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Original Research Article

Dimedone Derivative {2-[(4-Hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione} Plays an Important Role in Breast Cancer Treatment

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Abstract

Purpose: *To investigate the effect of 2-[(4-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohex (HPDH) on mammary carcinogenesis induced by 7,12-dimethylbenz(a)anthracene in female Sprague Dawley rats.*

Methods: *Among three groups of rats (50 each) used in the study, the control group was fed standard diet alone, ibuprofen group received standard diet containing 1200 mg/kg ibuprofen while HPDH group was administered standard diet containing 1200 mg/kg HPDH. The treatment was for 10 days for all the groups. All the animals received 20 mg of DMBA intragastrically by gavage. Clinical parameters for the rats were recorded weekly. Micrometer caliper was used to measure the diameter of all the tumors at the end of the experiment and tumor volume calculated. Histological evaluation was performed using hematoxylin and eosin (H&E) staining. High performance liquid chromatography (HPLC) was used to determine the level of HPDH and ibuprofen in the serum of the animals.*

Results: *The data revealed a significant decrease in the number of rats with mammary tumor, number of tumors/rat and tumor volume by 54, 72 and 75 %, respectively, in HPDH group compared to control group. The ibuprofen-treated rats also showed significant decrease in the number of rats with tumor, number of tumors/rat and tumor volume by 43, 55, and 59 %, respectively. Treatment of rats with HPDH increased the latency period of tumor induction significantly ($p < 0.005$). Median detection period (50 % of tumors) was 92, 83 and 56 days, respectively, in HPDH, ibuprofen and control groups, respectively, after DMBA induction.*

Conclusion: *These results demonstrate that HPDH possesses strong chemopreventive activity against mammary carcinogenesis.*

Keywords: *Carcinogenesis, Mammary tumor, Median detection period, Tumor, Latency period, Chemopreventive activity, Ibuprofen*

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INTRODUCTION

In Western countries breast cancer is a most frequent malignant neoplasm among women. There were 234,580 new breast cancer cases detected and 40,030 estimated deaths in the United States alone in 2013 [1]. Despite intensive cancer control efforts, it remains the second leading cause of cancer deaths among American women [2]. Owing to difficulty in predicting

metathesis development many women are over-treated and suffer toxic side effects of chemotherapy [3]. Recent epidemiological studies suggested the presence of an inverse association between regular intake of NSAIDs and the relative risk of breast cancer [4-6]. Animal studies have also demonstrated the effects of NSAIDs on mammary carcinogenesis [7,8] and in our laboratories, the common over-the-counter drug ibuprofen produced highly significant reductions in tumor size and tumor burden associated with inhibition of the genetic expression of COX isoforms [9-11].

Enamides are often present in natural products and drug candidates. In particular, enamides and dienamides are common in a number of anti-parasitic and anti-cancer natural products and pharmaceutical drug leads [12,13]. A typical example of the important role played by the enamide moiety in the biological activity of natural products is represented by the comparison of the IC_{50} of the potent anti-cancer agent salicylihalamide A, with two analogues. The derivative with intact enamide moiety retained its biological activity compared to that of the analog without enamide functionality. [14]. In view of the biological activity of salicylihalamide A, and similar functionality of test drug, the effect of HPDH (Fig 1) in mammary carcinogenesis in female Sprague Dawley rats was investigated in this study.

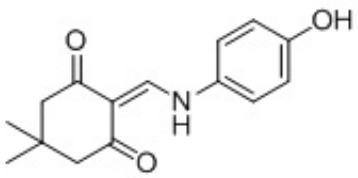

Fig 1: 2-[(4-Hydroxy-phenylamino)-methylene]-5, 5-dimethyl-cyclohexanone (HPDH)

EXPERIMENTAL

Reagents and chemicals

2-[(4-Hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexanone (HPDH), of 7,12-Dimethylbenz(α)anthracene (DMBA) and all other reagents of highest purity were purchased from Sigma Chemical Co. (St. Louis, MO). Oral Ibuprofen suspension was purchased as Motrin (100 mg/ 5ml; McNeil).

Dietary and tumor induction protocols

Female Sprague Dawley rats (150 in number) were purchased from Central Lab Animal Inc., Seoul, Republic of Korea. The rats were randomly assigned to three groups of 50 each. The control group of rats were fed standard diet alone, another group was fed standard diet containing 1200 mg/kg body weight ibuprofen while the third group received standard diet containing 1200 mg/kg body weight HPDH for110 days. All the animals received 20 mg of DMBA intragastrically by gavage. The experiment was terminated after 110 days and during this period general health of rats, weight gain and feed consumption were monitored. The animals were palpated twice a week for detection of mammary tumors after 28 days of DMBA treatment. Clinical parameters, including number of rats with tumor, time of first tumor appearance, number of tumors per rat, relative tumor size, and location of every tumor were recorded weekly. After end of experiment, a micrometer caliper was used to measure the diameters of all the tumors. From the diameter tumor volumes were calculated using the formula $V = 4/3\ \pi\ r^3$. The animals were sacrificed and gross examination of stomach, kidneys, and liver was performed. All tumors, stomach and both kidneys of each rat were resected and fixed in 10 % buffered formalin. Prior to histological evaluation samples were embedded in paraffin blocks. The histological evaluation was performed using H&E staining. HPLC was used to determine the level of HPDH and ibuprofen in the serum of animals after the completion of experiment.

Statistical analysis

Descriptive statistics on body weights, tumor latency, number of rats with tumor, number of tumors per rat, and tumor volumes were examined and compared among the control, HPDH and treatment groups. The statistical significance between the three treatment groups were obtained using students t test and one-way analysis of variance (ANOVA). $P < 0.05$ was taken to represent a statistically significant difference.

RESULTS

Body weight

There was no significant difference ($p < 0.05$) in the initial and final mean body weights of animals for control, ibuprofen and HPDH groups (Table 1).

Histopathology of mammary tumors

The histopathological evaluation of the tumors excised from the rats of control, ibuprofen and

HPDH group showed that all the 150 tumors from control and 72 tumors from ibuprofen groups were adenocarcinomas. Among 22 tumors from HPDH group, 17 were adenocarcinomas, and 3 were non-malignant fibroadenomas.

Mammary tumor data

The examination of rats revealed a significant decrease in number of rats with mammary tumor, number of tumors/rat and tumor volume by 54, 72 and 75 %, respectively, in HPDH group compared to control group (Table 2). Out of 50 rats in HPDH group, 17 had malignant tumors and 4 rats suffered from fibroadenomas. The tumor volume for these rats was very small. On the other hand, in control group all the 50 rats suffered from malignant tumors.

Most of the rats had multiple tumors and the tumor volume was larger. The ibuprofen treated rats also showed significant decrease in number of rats with tumor, number of tumors per rat and smaller tumor volumes by 43, 55 and 59 %, respectively. Treatment of rats with HPDH also increased the latency period of tumor induction significantly. Median detection period (50 % of tumors) was 92, 83 and 56 days, respectively, in the rats of HPDH, ibuprofen and control group respectively after DMBA induction (Table 2).

Pharmacological data

The mean HPDH level in serum was 4.3 mg/mL. However, the level of HPDH in rats without tumor was slightly more (4.6 mg/ml) than those with tumor (4.1 mg/mL). The ibuprofen levels in serum ranged from 5 – 11 mg/mL.

DISCUSSION

As the presence of enamide moiety plays vital role in the anticancer activity of salicylihalamide A [14]. Since HPDH also possesses similar functionality we devised an experiment to investigate the effect of HPDH in the treatment of mammary carcinogenesis. Our results demonstrate that HPDH is a potential chemopreventic agent against the development of chemically induced breast cancer. The observed chemopreventive effects of the HPDH exceeded those of the ibuprofen as well as other agents like retinoic acid 4-HPR and the glucuronidase inhibitor glucarate [16] that have shown significant antitumor effects in this animal model, but were slightly less than those of celecoxib [17]. The exact mechanism by which HPDH suppresses mammary tumor is not clearly known but it works similar to that of ibuprofen. Treatment of animals with HPDH did not lead to any toxic effect.

The animals were palpated twice a week for detection of mammary tumors after 28 days of DMBA treatment. The clinical parameters like number of rats with tumor, time of first tumor appearance, number of tumors per rat, relative tumor size, and location of every tumor were recorded weekly. A micrometer caliper was used to measure tumor diameters from which tumor volumes were calculated using the formula $V = 4/3\pi r^3$.

Table 1: Body weight of treated Sprague Dawley rats

Treatment group	Initial weight (g)	Final weight (g)
Control	166.3 ± 1.7	281.1 ± 2.4
HPDH	169.7 ± 1.5	283.6 ± 2.6
Ibuprofen	167.4 ± 2.9	281.6 ± 1.3

Values are mean weight ± SEM; no significant variation was observed in the means of treatment; (initial weights, $p = 0.17$; final weights, $p = 0.32$)

Table 2: Effect of HPDH and ibuprofen on the number of rats with tumor, number of tumors per rat, and tumor volume after DMBA-induced rat mammary tumors

Treatment group	Latency period (days)	No of rats with tumor (%)		No of tumors per rat[c]	Tumor volume (cc)[d]
		Cancer[a]	All tumors		
Control	58	100	100	3.2 ± 60.2	1.56 ± 0.4
HPDH	95[f]	32 (68)[e]	60 (40)[e]	1.5 ± 0.3 (52)[e]	0.66 ± 0.2 (57)[e]
Ibuprofen	86[f]	60 (40)[e]	60 (40)[e]	1. 6 ± 0.4 (52)[e]	0.7 ± 0.3 (57)[e]

[a] Frequency of animals that developed breast cancer; [b] All tumors include animals in the HPDH treatment group that developed fibroadenomas; [c] Mean number of tumors/animal 6 ± SE; [d] Mean tumor volume 6 ± SE; [e]Statistical significance relative to the control group at $P < 0.001$. % Reductions in the incidence rates, tumor burden, and tumor volume for the experimental diets relative to the control diet are given in parentheses

H&E staining was used for histological evaluation of the tumors and HPLC was used to determine the level of HPDH and ibuprofen in the serum of animals at completion of the experiment. All these results indicated that HPDH can act as effective therapeutic agent in the treatment of mammary carcinogenesis.

As the presence of enamide moiety plays vital role in the anticancer activity of salicylihalamide A [14]. We investigated the role of HPDH in the treatment of mammary carcinogenesis. Our results demonstrate that HPDH is a potential candidate chemopreventive agent against the development of chemically induced breast cancer. The observed chemopreventive effects of the HPDH exceeded those of the ibuprofen as well as other agents like retinoic acid 4-HPR and the glucuronidase inhibitor glucarate [16] that have shown significant antitumor effects in this animal model, but were slightly less than those of celecoxib [17]. The exact mechanism by which HPDH suppresses mammary tumor is not clearly known but it works similar to that of ibuprofen. Treatment of animals with HPDH did not lead to any toxic effect. All these results indicated that HPDH can act as effective therapeutic agent in the treatment of mammary carcinogenesis.

CONCLUSION

Administration of HPDH suppresses breast tumors induced by DMBA in female Sprague Dawley rats. The degree of inhibition is more pronounced with HPDH than with ibuprofen. These results suggest that HPDH may be an effective chemoprevention agent against human breast cancer.

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