Antitumor Effect of Fever Range Whole Body Hyperthermia with Curcumin in Breast Cancer-induced Mice

Hanim Saim, Maheza Irna Mohamad Salim, Khairunadwa Jemon

Curcumin is a hydrophobic polyphenol, a dietary phytochemical and a principle active ingredient derived from turmeric. It was first isolated by Vogel and Pelletier in 1815, its chemical structure and synthesis confirmed by Lampe et al. in 1910 and 1913 [4]. Chemically, it is a bis-α,β unsaturated β-diketone named (E,E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-5-one or commonly called diferuloylmethane [5]. Commercial curcumin is a mixture of curcuminoinds, containing approximately 77% diferuloylmethane (Curcumin I), 17% demethoxycurcumin (Curcumin II), and 5% bisdemethoxycurcumin (Curcumin III) [4].

Curcumin has chemopreventive and therapeutic activity against various breast cancer cell lines [6]-[8]. Besides, previous study of curcumin in vivo presented significant reduction of tumor multiplicity, prolongation of tumour-free survival inhibition of the tumor growth, reduced the tumor size and weight during the treatment [7]–[10]. Additionally, curcumin also used in combination with other modalities. The tumor growth in female CD1 nude mice suppressed by the combination treatment of curcumin (200mg/kg/day) and epigallocatechin gallate (25mg/kg/day) for 10 weeks [11]. Further, study reported that combination of curcumin (50mg/kg) and metformin (80mg/kg) in 14 days showed the highest significant reduction in the tumour size [12]. While the combination of taxol with curcumin in the BT-474 xenograft model had an antitumour effect comparable with taxol and herceptin treatment [10].

On the other hand, hyperthermia is broadly referred as a condition where mean body temperature higher than normal. In medicine, hyperthermia therapy (HT) or thermotherapy is the procedure to elevate the temperature of a part of or the whole body above normal for a definite period of time, using external and internal heating device. Hyperthermia treatment temperatures in clinical procedure range between 39–45 °C [13]. Hyperthermia is commonly applied as combination with various established cancer treatment modalities such as radiotherapy [14], chemotherapy [15], surgery and immunotherapy [16]. The temperature and duration of HT damages cells and enhances radiotherapy and chemotherapy sensitivity [17].

The cellular changes such as protein denaturation and aggregation, inducing cell cycle interruption and apoptosis, increasing membrane permeability, altering Ca2+ homeostasis and promoting intracellular accumulation of chemical agents show the efficiency of hyperthermia in cancer treatment.
As well as, the cytotoxic effects of hyperthermia is including activation of the immune systems against tumor, enhancing drug delivery and improvement of oxygenation [18], [19]. This study aimed to investigate the efficacy of curcumin combined with fever range whole-body hyperthermia in suppressing breast cancer tumor growth.

II. MATERIALS & METHODOLOGY

Animals

Animal care and use in this study was conducted according to standard ethical guidelines and experimental protocols approved by the Animal Ethics Committee of Universiti Kebangsaan Malaysia, approval number (UTM/2017/MAHEZA IRNA/22-NOV./884-NOV.-2017- MARCH-2019).

Female BALB/c mice (6-8 weeks old) [20] with 17-25g body weight was purchased from local university Animal Research Unit. Animals were housed in cage (max 5/cage) with 12-h dark and light cycles, fed standard laboratory diet and water ad libitum daily and bedding changed every two days. The mice allowed to acclimatize for at least 7 days before experiments.

Selection of curcumin dose

Dosage range between 50 ug/kg to 200mg/kg were effectively inhibit breast cancer growth as reported in previous studies on various animal model species [11], [21], [22]. However, curcumin dosage of 50mg/kg was used to Balb/c mice induced EMT6 breast cancer tumor size [12]. Therefore this study is used as reference.

Selection of temperature and duration of fever range whole-body hyperthermia (FRWBH) treatment

This study was initiated by the optimization of temperature and duration of FRWBH treatment conducted using water-filtered infrared-A (wIRA) by Hydrosun Medizintechnik GmbH device to observe the viability of mice under certain temperature of mild hyperthermia within the range of 39.0-40.0°C which commonly used in clinical procedure [16].

Tumor inoculation

EMT6 mouse mammary tumor cells were maintained in Waymouth’s MB752/1 Liquid Medium (1x) Thermo Fisher [23] supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS) Thermo Fisher and 1% antibiotics (100U/ml penicillin and 100μg/ml streptomycin). Cells were harvested using trypsin solution TrypLE™ Express Enzyme (1x) without phenol red, washed, centrifuged and re-suspended in Phosphate Buffered Saline (PBS), at a density of 5 X 10^6 cells/ml [20]. Cells count performed using haemocytometer plate and the cells re-suspended in PBS. Each BALB/c mouse received 5x10^5 EMT6 cells in 100μl PBS [24] injected subcutaneously at right flank.

FRWBH treatment

Whole body Fever Range hyperthermia (FRWBH) was conducted twice using water-filtered infrared-A (wIRA) by Hydrosun Medizintechnik GmbH device on day-17 and day-23 post inoculation. Mice were anesthetized with the mixture of ketamine (150 mg/kg) and xylazine (7.5 mg/kg) and hyperthermia treatment were conducted at 39°C (±0.5) maintained for up to 15 minutes. Rectal temperature monitored continuously in each mouse using small animal rectal thermostat probes [25] along the treatment (Figure 1).

General toxicity assessment

Mice body weight recorded every other day after inoculation as a general indicator of toxicity. Drug-specific toxicities such as liver toxicity, renal toxicity, neurotoxicity were not measured in this study.

Antitumor activity assessment

Mice were randomly distributed into four groups (n=5 for each group). Group 1 received no treatment (CONTROL). Group 2 received 50mg/kg bwt curcumin orally (CUR) using oral gavage daily for 14 days starting day-14 post inoculation until day-27 post inoculation. Group 3 received whole-body hyperthermia treatment conducted at 39°C (±0.5) maintained for up to 15 minutes on day-17 and day-23 post inoculation (FRWBH). Group 4 received both curcumin 50mg/kg bwt orally and hyperthermia treatment conducted at 39°C (±0.5) maintained for up to 15 minutes on day-17 and day-23 post inoculation (CUR+FRWBH). Summary of mice group allocation and the treatment received as shown in Table 1. Mice were sacrificed on day-28 post inoculation. Tumor size was measured and recorded every other day using electronic caliper to determine the diameter [26]. Tumor volume was calculated according to the formula ‘tumor volume = 1/2(ab2’ in which ‘a’ is the longest diameter and ‘b’ is the shortest diameter of the tumor.

Fig. 1 Mice rectal temperature recorded during FRWBH treatment. Median rectal temperature of the mice during hyperthermia treatment was 39.05°C and mean rectal temperature of the mice was 38.17 °C. Approximately 12 minutes required for the rectal temperature to reach 39.0 °C (±0.5).

![Image](image-url)
Table 1. Summary of mice group and the treatment received

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment received</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>No treatment received.</td>
</tr>
<tr>
<td>CUR</td>
<td>Orally 50mg/kg daily (day-14 to day-27 post inoculation).</td>
</tr>
<tr>
<td>FRWBH</td>
<td>Whole-body hyperthermia treatment conducted at 39°C (±0.5) maintained for up to 15 minutes on day-17 and day-23 post inoculation.</td>
</tr>
<tr>
<td>CUR + FRWBH</td>
<td>Orally 50mg/kg daily (day-14 to day-27 post inoculation and Whole-body hyperthermia treatment conducted at 39°C (±0.5) maintained for up to 15 minutes on day-17 and day-23 post inoculation.</td>
</tr>
</tbody>
</table>

Statistical Analysis

Data analyzed using Graph Pad Prism Windows 5.00 and presented as mean ± SEM (standard error of mean). One-way analysis of variance (ANOVA) was used to measure variations between different groups. Unpaired-samples t-test was used to examine the effects of different treatments on the tumor size and body weight. The level of significance was set at p < 0.05.

III. RESULTS

Selection of temperature and duration of fever range whole-body hyperthermia (FRWBH) treatment

Each of experiment group for temperature optimization consist of two mice (n=2). All mice (n=2) were dead immediately after completion of FRWBH treatment of 40.0 °C (± 0.5) for 30 minutes. The second group of mice is exposed to FRWBH treatment of 40.0 °C (± 0.5) for 15 minutes. One mouse (n=1) dead a day after the treatment and another one (n=1) survived up to 14 days after the treatment. The result was similar for mice received FRWBH treatment of 39.0 °C (± 0.5) for 30 minutes. However, all mice (n=2) which received FRWBH treatment of 39.0°C (± 0.5) for 15 minutes were survived more than 10 days after the treatment. Therefore, the temperature of 39.0°C (± 0.5) was selected as optimum temperature and duration of 15 minutes as duration method for FRWBH treatment of this study.

General toxicity assessment

Table 2 shows the result for mice body weight for each group. Mice body weights recorded every other day as an indicator of general health and treatment-induced toxicity. Overall result shows there is significant different of mean body weight among the groups (p<0.0001) and weight gain observed significantly in all groups within 28 days of experiment.

Table 2 Effect of 50mg/kg curcumin, FRWBH and combination of both on mice body weight.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean body weight (g)</th>
<th>p value</th>
<th>Body weight gain (g)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>24.21 ± 0.14</td>
<td></td>
<td>1.21 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>CUR</td>
<td>23.96 ± 0.13</td>
<td>0.1975</td>
<td>0.36 ± 0.13</td>
<td>0.0004</td>
</tr>
<tr>
<td>CUR + FRWBH</td>
<td>26.64 ± 0.13</td>
<td>&lt;0.0001</td>
<td>0.64 ±0.13</td>
<td>0.0089</td>
</tr>
<tr>
<td>FRWBH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Antitumor activity assessment

Tumor observed to continuous grow starting on day-7 and the time course for tumor volume changes after the initiation of treatment on day-14. Figure 2 shows results of the tumor growth profile for all groups during experiment. Mean tumor volume of the mice after 28 days was significantly different among groups (p=0.0042, p<0.01). Mice treated with CUR+FRWBH proved significantly delayed tumor growth as compared to curcumin and control. The tumor in control group grew progressively as compared to others group, while FRWBH demonstrated the greater tumor growth control among the group.

Tumor inhibition rate for all treatment groups is shows in Figure 3. CUR+FRWBH inhibition rate is higher than curcumin with 68.45% and FRWBH has the highest inhibition rate.
Antitumor Effect of Fever Range Whole Body Hyperthermia with Curcumin in Breast Cancer-induced Mice

Breast cancer is one of the main cancer among female population worldwide. Due to the elevated death rate related with cancer and severe side effects of chemotherapy and radiation therapy, many cancer patients seek alternative complementary and alternative medicines (CAM) as method of treatment. CAM have related with natural herbal medicines and herbal plant, which are alleged to various biological and molecular mechanisms by inhibiting the growth of cancer. Hyperthermia is commonly applied as combination with various established cancer treatment modalities such as radiotherapy, chemotherapy, surgery and immunotherapy, effectively increased antitumor effect. Thus, this study is conducted to search for new alternative treatment of breast cancer by funds of curcumin combined with hyperthermia treatment.

In this study, each group received single treatment of curcumin or hyperthermia and combination of both to see the efficacy of each treatment towards the tumor growth and animal health. Mean body weight of treated mice were higher than control mice and there were weight gained in all groups up to 28 days of experiment that may due to tumor growth. It is better than the decline in body weight due to toxicity [27]. General observation of animal health showed that the treatments were well tolerated by the mice and non-toxic to the mice.

The results of our study towards tumor volume of mice suggested that FRWBH was effectively reduced tumor size with the highest inhibition rate (86.14%). The findings are equivalent with previous studies which stated hyperthermia was cytotoxic to breast cancer cells [30], [31]. However, there was rapid tumor growth few days before experiment ended which reinforced that hyperthermia as single-treatment modality had low response rate and short duration limits [16], [32].

Even though the tumor inhibition rate in combined group was lower than FRWBH group, our results show the tumor growth in CUR+FRWBH group was plateau few days before end of experiment compared to others treatment group, indicating the combination treatment had better effect on tumor growth suppression compared to CUR and FRWBH treatments. The findings are in line with previous studies that combination of radiotherapy (RT), chemotherapy (CT) or RT plus CT treatment with hyperthermia were better, compared with the same treatment without hyperthermia in mice and human [13], [25], [33].

In summary, we demonstrated that FRWBH combined with curcumin was beneficial against solid tumor than treatment with FRWBH or curcumin alone, with respect to inhibition of tumor growth without general toxicity effect.

V. CONCLUSION

As from this perspective, we suggest that combination treatment with FRWBH and curcumin is actually useful as alternative antitumor treatment for breast cancer. However, further study with larger sample and various temperature and duration of whole body fever range hyperthermia should be conducted in the future in regards to explore the mechanism of action of this combination treatment.

VI. ACKNOWLEDGMENT

The authors' work was supported by grants from Ministry of Higher Education for Universiti Teknologi Malaysia (KPT-UTM RUG grant 4F274 15H87).

REFERENCES

Preclinical evaluation of Laromustine for use in cancer care.


