20 kV). The LE signal sensed by PMT was stored at 50 MHz sampling rate. The time $t = 0$ represents the moment when shock wave generator is switched on. The signals measured for $t < 0$ represent the back ground signals from each medium before exposure to shock pulses.

LE is hardly detected from the distilled water (for $t < 0$ in Fig. 3a) and is virtually equal to the dark noise of PMT (~0.02 V) obtained from air without light in the dark room. However LE was enhanced in the luminal solution (for $t < 0$ in Fig. 3b) to the back ground level (BGL ~0.09 V). This would be attributed to SCL that the luminol chemically reacts with OH$^-$ FR already existing in water. Note that the dark noise would be originated from PMT itself as well as caused during signal transmission.

LE increases significantly when the shock pulse was irradiated to the media. A number of large LE pulses appeared in a very short time for a several hundred microseconds. The LE pulses result from SL that occurs during the rapid compression or violent inertial collapses of the bubbles produced by the shock pulses [2,24,25].

When the temperature and pressure inside the bubble rise rapidly due to strong bubble rupture, the chemical bond of the gas inside the bubble is broken. SL is a phenomenon in which the energy difference generated at this time is emitted as light. The LE pulses emitted in the $t = 150–600$ µs section where the SL phenomenon occurs exceeds the maximum measurable value set by the sensor (~8 V) in both water and luminal solution. Fig. 3 shows the signal amplitude up to ~0.4 V in order to maintain the detail of the low signal, which occupies most of the signal.

After the burst of the bubble mostly ends about 600 µs, the SL-induced strong light is no longer emitted (Choi & Song 2020). The LE signal emitted from the water returns to the background noise (dark noise) state before the shock wave occurs after the SL phenomenon ends. Unlike water, in luminol solution, after the SL phenomenon is completed, LE pulses are continuously emitted due to the chemical
reaction between OH* FR generated in the SL process and luminol. The magnitude of the LE pulse emitted as a result of SCL is very small compared to the LE pulse signal emitted during the SL process, and is slightly larger than the magnitude of the SCL signal emitted from luminol before shock wave irradiation.

However, since the number of LE pulses per unit time is much higher than before the shock wave irradiation, it can be seen that the FR generated by the shock wave is emitting light through the SCL phenomenon. The repetition of LE pulse per unit time recorded gradually decreases over time.

The chemical reaction of OH* FR takes place for a very short time, and the light signal emitted during this process has a very short duration (<\sim10\text{ ns}). Since the sampling time of 20 ns (frequency 50 MHz) used in Fig. 3 is not small enough compared to the LE pulse width (several ns), it was down-sampled during recording the signal with an oscilloscope. Therefore, it is difficult to visually detect the change of temporal density of LE pulses emitted by SCL.

Fig. 4 shows, on a more detailed time scale, the density and magnitude of LE pulses measured for 1 ms at nine different time-point A to I marked in Fig. 3: A $= 5$ ms, B $= 0$ ms, C $= 5$ ms, D $= 10$ ms, E $= 20$ ms, F $= 50$ ms, G $= 100$ ms, H $= 200$ ms. In the case of water (Fig. 4a), the signal recorded for 1 ms at the time of shock wave generation ($t = 0$) is a large LE signal due to the SL phenomenon, but the pulse density is not high. At the same time point, the dense LE signal by SCL as well as the large LE signal by SL are recorded in luminol case (Fig. 4b). In Fig. 4b, it is easy to understand the change in the LE pulses density gradually decreasing over time, while it is not clearly observed in Fig. 3. It takes more than 500 ms for
the LE pulses density to decrease similarly to before the generation of shock wave. This required time represents the persist time of the FR generated by the shock wave. If data is collected for a long time (1 s using 20 GHz sampling time) to observe the persist time of FR, the capacity that can be processed by the oscilloscope is exceeded. Therefore, in this study, the sampling frequency was set to 50 MHz, which is the maximum frequency that can be processed.

The signals shown in Figs. 4 and 5 represent the LE pulses emitted whenever the shock wave-induced SL (<1 ms) and SCL caused by the reaction of FR generated in the SL process with luminol occurred. The amount of FR generated by the shock wave is expected to be proportional to the number of SCL LE pulses. Therefore, the number of pulses per unit time (temporal LE pulse density) or the amount of LE per unit time can be used as a variable for estimating the amount of FR generated by the shock wave.

Fig. 5a shows the amount of LE per unit time (1 ms) that was converted from the value of the LE pulse recorded in Fig. 3 for 1 ms at 1 ms intervals. Fig. 5a shows high variability as a result of a single measurement. In order to reduce the variability, a signal with low variability were obtained by averaging the results of 10 repeated measurements as shown in Fig. 5b. Although the timing and magnitude of the LE pulse cannot be seen in Fig. 5b, the change in the amount of LE by the SCL of FR over time can be easily observed.

As shown in Fig. 5, the amount of light emitted per unit time reaches a maximum immediately after the shock wave is generated, and decreases rapidly exponentially. The amount of light emitted after the shock wave first reaches the value of MBGL (max BG level ~0.1094 V us), the maximum value of the background signal before the shock wave, around 200 ms. The amount of emitted light repeatedly exceeds or falls below the max BG level for a certain period of time, and then falls below the max BG level (Fig. 5b).

In Fig. 5b, the total amount of light exceeding the max BG level (hatched area) indicates the amount of light emitted by the reaction of all shock wave-induced FR with luminol. It is expected that this value can be used as an index to estimate the amount of OH* FR generated by the shock wave.

Fig. 6 shows the corresponding time from the 1st crossing (n = 1) to the last crossing (nmax). The persist time tp, which is the period that the active FR generated by the shock wave is maintained, has a value in the range of t1 to t48. Note that tn represents the nth crossing. As shown in Fig. 6, they are grouped into four time domains (T1 – T4) based on the period in which no crossing occurs for a relatively long time. Each of the four time domains represents T1 from n = 1 to 32, T2 from n = 33 to 44, T3 from n = 45 to 46, and region T4 from n = 47 to 48, respectively. For about 250 ms between T3 and T4, the amount of LE maintains below the MBGL with no crossing, then shows the last crossing at about 600 ms.

Fig. 7 shows the result of measuring the amount of LE for 1 ms over time by changing the output setting of the shock wave generator (in the range of 1 to 9). In order to observe when the LE reaches the MBGL, the scale of the vertical axis in Fig. 7 is enlarged to show the low signal in detail. As the output setting lowered from 9 to 1, the amount of LE by the SCL decreases and t1 becomes faster.

Fig. 8 shows the persist time of FR (tp = t1 – tmax) and the total LE by the SCL for t1 – tmax (TLE(tp)) using the signal measured 10 times in each setting. In each figure, the 10 measured data are presented as a scattergram on the left, and symbolic presentation on the descriptive statistical parameters (outlier (min, max), quartile (upper, lower), mean, median) are shown on the right. As expected in general, the persist time of FR and TLE by SCL of FR increase as the output setting increases (Fig. 8). The t1 value decreases

Fig. 5. Temporal history of the extent of LE measured in the luminol solution at the maximum output settings of 9. The vertical axis represents the quantity of light calculated by integrating the LE pulses above the dark noise level of 0.02 V for 1 ms: (a) single measurement, (b) average with 10 repeated records. Note that the persist time ranges from the 1st crossing to the last crossing of the signal to the max BG level (~0.1094 V us).
to below 100 ms with high correlation as the output decreases. On the other hand, the maximum time during which FR is maintained (tnmax) reaches a maximum of 1000 ms and it does not show a high correlation with the setting of the output value (Fig. 8a). TLE(tp), which is highly correlated with the amount of FR generated by the shock wave, changes very sensitively depending on the output value setting and non-linearly increases as the set value increases (Fig. 8b).

At 169 µs after the shock wave is generated, strong energy is accumulated at the focal point, and a large amount of cavitation bubbles are created. The strong negative pressure (down to ~20 MPa) of the shock wave exceeds the tensile strength of the propagation medium (water) and creates a large amount of air bubbles. Most of these bubbles immediately contract or burst, but some of the contracted bubbles rebound and grow into large bubbles for a relatively long time (< ~600us) then burst violently.

The contraction or rupture of the bubble causes high temperature and pressure in the bubble, resulting in a sonoluminescence phenomenon in which light is emitted from the bubble. At the same time, it also leads to the generation of FR in these environments.

4. Discussion

In order to measure the degree of occurrence and persist time of OH* FR generated by the shock wave used in ESWL, the LE signal emitted from the luminol solution was observed after irradiating the shock wave. Immediately after the shock wave irradiation, a very large signal is emitted for a short time, which is emitted as an SL phenomenon in the course of strong bubble contraction or rupture. After that, LE pulses with a small signal size but high temporal density were observed due to the SCL phenomenon, which emits light by reacting luminol with FR generated in the SL process. Since bubble bursts are all terminated within ~600 us, LE after 1 ms is not related to SL, and is interpreted as the result of reaction between luminol and OH* FR generated in the SL process.
The generation of FR depends on the cavitation, and the cavitation is affected by the shock wave generation method and the conditions of the medium to which the shock wave is irradiated [1, 7, 26]. In this study, the FR generated in luminol solution was observed using an EM-type shock wave generator. Among the shock wave generation methods, the electrohydraulic (EH) method is known to induce the strongest cavitation because of its low fundamental frequency and high energy at low frequencies, and is expected to generate the most FR. The polyethylene (PE) shock wave generation method is expected to generate the least FR because the fundamental frequency is relatively high and the pulse waveform is relatively long. In this study, OH\(^*\)FR generated in luminol solution was considered, but the correlation with various FRs generated in the living tissue environment is not yet clear. Several in vitro experiments on shock waves have reported FRs produced between cells [10]. Since there are several factors that inhibit cavitation in living tissue, the generation and duration of FR in living tissue is expected to be smaller than the results measured in this study conducted on OH\(^*\) generated by shock waves irradiated in luminol solution [9]. In addition, the cavitation conditions of living tissues are physically and chemically different from the experimental conditions considered in this study. Therefore, in order to more clearly understand the effect of the FR in clinical practice, systematic in vivo studies on FR induced by the shock waves in tissues are necessary.

In general, FR is chemically unstable and reacts immediately after formation and exists for a very short time [27]. Although studies on the generation of FR by shock waves and the increase in the amount of FR generation according to the setting of the shock wave generator have been reported [27], there is no measurement technique with sufficient time resolution to observe the formation and disappearance of very rapidly changing FRs. Therefore, it is not easy to obtain information about the persist time of FR.

As observed in this study, the FR induced by the shock wave from the generator used in this study survived up to 1000 ms. In order to minimize the harm of FR caused by shock wave irradiation, the interval of the shock wave irradiation should be sufficiently larger than the FR persist time. In the ESWL procedure, the shock wave irradiation interval is usually set to 1 s, but the recent years, there is a trend to reduce the irradiation interval to shorten the treatment time. If the irradiation interval is too short, the exposure efficiency of shock wave energy is reduced because not only the harmfulness of FR but also the physical environment of the tissue changed by the preceding shock wave affects the propagation of the subsequent shock wave.

Therefore, additional researches on the generation and the persist time of FR according to the shock wave irradiation interval is required to minimize the harmfulness of FR while maintaining the shock wave exposure efficiency in the actual treatment environment.

This study was not limited to a specific FR, but designed to obtain information on the generation and persist time of OH\(^*\)FR by measuring the amount of SCL emitted by the reaction of luminol with OH\(^*\)FR produced as a result of cavitation by shock wave. Therefore, various production conditions of OH\(^*\)FR were not considered in this experiment. OH\(^*\)FR can be produced by the chemical reaction of H\(_2\)O\(_2\) and O\(_3\) produced by cavitation [16] or the reaction of H\(_2\)O\(_2\) with metal ions [19]. In addition, the LE measured in the experiment is mainly understood as a result of the reaction between OH\(^*\)FR and luminol, but since H\(_2\)O\(_2\) generated by cavitation can react directly with luminol to show sonochemiluminescence [19], SCL from the reaction of H\(_2\)O\(_2\) and luminol might be included in the measured LE signal. Based on these
information, it is suggested as an additional study to apply the FR generation and persist time measurements presented in this study to specific OH•FR generation conditions. In particular, for OH•FR generated by the reaction of H₂O₂ with metal ions, it is recommended to add EDTA to the solution to reduce the chemical reaction involving metal ions.

5. Conclusions

The measured persist time of FR ranged from 75 to 1000 ms and as the setting of the shock wave generator increases, the minimum or average FR persist time increases, but the maximum value does not show a high correlation with the setting. The amount of FR generation is highly correlated with the shock wave setting and it increases rapidly, non-linearly and very sensitively, with increasing the setting. In order to reduce the risk of FR to lithotripsy patients, the amount of FR generation during the procedure should be minimized, and the irradiation interval of shock wave should be sufficiently larger than the persist time of FR. The recent trend to increase the output and shorten the shock wave irradiation interval to reduce the procedure time of ESWL is expected to have negative consequences for patient safety related to FR. For the purpose of formally raising this risk, additional studies on the generation and persist time of FR according to the method and setting of shock wave generation in living tissues or similar environment are proposed as the next step.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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