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The Xihuang Pill in the Treatment of Breast Cancer MCF7 Cell

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Abstract

Purpose: Treating cancer victims with triple negative is difficult because of a deficit in treatment alternatives, aggressive characters, and inappropriate diagnosis. A common anti-tumor impacts used in China is Xihuang Pills (XHP) and it an example of a Chinese traditional therapy. The research paper explores the anti-cancer influences of Xihuang pill particularly on MCF7 cells and also looks into its molecular processes in treating cancer. Method: Several tests for cell death (apoptosis), cell cycle dispersal, and mitochondrial membrane potential will be done and analyzed. Western blot assay shall also be conducted and finally a tumor xenograft form will be applied in studying the impacts of Xihuang pill in vivo. Possible Results: Results of the MTT test indicate that feasibility of MCF7 cells is significantly hindered by Xihuang in a dose and time reliant form. The hinderance impact of the pill on the feasibility of MCF7 cells is higher than that of MCF-10A cells. After administering with 4, 8 and 14 mg/ml Xihuang from MCF7 cells, increased apoptosis and disintegration of mitochondrial membrane potential were realized. Protein expression of cleave caspase-3 increased 1.62, 2.13, and 2.19-overlap equated to untreated experiments, with minimum effect on manifestation of B-cell lymphoma 2 (Bcl-2) or the Bcl-2 related protein X (Bax) will be portrayed. Cell cycle distribution assay analysis results indicate that administration of Xihuang pills disrupt cells in G2 / M phase. Conclusion: The viability of MCF7cells will be hindered by Xihuang in a dose and time-reliant and particular way for cells in vitro, and the possible related processes can include mediated cell death together with cell cycle arrest in the G2/ M phase. XHP can initiate death of MCF7cells by the intrinsic channel, not involving the Bcl -2/ Bax ratio. The G2 / M phase arrest can have resulted because of the consolidated activity of reduced expression of cyclin A and raised expression of p21Cip1. Furthermore, Xihuang inhibit the thriving of xenograft cancer without weight loss in vivo.

Keywords

Cancer therapy; Traditional Chinese Medicine; MCF7; Caspase 3/8/9; PARP Cleavage.

1. Introduction

Breast cancer is one of the prevailing kinds of malicious malignancy in women globally, and the major reason of cancer demises among women world-wide. It accounts for about 25.5% of all cancers in the world. For instance, in the U.S, about 253,720 recent instances of encroaching breast cancer and 41,590 breast tumor deaths are anticipated to happen in women in the U.S. in 2007[1,2]. The cases of breast tumor have raised in the developing due to several reasons ranging from growing urbanization, embracing a stressful modern way of life to extended life expectancies. Early diagnosis of breast cancer can result in a significant prognosis and high chances of survival. Treatment of cancer has been a hectic task though there have been several vital treatments currently being applied. These treatments include; surgery, endocrine therapy, immunotherapy, molecular targeted therapy, and chemotherapy [3]. However, according to many studies, these therapies are connected to many postoperative side effects such as significant vein thrombosis, harmfulness to the liver and renal

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functioning, complications, myelosuppression, severe gastrointestinal tract activities, upper limb swelling, syndromes associated with menopause, peripheral neurotoxicity, and also heart attack.

Hence, it is crucial to discover a potent therapy with minimum toxicity and lesser terrible side effects for the treatment of breast cancer. The Chinese traditional medicine (Xihuang pill) is an actual example of the alternative treatment that has been in use in China for about 3000 years ago due to its particular system of medical hypothesis about pathogens and therapeutic way. This pill was initially noted in the former Chinese medicine book Wai Ke Sheng Ji [1]. The Xihuang pill is prepared from extracts of rare herbs; myrrh, musk, frankincense, and bezoar. Each ingredient used to manufacture this pill has a significant advantage. The musk is known to activate blood stagnation, and bezoar acts as a detoxicant. Experiments have shown that Xihuang pills can impede cancer proliferation yet encouraging apoptosis of human cancer cells and also boost immunity [4].

Xihuang Pill (XHP) is commonly administered to manage breast cancer. It enhances the effectiveness of chemotherapy and reduces chemotherapy-triggered harmfulness in patients with breast malignancy. However, the various molecular processes of Xihuang pill in the treatment of breast cancer have been explored by few researchers. This paper analyzes the prediction that increasing amounts of Xihuang pills kill breast cancer cells or rather breast cancer cell xenograft. In the various experiments in this paper, I will treat MCF7 cell with the Chinese traditional medicine (Xihuang Pill) and also have a control experiment using taxol positive control and then measure MTT, cell counts, colony formation assay and western blot for caspase 3/8/9 and finally PARP cleavage determination.

2. Main Body

2.1 Materials

2.1.1 Drug Pedagogy

A total of 4 g of Xihuang will be put in 15 ml of cold distilled water and combined with a rotary test tube mixer for three hours at 5 ° C. The Xihuang will then be disintegrated by use of an oscillator. ultrasound (40 kilohertz) for 3 hours at 36 degrees Celsius and the specimen will be centrifuged at 1500 xg for 6 minutes at 5 degrees Celsius. The supernatant will again be centrifuged at 4800 xg for 20 min at 5 ° C, then the eventual supernatant will be sieved through sterile porous membrane (0.25 µm in diameter) prior being stored at -25 degrees Celsius. Xihuang will be mixed to desired concentrations in RPMI-1640 solution before processing the cells [1,5].

For in vivo experiments, 4 g of Xihuang pill will be mixed in 23 or 45 ml of cold distilled liquid and centrifuged at 4 ° C for 3 hours. The XHP will then be disintegrated with an ultrasonic oscillator (40 kilohertz) for 3 hours at 36 degrees Celsius and stored at -25 degrees Celsius up to use. Xihuang will be warmed to room temperature and shaken with the XHP solution before intragastric administration to nude mice.

2.1.2 Cell Culture [6]

It will be obtained the human breast cancer cell line MCF7 from the Cell Resource Center of Peking Union Medical College. The cells will be grown in RPMI-1640 environment having 15% fetal bovine serum. The cells will be grown in a moistened section at room temperature and 7% carbon dioxide [6,7].

MCF-10A Human Breast Epithelial Cells will be offered by a donor from a cancer hospital. Cells will be grown and processed in Dulbecco / F-12 Modified Eagle's environment provided an epidermal thriving element of 22 ng per milliliter, human insulin of 10 ng per milliliter, hydrocortisone of 0.6 microgram per milliliter, cholera toxin of concentration 100 ng per milliliter, five per cent horse serum, 100 microgram per milliliter of streptomycin and also penicillin [6,7].

2.1.3 In vivo cancer xenograft version

Female BALB / c nude mice will be caged in laminar wind cabins with no viruses around and subjected to 10 hours of light and 10 hours of darkness. The mice will be provided by Vital River Laboratory Animal Technology Co. Limited. The mice will receive autoclaved general food and

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water. The specimen testing authority will be authorized by the Animal Research Committee of Peking University and will be done according to the rules of the Guide for the Care and Use of Laboratory Animals. Living MCF7 cells will be mixed in 0.1ml RPMI-1640 medium, mixed 1: 1 with Matrigel to obtain the percentage of Matrigel 60% (Pan et al.,2013, pp.770-778). The cells will be immunized subcutaneously with 2 x 104 cells / mouse on the right side of the mice [5].

Swelling will be recorded and tumor volume will be evaluated using: Tumor volume (mm3) = 0.5 x length x width. After the swelling size reaches 6-0100 mm3, the mice will be assigned to three various management groups where n = 10 per group: refined water per control group, 20 milligrams per day group, and 40 milligrams group per day. The mice will be given refined water, 30 milligrams per day Xihuang pill together with 30 milligram per day Xihuang pill intragastrically specifically. The overall quantity of the therapy will be administered two times in 14 hours for 14 days. Also, after the mice Xihuang pills, the body size will be recorded and this will happen after every four days. The mice will be offered 14 days after giving of XHP with 1.6% sodium pentobarbital and swelling tissues will be preserved at -75 ° C for analysis. protein modification.

2.2 Methods

2.2.1 MTT assay

MCF7 cells will be inoculated into 96-well plates and administered the next day with different quantities of Xihuang ranging from 0, 4, 8, 12, 16 milligrams per milliliter for 6, 12, 24 hours or two days hours. MCF-10A cells will be inoculated into 96-well plates and treated with Xihuang (14 mg / ml) for 14 hours the next day. The MTT assay will be done applying the procedure previously explained. Afterwards recording of the optical density will be done at 570 nanometers utilizing a 96-well microplate reader [1]. Because the decrease of MTT occurs only in metabolically live cells, the optical density records will be applied to give an evaluation of cell feasibility [5]. The percentage of cell feasibility will be determined using the technique: (Optical Density treatment / Optical Density control) x 100.

2.2.2 Apoptosis assay

MCF7 cells (3.2 x 105) will be plated on 40 mm culture plates. The next day, cells will be administered with a range of 0, 4, 8 or 12 milligrams per milliliter of Xihuang for the whole day. Afterwards, cold PBS (Phosphate buffer saline) will be used to clean the cells and then stained with annexin V per fluorescein isothiocyanate per propidium iodide staining tool. The cells will be recognized by movement cytometry evaluation on a BD FACSCalibur flow cytometer and the outcome will be reviewed using ModFit software [8].

2.2.3 Mitochondrial Membrane Potential Assay

Disintegration of mitochondrial membrane potential will be realized through a mitochondrial membrane potential assay. MCF7 (3.2 x 105) cells will be concepted in 30-millimeter culture plates. The next day, the cells will be administered with 0, 4, 8 and 12 milligrams per ml Xihuang during the entire day. The cells will later be trypsinized and stained for 20 minutes with JC-1 at 36 degrees Celsius. The cells will then be cleaned twice with a staining buffer (JC-1) and immediately assessed by flow cytometry using a BD FACSCalibur flow cytometer, and the final outcome will be reviewed with software version [8].

2.2.4 Cell cycle distribution assay

MCF7 cells (8 x 105) will be seeded in 40 mm culture plates. The next day, the cells will be administered with a range of 0, 4, 8 and 12 milligrams per milliliter Xihuang for two days. After the cells have been trypsinized, cleaned using PBS and then mixed in 1 milliliter of cold 72 per cent ethanol throughout the night at a temperature of 5 degrees Celsius, the cells will be centrifuged (1000 xg, 8minutes, 5 degrees Celsius), cleaned with cold and processed PBS with PI/ RNase staining buffer following manufacturer's guidelines for 20 minutes at normal temperature with no light [6].

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Cell cycle division will be realized through movement cytometry by a BD FACSCalibur flow cytometer. To calculate the percentage of cells in the G1, S or G2 / M phases, ModFit version 6.0 software will be utilized.

2.2.5 Western blot analysis

MCF7 cells (3.06 × 10 6) will be seeded in 100 mm culture medium. The day after, cells will be administered with 0, 4, 8 and 12 mg per ml Xihuang pill for about a whole day. The cells will later be gathered, cleaned with cool PBS and integrated with radioimmunoprecipitation assay buffer. In a mouse-mouse xenograft type, after the mice will be killed, cancer tissues will be preserved at -75 degrees Celsius for protein expression review. After the protein removal, cancer tissues will be amalgamated with radioimmunoprecipitation assay lysis buffer. The concentration of overall protein concentrates will be recognized using a bicinconic acid protein assay tool. The protein tests will then be combined five times with loading buffer and heated for 8 minutes. Protein tests (35 µg) will be distinguished by 10% SDS-PAGE and moved to polyvinylidene difluoride membranes. The membranes will be obstructed with 10% skimmed milk or bovine serum albumin, 10 mM Tris-HCl, 0.1 M NaCl and 0.1% Tween-20 in Tris-buffered saline for three hours at normal temperature. -Tween-20 (TBST). 7.4.) The membranes will later be hatched with particular main antibodies throughout the night at 5 degrees Celsius with calm mixing. After cleaning with cold TBST, the membranes will be covered with secondary antibodies bound to horseradish peroxidase for 45 minutes at ordinary temperature. The protein bands will be observed using HRP Immobilon Western Chemiluminescent Substrate. The strength of the protein band will be recorded with the ImageJ software version 2.0, and assimilated for β-actin and equated to the control [1].

Table 1. Different Breast Cell Lines Used in This Study

Cell Line Organism type	Description
MDA-MB231 Human	Commonly used to late- stage breast cancer [5]
MCF-7 Human	Breast cancer cell line with glucocorticoid receptors, estrogen and progesterone [6,7]

2.3 Data analysis

Outcome is shown as the mean ± standard deviation of at least three self-supporting practicals. The numerical information will be reviewed directly by assessment of variance and Tukey's post-hoc test with SPSS version 13.0. $P < 0.05$ is perceived as a sign of a statistically important variation.

2.4 Results

Possible Result 1: XHP inhibits the sustainability of MCF7 cells

Assessing the cytotoxic impacts of Xihuang pill on MCF7 cells, the feasibility of cells administered with various volumes (0-16 mg per ml) of Xihuang pill will be recorded using the MTT assay. The outcome show that Xihuang pill minimizes cell feasibility in a dose and time reliant form. Hinderance of the feasibility of MCF7 cells after subjection to Xihuang pill is observed as early as five hours after subjection. The percentage of cell feasibility after subjection to Xihuang pill for 6, 12, 24 and 48 hours at the highest volume is greatly minimized from 100% to 85.55 ± 4.03%, 75.39 ± 12.42%, 33.13 ± 8.04% and 4.24 ± 2.36%, respectively. In contrary, no numerical variation is realized between the control group and the 4 mg per ml Xihuang pill group all through stages. Therefore, the use of MTT assay can support the hypothesis in this paper that increasing amounts of Xihuang pills kill breast cancer cells or rather breast cancer cell xenograft.

Possible Result 2: XHP induces apoptosis in MCF7 cells

To determine if the antiproliferative impacts of Xihuang pill in MCF7 cells can include triggering of cell death, the annexin V / propidium iodide double coloring technique will be used in combination with flow cytometric analysis. After administering with 4, 8 and 12 milligrams per milliter Xihuang pill for the entire day, the percentage of cells at initial cell death shot from 3.7% (untreated cells) to

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4.7, 5.5 and 9.9%. while the percentage of cells in final cell death shot from 8.3% (untreated cells) to 12.9, 16.7 and 22.6%, specifically. A numerical great rise in the speed of apoptosis is seen after administering cells with 10 and 12 mg / ml Xihuang pill equated to controls. Hence, Xihuang pill dose-dependently trigger cell death in MCF7 cells. Therefore, the use of apoptosis assay can support the hypothesis in this paper that increasing amounts of Xihuang pills kill breast cancer cells or rather breast cancer cell xenograft.

Possible Result 3: XPH induces the reduction of mitochondrial membrane potential in MCF7 cells

Movement cytometric review of MCF7 cells administered with different amounts of Xihuang pill will be done to record changes in potential membrane mitochondria in order to find out a rise in cell death of MCF7 cells is related with decrease of mitochondrial membrane potential. After administering with Xihuang (4, 8, and 12 milligrams per milliliter) for the entire day, the percentage of low potential mitochondrial membrane cells reduced from 5.3% (untreated cells) to 7.0, 19.5 and 20.2%, specifically. Xihuang pill triggers great reduction of mitochondrial membrane potential in MCF7 cells after is administered with 12 mg per milliliter of Xihuang pill. The outcome shows that Xihuang pill causes a dose-reliant decrease in mitochondrial membrane potential. Thus, the use of mitochondrial membrane potential assay could support the hypothesis in this paper that increasing amounts of Xihuang pills kill breast cancer cells or rather breast cancer cell xenograft.

Possible Result 4: Xihuang triggers apoptosis through the intrinsic channel

Cell death(apoptosis) is performed through two main routes called intrinsic and extrinsic routes. These pathways lead to the activation of caspase-3. The caspase group is the main group of proteases involved in cell death and is divided into two operational groups. Initiators of cell death (Caspase-8, -9 and -10) and executors of cell death (Caspase-3, -6 and -7).

Bax is a common pro-apoptotic protein and Bcl-2 is an anti-apoptotic protein, and the Bcl-2 / Bax ratio plays a crucial function in the triggering of cell death. To find out whether the proteins caspase-3, caspase-8, Bax and Bcl-2 participate in triggering apoptosis, the expression of these proteins in MCF7 cells will be administered with Xihuang pill by Western blot technique. The outcome show that the cleaved caspase-3 protein expression quantities increased 1.62, 2.13 and 2.19-fold after XHP treatment of 4, 8 and 12 mg per millilter, respectively in one day. Nevertheless, the expression extents of cleaves caspase-8, Bcl-2, Bax and Bcl2 / Bax ratios in MCF7 cells did not differ greatly between cells administered with 0, 4, 8 and 12 mg per milliliter Xihuang pill. These outcome indicate that Xihuang pill can intrinsically and not extrinsically induce apoptosis, which is linked to the Bcl-2 / Bax ratio. Therefore, the use of Western blot technique could support the hypothesis in this paper that increasing amounts of Xihuang pills kill breast cancer cells or rather breast cancer cell xenograft.

Table 2. Possible Results on Cell Proliferation

Cell Lines	Result 1	Result 2	Result 3	Result 4	Result 5
MTT assay	+	-	-	-	-
Apoptosis assay	-	+	-	-	-
Mitochondrial Membrane Potential Assay	-	-	+	-	-
Cell cycle distribution assay	-	-	-	-	+
Western blot analysis	-	-	-	+	-

Note. "+" represents a significant decrease in cell proliferations. "-" represent not significantly different from negative control.

Possible Result 5: Xihuang pill induces cell cycle arrest at the G2/M stage

Regulation of the cell cycle is essential for cell multiplication. Many anti-tumors medicine trigger cell cycle hinderance by preventing cell multiplication. To analyze if the anti-multiplication impacts of Xihuang pill are linked with cell cycle arrest, the cell cycle dispersal of MCF7 cells administered with XDA will be examined. G2 / M phase cells rose from 11.43% in the untreated group to 17.72,

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20.65 and 26.33% after administration with 4, 8 and 12 mg per milliliter Xihuang pill, in the following order. In line with these changes, the percentage of cells in the G1 or S phases will decline. The outcome will show that the Xihuang pill therapy greatly affects the cell cycle dispersion of MCF7 cells, resulting to cell cycle arrest in the G2 / M phase in a dose-reliant form.

Hence, the use of cell cycle distribution assay could support the hypothesis in this paper that increasing amounts of Xihuang pills kill breast cancer cells or rather breast cancer cell xenograft.

2.5 Discussion [9]

The study aims at predicting the anti-tumor(cancer) effects of Xihuang pill in cancer cells in vitro and in vivo, together with the possible molecular processes entailed. Hence, MTT, cell death, cell cycle division, and Western blot assays is performed and a nude mouse xenograft cancer type is launched.

Xihuang pill hinders the feasibility of MCF7 cells through a drug-reliant and time form, and this has been shown by the outcome of MTT assay. Regarding on the difference in feasibility of MCF7 and MCF-10A cells after treatment with XHP, it is likely that Xihuang selectively hinders the feasibility of MCF7 cells for cells, which is a crucial aspect in cancer management.

To comprehend the process relating to the antiproliferative impacts of Xihuang in vitro, the degree of cell death and the cell cycle division of MCF7 cells administered with varying volumes of Xihuang pill is realized, and the outcome indicates that Xihuang triggers cell death and cell cycle arrest in the G2 / M phase in MCF7 cells, and is in agreement with the outcome of the MTT assay. A past research with the human TNBC Hs578T [4] cell line showed same impacts of Xihuang pill in vitro, with Xihuang pill hindering the feasibility of Hs578T cells, bringing about cell death and cell cycle arrest in S phase, not G2 phase [10]. Also, past research studies have shown the anti-cancer impacts of Xihuang pill, in which Xihuang pill blocked the multiplication of human cancer cell lines. All of these analyses, including my study, show that Xihuang pill has anti-cancer action. To elaborate the process of the antiproliferative impacts of Xihuang pill, further research was done, and on top of the cell death and cell cycle arrest found in my research, the elucidated anti-cancer processes such as killing of cancer cell invasion, movement and metastasis, hinderance of angiogenesis and development of the cancer immune micro surrounding.

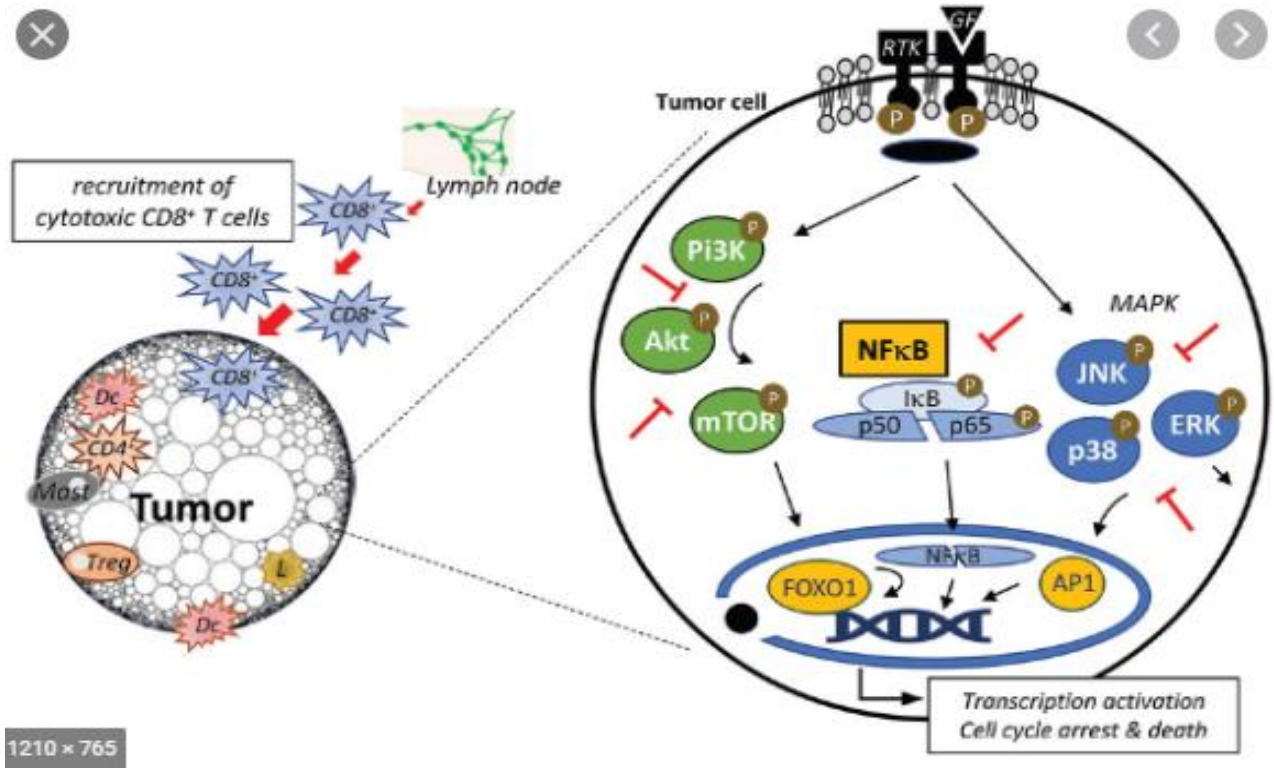

Figure 1. Mechanism of action of the Chinese Traditional Medicine (Xihuang Pill) [10]

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3. Conclusion

The feasibility of MCF7 cells is greatly hindered by treatment with Xihuang in a dose-reliant, temporal and cell-choosy form in vitro. Possible underlying mechanisms may include triggering of cell death and cell cycle arrest in the G2 / M phase. Xihuang pill instigates MCF7 cells death by the intrinsic channel, that is not linked with changes in Bcl 2 / Bax ratio. The cell cycle arrest observed in the G2 / M phase may result from the integrated process of declined expression of cyclin A and high expression of p21Cip1. Additionally, Xihuang pill stopped the thriving of xenograft cancer in nude mice without reducing the size of the body in vivo. Hence, the results of my study indicate that Xihuang pills exhibits anti-tumor impacts on the TNBC MCF7 cell line, providing proof of using Xihuang pill for treatment of breast cancer.

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