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Oral artemisinin prevents and delays the development of 7,12-dimethylbenz[a]anthracene (DMBA)-induced breast cancer in the rat

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Abstract

Artemisinin, a compound isolated from the sweet wormwood *Artemisia annua L.*, has previously been shown to have selective toxicity towards cancer cells in vitro. In the present experiment, we studied the potential of artemisinin to prevent breast cancer development in rats treated with a single oral dose (50 mg/kg) of 7,12-dimethylbenz[a]anthracene (DMBA), known to induce multiple breast tumors. Starting from the day immediately after DMBA treatment, one group of rats was provided with a powdered rat-chow containing 0.02% artemisinin, whereas a control group was provided with plain powdered food. For 40 weeks, both groups of rats were monitored for breast tumors. Oral artemisinin significantly delayed ($P < .002$) and in some animals prevented (57% of artemisinin-fed versus 96% of the controls developed tumors, $P < .01$) breast cancer development in the monitoring period. In addition, breast tumors in artemisinin-fed rats were significantly fewer ($P < .002$) and smaller in size ($P < .05$) when compared with controls. Since artemisinin is a relatively safe compound that causes no known side effects even at high oral doses, the present data indicate that artemisinin may be a potent cancer-chemoprevention agent.

Keywords: Artemisinin; Daily oral intake; Breast cancer; Prevention

1. Introduction

Artemisinin and its analogs are now widely being use as antimalarials. These compounds contain an endoperoxide bridge that forms a carbon-base free radical, when encounter an iron atom [1,2]. Free radicals, when formed intracellularly, cause molecular damages and could lead to cell death. Artemisinin and analogs are effective antimalarials because malaria parasites contain a high amount of intracellular heme iron.

Due to their rapid rate of division, most cancer cells have high rates of iron intake [3] and express a high cell surface concentration of transferrin receptors [4], which are involved in the transport of iron into cells. In general, the aggressiveness of a tumor is positively correlated with transferrin receptor concentration of its cells. For example, breast cancer cells have 5–15 times of transferrin receptors on their cell

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surface than normal breast cells [5], transferrin receptors are expressed on cell surface of breast carcinoma cells but not on benign breast tumor cells [6], and breast cancer cells do take up more iron than normal breast cells [7]. High cell surface concentrations of transferrin receptor are also found in leukemic cells [8,9]. Thus, artemisinin is also selectively toxic to cancer cells because of their high iron content. We have shown that artemisinin, in the presence of iron in vitro, induces apoptosis [10], and is lethal towards human leukemia [11] and breast cancer [12] cells. Normal cells pick up less iron and have better intracellular regulation of iron content, they are significantly less susceptible to artemisinin. For example, we have shown that artemisinin is approximately 100 times more potent in killing human leukemia cells than normal lymphocytes.

Since artemisinin can effectively kill cancer cells, it may also be toxic to pre-cancerous cells. In the present study, we investigated whether daily oral intake of artemisinin could prevent the development of cancer in the rat. In the research, rats were orally administered a single dose of the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) to induce breast cancer [13,14]. They were then provided with a powdered food mixed with 0.02% artemisinin. Incidence and time of breast cancer development were recorded and compared with animals that were similarly treated with the carcinogen and given a normal powdered rat chow.

2. Materials and methods

2.1. Animals

Female Sprague–Dawley rats (150 g at the start of the experiment), purchased from Charles River Laboratories (Wilmington, MA), were used in the experiment. Animals were housed three to a cage in a specific-pathogen free vivarium maintained in a 12-h light–dark cycle (light on 6 a.m.–6 p.m.) and at an ambient temperature of 22 °C and relative humidity of 65%. Animal-use protocol of this experiment was approved by the Animal Use and Care Committee of the University of Washington.

2.2. Experimental procedures

Rats were orally intubated with 50 mg/kg of 7,12-dimethylbenz[a]anthracene (DMBA, Sigma Chemicals, St Louis, MO) using a 8-french feeding tube (Burns Veterinary Supply, Inc., Vancouver, WA). DMBA was suspended in olive oil (Sigma Chemicals, St Louis, MO) and given in a volume of 2 ml/kg. On the day after DMBA treatment, animals were randomly divided into two groups. The first group ($N=21$) was given a powdered rodent chow (TestDiet 5001, Nutrition International, Brentwood, MO) mixed with 0.02% of artemisinin (Holley Pharmaceuticals, Fullerton, CA). The second (control) group ($N=23$) was given the powdered rodent chow alone. Water was provided ad libitum. Based on the assumption that a 250 g female rat consumes ~10 g of food per day [15], the daily intake of artemisinin would be ~8 mg/kg. Animals were palpated for tumor weekly and the location and size of tumor were recorded. Tumor size were calculated using the formula: $\pi/6\times$ length (cm) $\times$ width^2 (cm). Animals were sacrificed when the calculated total weight (assuming a density of 1 g/ml) of the tumors in an animal exceeded 10% of its body weigh. Measurements were made weekly for 40 weeks after the administration of DMBA.

2.3. Data analysis

Cumulative percentage of animal that developed tumor was plotted against time (weeks) after carcinogen treatment. Data were analyzed using the Kaplan–Maier Analysis followed by the Log-Rank test. The χ^2-test was used to compare the proportion of animals that developed tumors in the two treatment groups at the end of the 40th week. Latency of tumor development was compared by assigning the week when a tumor was first detected in an animal and data between the two treatment groups compared by the two-tailed Mann–Whitney *U*-test. Only animals that developed tumor (artemisinin-fed, $N=12$; control, $N=22$) were used in the analysis. In addition, number of tumors developed and the total volume of tumor in each animal were calculated. The Mann–Whitney *U*-test was used to compare the number of tumor per animal and the two-tailed Student's *t*-test was used to compare total tumor volume between the two

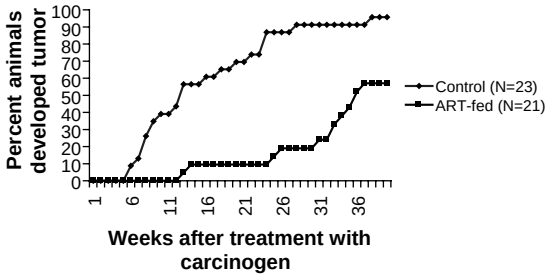

Fig. 1. Culmulative percentage of animals that developed tumors in the artemisinin-fed (ART-fed, $N=21$) and control ($N=23$) groups over the 40-week experimental period.

treatment groups. A difference is considered significant at $P<.05$.

3. Results

Cumulative percentages of animal that developed tumors in the artemisinin-fed and control groups over the 40-week experimental period are shown in Fig. 1. The two curves are significantly different (Kaplan–Meier analysis and Log-Rank test, 22.40, df = 1, $P<.0001$). The latency of tumor development was also significantly longer for the artemisinin-fed rats compared to that of the controls (an average of 29.4 versus 15.3 weeks, respectively; U (12,22) = 31.5, $P<.002$). At the end of the 40th week, there were significantly less animals that developed tumors in the artemisinin-fed group than in the control group ($\chi^2=7.21$, df = 1, $P<.01$). Twelve of the 21 (57%) artemisinin-fed animals, and 22 of 23 rats (96%) in the control group developed tumors. Animals that did not develop tumor appeared healthy and normal.

Fig. 2 is a distribution plot of number of tumors in each animal. The average numbers of tumor ($\pm$SEM) per animal in the artemisinin-fed and control groups were 1.1 ± 0.3 ($N=21$) and 3.7 ± 0.4 ($N=23$), respectively. Artemisinin-fed animals developed significantly less multiple tumors than the controls ($U=71$ (21,23), $P<.002$).

Fig. 3 shows the distribution of total volume of tumors in an animal in the two treatment groups. The average total tumor volume (in $cm^3\pm$SEM) for the artemisinin-fed and control groups were 14.07 ± 6.07 ($N=14$) and 37.6 ± 9.1 ($N=22$), respectively. Thus, the artemisinin-fed animals had significantly smaller tumor volume than the controls ($t=1.89$, df = 34, $P<.05$).

4. Discussion

DMBA-induced mammary gland tumor in rodent has been widely used as an animal model for development of chemopreventive drugs for breast cancer in humans [13,14]. Data of the present experiment indicate that daily oral intake of artemisinin could prevent or delay the development of breast cancer in the rat. The effects are highly significant, because, first, significantly fewer of the artemisinin-fed animals than the controls developed tumor within 40 weeks after DMBA administration (57 versus 96%), and second, for the artemisinin-fed rats that developed tumor, it took almost twice the time to develop the first tumor than the controls (29.4 versus 15.3 weeks). Artemisinin also decreases the number and size of tumors induced by the carcinogen.

Artemisinin may affect cancer development and growth via various mechanisms. A possible mechanism is that it selectively kills pre-cancerous cells. Artemisinin reacts with iron to form free radicals that kill cells. Various studies have suggested the involvement of iron in the development of pre-cancerous lesions. For example, iron accumulation preceded tumor formation in polycholinated biphenyl-induced liver tumor [16]. Over expression of transferrin receptors is observed in the pre-malignant columnar-lined esophageal cells [17]. Iron also plays a role in pre-neoplastic liver lesion in rats exposed to a

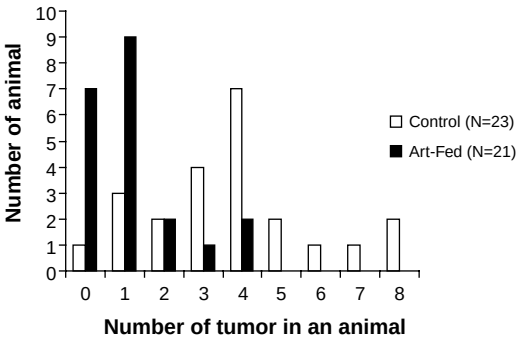

Fig. 2. Distribution plot of number of tumors per animal in the artemisinin-fed (filled bars) and control (open bars) animals.

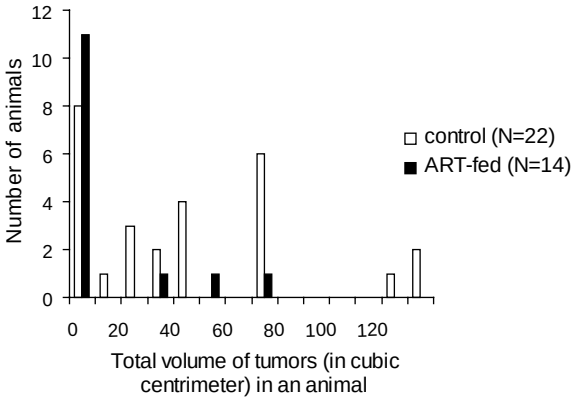

Fig. 3. Distribution of total volume of tumors in an animal in the artemisinin-fed (filled bars) and control (open bars) animals.

choline-deficient L-amino acid defined diet [18]. The iron chelator deferoxamine decreased preneoplastic lesion in chemically induced hepatocarcinoma [19]. On the other hand, iron deficiency has an inhibitory effect on preneoplastic foci in liver of rats induced by diethylnitrosamine and phenobarbital [20]. Another possible mechanism is that artemisinin directly kills cancer cells as shown in our previous experiments [11,12]. In both cases, artemisinin could prevent the appearance of tumors after carcinogen treatment. However, it is puzzling that 60% of the animals developed tumors, in spite of daily artemisinin intake, even though in general, the appearance of tumor was significantly delayed.

Artemisinin has also been shown to impede angiogenesis [21–26]. This has been shown in chicken chorioallantoic membrane, human ovarian tumor implanted in nude mice, mouse embryonic stem cell-derived embryoid bodies, and tube formation of human umbilical vein endothelial cells. The effect apparently involved down regulation of vascular endothelial growth factor-related processes and free radicals. This may explain the smaller sizes of tumor in artemisinin-fed animals. Angiogenesis inhibitors have been shown to retard tumor growth during the early stages of DMBA-induced tumorigenesis [27,28]. It is not clear whether artemisinin's effects on the immune system play a role in cancer prevention. Both stimulation [29] and suppression [30,31] of immune functions by artemisinin analogs have been reported.

The toxicology and pharmacokinetics of artemisinin and some of its analogs have been well studied and documented [32–36]. In general, no toxicity has been reported with oral artemisinin administration in humans for malaria treatment [37]. Toxicology of chronic intake of artemisinin, as necessary in the case of cancer prevention, has not been investigated. However, since rats in the 'artemisinin-fed' group that did not develop cancer were healthy and normal after 40 weeks of artemisinin intake, this indicates that chronic oral daily consumption of artemisinin (~8 mg/kg/day) may be safe.

An agent could prevent cancer development by either killing or stabilizing pre-malignant cells before they become invasive and metastatic [38]. Artemisinin could play both 'cytotoxic' (by selective killing precancerous/cancer cells) and 'cytostatic' (e.g. by inhibiting angiogenesis) roles in cancer prevention. Artemisinin is selectively toxic to cancer cells, relatively inexpensive, and effective orally. It is also highly efficacious. In another study, we have found that artemisinin, given orally once per week at a dose of 10 mg/kg, is sufficient to retard breast cancer development in DMBA-treated rats (unpublished results). Thus, it is an attractive drug candidate for cancer prevention. Further studies have to be carried out to investigate whether the breast cancer prevention property of artemisinin can be generalized to other types of cancer.

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