Sonosensitization Mechanism of ATX-70 in Sonodynamic Therapy

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Abstract: Sonodynamic therapy (SDT) is an effective method to cure tumors, but the mechanism is not clear up to now. In this work, the mechanism of SDT was analyzed by studying the reactions of gallium-porphyrin analogue (ATX-70). Results showed that the ATX-70 might play two roles in SDT. One was that the high temperature produced in the bubbles at collapse promoted ATX-70 into excited states, and reacted with the dissolved oxygen in liquid and produced oxygen free radicals, which was thought as an effective killer for cancer cells. The other was that ATX-70 played a role as surfactant in the process of cavitations and eased the cavitations, leading to produce more high-energy hydroxide radicals. [Life Science Journal. 2006;3(4):85-89] (ISSN: 1097-8135).

Keywords: sonodynamic therapy; ultrasound; ATX-70; cavitations

Abbreviations: ATX-70: gallium-porphyrin analogue; CL: chemiluminescence; DMSO: dimethylsulfoxide; ICCD: intensified charge coupled device; FCLA: luuoresceinyl cypridina luminescent analog; PDT: photodynamic therapy; SDT: sonodynamic therapy; SL: sonoluminesenece

1 Introduction

Sonodynamic therapy (SDT) is a new method to cure tumors[1-13]. This method, compared to the photodynamic therapy (PDT), has two virtues for clinical application. One is that ultrasound has a deeper penetrability, and the other is that it needn't avoid light in the whole process of treatment. The gallium-porphyrin analogue (ATX-70) is a widely used sonosensitive in SDT[3]. It can selectively gather in the tumors and enhance the effect of therapy. However, the mechanism was unknown[4].

There are different opinions on the mechanism of SDT. Umemura firstly thought the sonoluminescence (SL) excitated the ATX-70 to produce $^1O_2$ which can kill the tumor cells, the same mechanism as PDT[5]. The study of Kessel *et al* pointed that the sonosensitive function of ATX-70 was relative to the cavitation[6]. Miyoshi[3] studied the mechanism thoroughly and found that the oxygen content in the gas bubble was important to the cavitation. 20% O_2 was necessary for sonosensitive process, and the ATX-70 maybe acted as surface activator to strengthen the cavitations. He further indicated that the sonosensitive reaction did not cause from the SL excitated by the sonosensitiver, but has its own mechanism. There are always different viewpoints on whether the $^1O_2$ takes part in the sonosensitive process. The research of Sakusabe *et al*[7] suggested that the sonosensitiver can kill tumor cells by enhancing the yield of $^1O_2$ and other active oxygen in the process of cavitation. Yumita *et al* also thought that the active oxygen produced in the sonosensitive process was crucial in SDT[5]. However, in sonosensitive experiment, Miyoshi[8] *et al* did not find $^1O_2$ by EPR. In addition, it's not sure whether the ATX-70 can be used repeatedly in SDT. At present, there are no detailed reports about these problems.

In this paper, mechanism of the SDT was studied by optical method. In the experiments, the selective probe of active oxygen named fluoresceinyl cypridina luminescent analog (FCLA) and un-selective chemiluminescence (CL) probe named luminal were to detect whether the active oxygen produced in the sonosensitive process timely and directly.

2 Materials and Methods

2.1 Reagent preparation

FCLA (Sigma, USA) was diluted to 50 μmol/L by distilled water. It has been known that FCLA only reacts with $^1O_2$ and O_2^-[14, 15]. Luminol (Sigma, USA) which can react with many

sorts of free radicals and emit photons[16], was diluted to 50 μmol/L by distilled water. ATX-70 (Toyohakka Kogyo, Japan), which was thought as the best sonosensitizer[3], was also diluted to 50 μmol/L by distilled water. SOD (Sigma, USA) selectively consuming O_2^-, was diluted to 10 μmol/L by distilledwater. NaN_3, a sort of medicament to reacting with $^1O_2$ selectively, was prepared with 10 mmol/L[17], and DMSO, reacting with ·OH selectively, was prepared with 10 mmol/L.

When the experiment began, injected some distilled water in the glass firstly, and then added the needed reagents, made the whole volume 2 ml and the concentration of FCLA, luminol, ATX-70, DMSO, SOD and NaN_3 was 1 μmol/L, 1 μmol/L, 2 μmol/L, 2 mmol/L, 1 μmol/L and 2 mmol/L, respectively.

2.2 Apparatus and methods

The experiment setup was shown in Figure 1. The SL or CL was detected by: intensified charge coupled device (ICCD) image system(Princeton Instruments, ICCD-576-S/1, −40 ℃). The ST-130 controller(Princeton Instruments, USA) controlled the ICCD and put the optical signal into computer. The luminescence intensity could be caught by the software of WINVIEW. The ultrasound field made the reagents mixed equably and quickly.

The signal function (AFG320, SONY, Japan) brought sine signal with 500 kHz and fed the signal to power amplifier (ENI CO. Ltd, 2100L, 50-dB). The 50 W signal from the amplifier was used to driver the transducer (diameter: 5 cm, Mingzhu, Guangzhou, China) to emit ultrasound. To avoid the influence of idle photons, the black ink was used as the transmit media of ultrasound, and the temperature was kept 25 ± 1 ℃.

The absorb spectrum of ATX-70 was collected by absorb spectrum system with the split of 10 nm, which usually was used to highly sensitive detection.

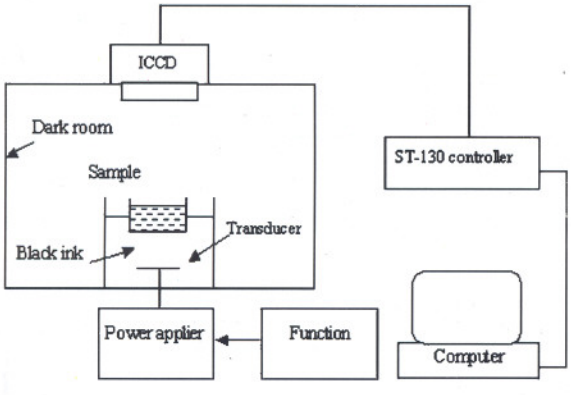

Figure 1. The setup for CL detection

3 Results and Discussion

Figure 2 is the detected curve of CL intensity in different solutions, with the power of ultrasound 50 W, the frequency 500 kHz, and the total time 50 seconds. In the first five seconds, the background intensity of ICCD is about 50 cps, and when the FCLA was added (the second five seconds), the intensity increased to about 450 cps. It can be taken as the experiment background. After that, when the ultrasound began to act on the solution, the CL intensity increased to about 15,000 cps. When ATX-70 was added in, the intensity increased to about 45,000 cps immediately. However, with the SOD was added in, the intensity decreased to about 21,000 cps, and it would decrease ulteriorly to about 2,600 cps when NaN_3 was added in. The experiment was repeated three times, and the standard deviation at each time point was lower than 9%.

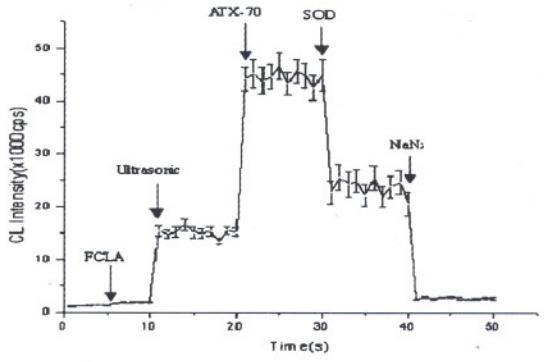

Figure 2. The CL intensity of saturated air liquid in 50 seconds. The data was means ± S.D. (n = 3)

As we know, FCLA can react with active oxygen selectively and emit photons. When the ultrasound appeared, added the ATX-70 to the FCLA solution, and the CL intensity increased obviously. It was believed that it was ATX-70 that accelerates the production of active oxygen radicals. When the SOD was added in the solution, the CL decreased but not disappeared, which indicated that not only O_2^- but also other active oxygen radicals were produced in the process, for the SOD only react with O_2^- and decrease the CL. The CL intensity continuously decreased when the NaN_3 was added in, which further proved that the $^1O_2$ radicals were produced in the ATX-70 solution. The final intensity was 2,600 cps not 450 cps, which indicated that the ATX-70 not only participated in the produce of active oxygen but also promoted the produc-

tion of sonoluminescence directly.

Figure 3 showed the dependence of CL intensity on the gas component in liquid. The left showed the CL intensity under saturated air condition and the right under saturated N_2 condition. The (□) and (■) represent the CL intensity of FCLA and FCLA + ATX-70 solutions, respectively. As shown in Figure 3, the CL intensity of FCLA + ATX-70 solution under N_2 saturated condition was only about 1,000 cps, much lower than that in the air saturated solution, which was reached to about 50,000 cps. It is because that in the air saturated solution, ATX-70 reacted with the dissolved O_2 and produced $^1O_2$ and O_2^-. However, in the N_2 saturated solution, the ATX-70 can't react with O_2, and no active oxygen could be produced. It indicated that, in the cavitations, ATX-70 can't produce the active oxygen free radicals solely, without the dissolved oxygen. Thereby the content of oxygen would affect the CL intensity.

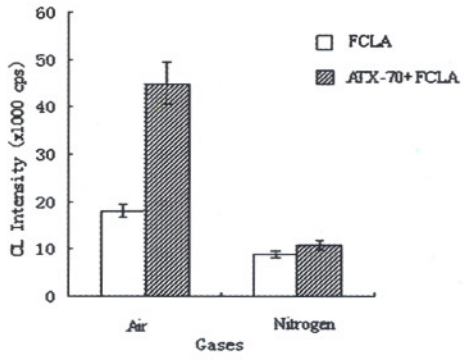

Figure 3. The CL intensities of 1 μM FCLA(□) and FCLA + ATX-70 (■) solutions which were detected in the air saturated (left) and nitrogen saturated conditions (right) respectively. The data was means ± S.D. (n = 3)

Figure 4 showed the absorb spectrums of ATX-70. Curve A represents the spectrum which was detected before the ultrasound and curve B represents the spectrum 20 seconds after the ultrasound. Before and after the ultrasound, the two absorb spectrums of ATX-70 were similar. The peak values of the both spectrums are about 400 nm, and the subordinate values are about 600 nm. The result showed that, in spite of the ATX-70 accelerated the active oxygen produced in cavitations, however, its molecular structure was not destroyed. In Figure 4, the intensity of curve B decreased more slightly than curve A at 400 nm. The reason is that ultrasound makes the ATX-70 redistribute in the solution and leads the scan light of spectrum system not to irradiate them completely.

From the above experiments, it can be concluded that, with the dissolved oxygen, ATX-70 promotes the production of active oxygen radicals in the process of cavitations. To describe how the ATX-70 acts in cavitations, luminal (mainly reacts with ·OH) was added in the following experiments. The sequence of the reagents was shown in Figure 5.

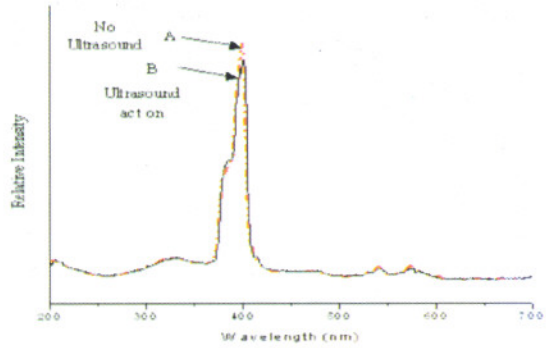

Figure 4. The absorb spectrums of ATX-70 before and after ultrasound act. curve A: spectrum of ATX-70 before ultrasound act; curve B: spectrum of ATX-70 after ultrasound act 20 s

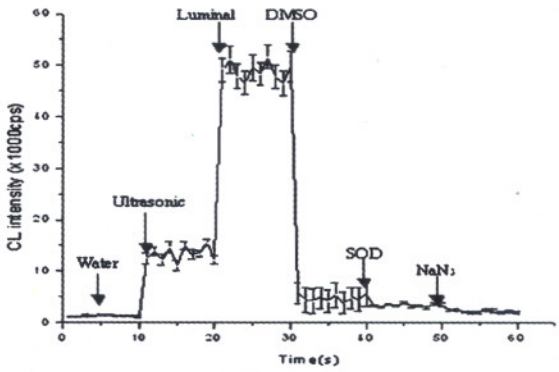

Figure 5. The change of CL intensity in luminal solution for prove the production in cavitation. The data was means ± S.D. (n = 3)

When the luminal was added in the water, with the ultrasound, the CL intensity increased obviously. However, when DMSO was added in, the CL intensity decreased quickly, just above the background. Then, adding the SOD and NaN_3 in order, the CL intensity did not almost change. It indicated that it was ·OH that was mainly produced in the process of cavitations, but the yields of $^1O_2$ and O_2^- were very low. When adding ATX-70 into the luminal solution, as shown in Figure 6 (the experiment was repeated three times), the CL

intensity decreased from about 51,000 cps to about 42,000 cps. Adding FCLA into the mixed solution, however, the CL intensity increased again. It indicated that the ATX-70 can react with the ·OH radicals and decrease the concentration of the ·OH in the cavitation, leading the CL intensity to decrease and more active oxygen to produce.

Based on the above results, the main reaction could be as follows:

$$2H_2O \xrightarrow{caviation} 2\cdot\dot{O}H + 2H + photons \quad (1)$$

$$\dot{O}H + ATX\text{-}70 \rightarrow \cdot OH + A\dot{T}X\text{-}70 \quad (2)$$

$$A\dot{T}X - 70 + 2O_2 \rightarrow {}^1O_2 + O_2^- + ATX\text{-}70 \quad (3)$$

In the experiments, another phenomenon was also found. It is the ATX-70 that can reduce the threshold of sonoluminescence. Experiments showed that when the power was 35 W, sonoluminescence began to appear in the liquid without ATX-70. However, the power decreased to about 28 W when ATX-70 was added. So ATX-70 makes the cavitations become easier. The ATX-70 maybe act as some sort of surface activator to strengthen the cavitations, just like described by Miyoshi[3].

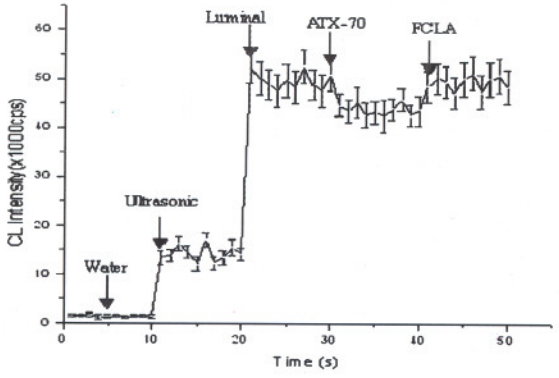

Figure 6. The change of CL intensity for prove the production of active oxygen. The data was means ± S.D. ($n = 3$)

4 Conclusion

In this paper, the main work is to discuss the role of ATX-70 in the process of cavitations. The results showed that the active oxygen, which was used to kill the tumor cells in SDT, was mainly produced by the chemical reaction in the process of cavitations instead of coming from cavitations directly. The main function of ATX-70 in cavitations is to transfer the energy of ·OH to the dissolved oxygen in the liquid, and turn the oxygen into $^1O_2$ and O_2^-. Though ATX-70 increases the yield of active oxygen, its molecular structure does not change, just like some sort of catalyze. The result also showed that ATX-70 is a highly efficient sonosensitizer in SDT.

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