A study of ovarian cancer biomarker amplification using ultrasound for early stage detection ☆

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ABSTRACT

The application of serum biomarker to ovarian tumors for early stage detection and clinical diagnosis is a rapidly expanding research area. The problem with conventional markers is that they are often released too late or at too low a level to be detected in time to trigger effective treatment. Ultrasound has been used to influence bio-effects in living cells, but there is only one reported case of the use of ultrasound to enhance the release of a biomarker (Carcinoembryonic antigen CEA). In this study we report the use of ultrasound to enhance the release of a combination of ovarian cancer biomarkers (CA125 and CA19-9) to help in the diagnosis of ovarian cancer at an early stage. The results indicated that after 5 min sonication at a frequency of 1 MHz and intensity of 0.3 W cm^{-2}, the CA125 and CA19-9 levels were increased by 2.02 and 4.21-fold respectively. These findings suggest that ultrasonic treatment can be used to enhance the release of serum biomarkers from ovarian tumors.

1. Introduction

Ovarian cancer is one of the leading causes of cancer deaths among women but if the disease is treated at an early stage there is an excellent prognosis for survival following treatment. However some 70% of patients are diagnosed at an advanced stage and in this case there is a poor survival rate of only 10–30% therefore it is important to detect ovarian cancer at early stage [1,2]. Current diagnosis methods for ovarian cancer are the CA-125 blood test and transvaginal ultrasound [3]. But neither of these tests are sufficiently sensitive or specific [4]. Recently, biomarkers such as CA125 and CA19-9 have been used to help in the early screening and diagnosis of ovarian cancer i.e. at a stage when it is the most treatable and before it has had a chance to grow and spread [5–8]. CA125 is the most widely used tumor marker in ovarian cancer but its sensitivity and specificity are not ideal because the levels of this marker are raised to approximately 80% of all epithelial ovarian cancers (EOC) and in only 50% of stage I EOC. The sensitivity and specificity rates of another biomarker CA19-9 are both lower than those of CA125. From this it can be argued that the results from individual existing markers are not specific

enough because they can be affected by other malignancies, benign conditions such as diverticulitis, liver cirrhosis and also by physiological conditions including menstruation and pregnancy. In addition the concentration of a biomarker signal in the blood is often very low and so is difficult to detect. In order to be used in screening for early stage cancer the tumor marker must be sufficiently detectable to provide a positive result. Research [9–16] suggests that the use of a combination of biomarkers improves sensitivity for the detection of ovarian cancer.

Ultrasound has been shown to be effective in a range of cancer therapies including the direct killing of cancer cells, enhanced drug delivery and for improving membrane permeability for the uptake of drugs. In 2009, researchers [17] reported the use of ultrasound to increase the release of biomarker Carcinoembryonic antigen (CEA). In this study, ultrasound at 1 MHz with an intensity of 0.3 W cm^{-2} was applied in cell culture experiments using the human colon cancer cell line LS174T. The biomarker CEA concentration was measured before and after exposure of the tumor cells to ultrasound. After 30 min treatment there was a 4-fold increase in the CEA levels when compared with untreated control samples. Mouse tumor xenograft models were also tested using 1 MHz ultrasound at an intensity of 2 W cm^{-2} applied directly to the site of the tumor for 6 min. The CEA level was increased by more than 10-fold, which may have been due to the somewhat higher intensity used. The ultrasonic transducer applied in this work was a commercial device (Sonitron 2000, Belgium), which can provide a well collimated beam penetrating deep into the tissue. This work indicated the possibilities that the concentration of tumor

biomarkers in blood could be increased leading to a more sensitive and specific diagnosis. As an extension of this it would be of interest to determine whether ultrasonic treatment can enhance the release of two other ovarian tumor biomarkers CA125 and CA19-9 to a concentration enabling early stage screening and a consequent improvement in patient survival rate. One of the bio-effects of ultrasound is to induce a temporary increase in the permeability of the cell membrane. 1 MHz, continuous-wave ultrasound ($\sim$8 W cm^{-2}) for 30 s treatment has been suggested to induce transient pores in the cell membranes of some surviving cells [18]. This was the driving force for our work which was to use this effect to provide a safe but effective ultrasonic treatment for the enhanced release of ovarian tumor biomarkers without causing permanent damage to the human cells involved.

2. Materials and methods

2.1. Cells

Human ovarian carcinoma cell lines, SKOV$_3$ were cultured in McCOY's 5A medium (12400024, Gibco, Life Technologies, USA) supplemented with 10% fetal calf serum (SV30087.02, Hyclone Laboratories Inc., USA) at 37 °C and under 5% CO$_2$ concentration. Cells grew in incubator (SERIES 8000 WJ, Thermo Scientific, USA).

2.2. Ultrasonic equipment

The parameter settings of the HM-1 ultrasonic equipment (Jiangsu Hanmei Science and Technology Co., Ltd., Taizhou, China) were: frequency of 1 MHz, intensity of 0.3 W cm^{-2} and 50% duty cycle. The diameter of the ultrasonic probe was 21 mm. An ultrasound power meter (Model UPM-DF-1E, Ohmic Co., Ltd., USA) was used for intensity measurement and an oscilloscope (model TDS1000B, Tektronix Co., Ltd., USA) for the measurement of frequency.

2.3. Ultrasonic treatment of cells

Cells were seeded at 5×10^6 in culture flasks (Gibco, USA), and grown overnight in complete media forming a 100% confluent monolayer of cells. On the following day, the media was removed and the cells were rinsed three times with 2–3 mL fresh media. 5 mL cells were then added into culture flasks immediately before sonication. Ultrasonic treatment was applied from the bottom of the flask for 5 min. To achieve good sonication, ultrasound coupling gel (Shengyou, Taizhou, China) was used between the bottom of the flasks and the ultrasonic probe, with the probe placed flush against the bottom of the flasks (Fig. 1). Control samples were run in parallel without sonication. Immediately after treatment 0.5 mL samples of suspension were removed for analysis.
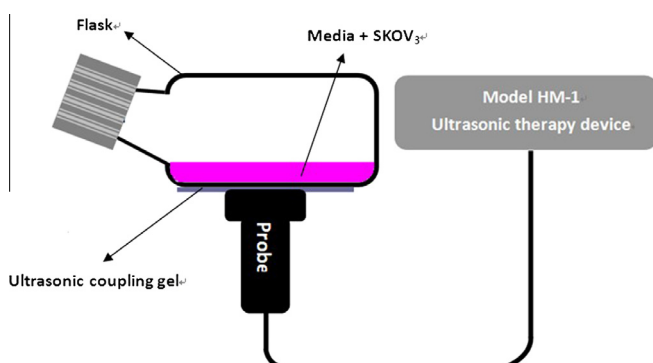

Fig. 1. Schematic diagram of experimental set-up.

2.4. CA125/CA19-9 quantification

CA125 and CA19-9 concentration in media were determined using a Roche automatic electrochemiluminescence immunoassay analyzer (COBAS6000, Switzerland). The sensitivity of this method was 0.6 U/mL.

2.5. Cell death detection

Cell death was determined immediately after sonication. The media was removed, the cells were harvested and cell death was determined by adding 10% Trypan blue to the cell suspension. Only dead cells with disrupted cell membranes are stained by the dye. The percentage of dead cells was counted using an inverted microscope (1X51, OLYMPUS, Japan) within 3 min after staining.

2.6. Statistical analysis

Statistical analyses were done with SPSS17.0. All data are presented as the mean ± standard deviation (SD). Analysis of variance (Univariate ANOVA) and t test were adopted. A significance level of 0.05 was used.

3. Results and discussion

3.1. Time release of CA125 from ovarian cancer cells in culture treated with ultrasound

To study the time release of biomarkers, cells were sonicated using the ultrasonic probe system described above. A significant increase of CA125 was observed from a treatment time of 5 min and the biomarker concentration ($P < 0.01$) continued to increase slowly over the remaining 30 min sonication. The average concentration of CA125 after sonication was 6.26 U/mL, which was a 3-fold increase in CA125 levels compared with control samples (2.15 U/mL). The release of CA125 was also seen to increase slightly ($P > 0.05$) without sonication but this was significantly lower compared with sonicated samples (Fig. 2). Similar reports of ultrasonic effects on CEA were published by D'Souza et al. [17]. In our study we found that the major increase occurred after 5 min treatment but after this the continued increase of CA125 concentration was slow ($P > 0.05$). Thus, we decided to set the sonication time in all further experiments for 5 min.
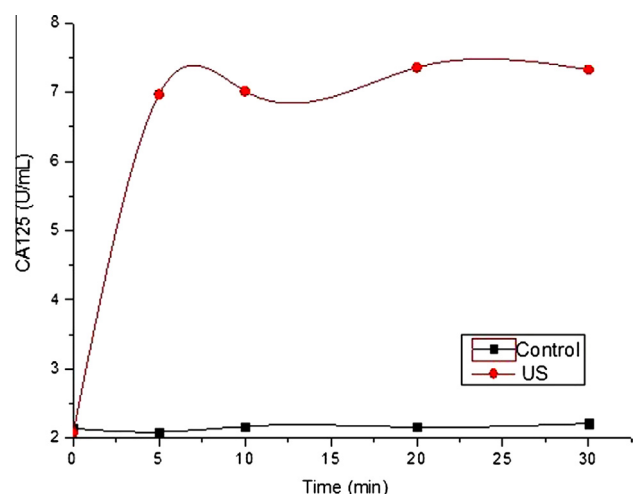

Fig. 2. Time release of CA125 from ovarian cancer cells in culture in the presence and absence of ultrasound.