Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 September 14; 13(34): 4586-4588
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Hypoestoxide inhibits tumor growth in the mouse CT26 colon tumor model

Emmanuel A Ojo-Amaize, Howard B Cottam, Olusola A Oyemade, Joseph I Okogun, Emeka J Nchekwube

Emmanuel A Ojo-Amaize, Howard B Cottam, Olusola A Oyemade, Joseph I Okogun, Emeka J Nchekwube, Immune Modulation, Inc., 2273B South Cactus Avenue, Bloomington, California 92316, United States
Supported by Immune Modulation, Inc., United States
Correspondence to: Dr. Emmanuel A Ojo-Amaize, Immune Modulation, Inc., PO Box 998, Bloomington, CA 92316-0998, United States. ojoamaize@aol.com
Telephone: +1-909-8774579 Fax: +1-626-9141575
Received: 2007-05-24 Accepted: 2007-06-30

Abstract

AIM: To evaluate the effect of the natural diterpenoid, hypoestoxide (HE) on the growth of established colon cancer in mice.

METHODS: The CT26.WT mouse colon carcinoma cell line was grown and expanded *in vitro*. Following the expansion, BALB/c mice were inoculated s.c. with viable tumor cells. After the tumors had established and developed to about 80-90 mm^3, the mice were started on chemotherapy by oral administration of HE, 5-fluorouracil (5-FU) or combination.

RESULTS: The antiangiogenic HE has previously been shown to inhibit the growth of melanoma in the $B16F_1$ tumor model in C57BL/6 mice. Our results demonstrate that mean volume of tumors in mice treated with oral HE as a single agent or in combination with 5-FU, were significantly smaller (> 60%) than those in vehicle control mice (471.2 mm^3 *vs* 1542.8 mm^3, $P < 0.01$). The significant reductions in tumor burden resulted in pronounced mean survival times (MST) and increased life spans (ILS) in the treated mice.

CONCLUSION: These results indicate that HE is an effective chemotherapeutic agent for colorectal cancer in mice and that HE may be used alone or in combination with 5-FU.

Key words: Hypoestoxide; 5-Fluorouracil; Colon, Cancer; Mice

Ojo-Amaize EA, Cottam HB, Oyemade OA, Okogun JI, Nchekwube EJ. Hypoestoxide inhibits tumor growth in the mouse CT26 colon tumor model. *World J Gastroenterol* 2007; 13(34): 4586-4588

http://www.wjgnet.com/1007-9327/13/4586.asp

INTRODUCTION

The clinical usefulness of chemotherapy against advanced solid tumors is limited by host toxicity and tumor resistance. Combination chemotherapy is an approach to meeting this challenge. Colorectal carcinoma is the second most common cause of cancer deaths in the United States and is responsible for the deaths of over 55 000 patients annually[1]. 5-fluorouracil (5-FU) has long been recognized as standard chemotherapy for colorectal cancer[2] and has been used either alone or in combination with newer chemotherapeutics such as irinotecan and oxaliplatin[3]. Both these drugs are believed to increase efficacy but also cause more toxicity. The predominant side effects of 5-FU are diarrhea, anorexia, enteritis, hand-foot syndrome and myelosuppression. While the combination of other drugs with 5-FU has improved overall patient survival, it has also been associated with more severe side effects[4]. Modalities that include low doses of 5-FU and combination with low doses of an effective nontoxic drug which decrease or eliminate toxicity and at the same time enhance overall efficacy, would greatly advance the treatment for colorectal cancer and other malignancies. To this end, HE is an ideal candidate.

HE is a novel natural diterpenoid with anti-tumor[5], anti-inflammatory[6], and anti-parasitic activities[7].We examined the effect of HE alone or in combination with a very low dose (25 mg/kg) of 5-FU and a low dose of HE (1 mg/kg) upon efficacy in 5-FU therapy for CT26, a mouse colon cancer model.

MATERIALS AND METHODS

Experimental animals

Female BALB/c mice were purchased from Charles River Laboratories, Wilmington, MA. The thirty-five female 6-8 wk old mice were maintained on a standard laboratory chow and under pathogen-free conditions according to institutional regulations in facilities approved by the American Association for Accreditation of Laboratory Animal Care.

Reagents

HE was prepared in our laboratory with modifications, as previously reported[8]. 5-FU was purchased from Sigma-

Table 1 Oral administration of HE inhibits the growth of s.c. implanted CT26 colon tumor in BALB/c mice

Drug	Tumor volume (mm^3)			ILS (%)
	Dose (mg/kg per day)	(d 18) (mean ± SE)	MST (d) (mean ± SE)	
"Vehicle control (PBS/DMSO)"	0	1542.8 ± 330	34.0 ± 6.2	0
HE	1 × 10	738.7 ± 158	43.0 ± 1.2	25
HE	5 × 10	582.3 ± 116	77.0 ± 4.2	126
HE	50 × 1	288.6 ± 188	52.7 ± 3.0	54
5-FU	25 × 1	911.1 ± 144	48.0 ± 3.6	40
5-FU	100 × 1	486.7 ± 171	59.7 ± 4.8	75
5-FU + HE	(5-FU) 25 × 1 + (HE) 1 × 10		57.7 ± 4.0	69

PBS/DMSO: phosphate buffered saline/dimethyl sulfoxide; MST: mean survival times; ILS: increased life spans. MST: 75.0 ± 3.8 (HE-treated mice at 5 mg/kg × 10) *vs* Vehicle control (30.0 ± 4.1), ILS: 150% (HE-treated mice) *vs* 0% (Vehicle control mice), $P < 0.01$.

Aldrich (St. Louis, MO). Phosphate buffered saline (PBS), Dulbecco's minimal essential medium (DMEM), and other culture media components were purchased from Irvine Scientific (Irvine, CA).

Cell line

CT26.WT mouse colon carcinoma cell line was purchased from ATCC, Manassas, VA. The cell line was grown as monolayer in DMEM culture medium containing 10% FBS and 1% L-glutamine. Full grown monolayer cultures were trypsinized for 15 min (0.25% trypsin-EDTA), harvested and passaged several times for expansion.

Experimental procedure

Following the growth and expansion of the cell line *in vitro*, trypsinized cells were harvested, washed, counted by trypan blue dye exclusion method and cell density was adjusted to 10×10^6/mL in PBS. A suspension of 2×10^6 viable tumor cells in 0.2 mL PBS was inoculated s.c. into the left flanks of 35 mice. After tumors developed to about (80-90) ± 10 mm^3 volume, the mice were randomized into seven groups as depicted on Table 1. They were treated with varying doses of HE alone, 5-FU alone or combination of lower doses of HE (1 mg/kg) and 5-FU (25 mg/kg) *via* oral administration with a gavage needle attached to a 1.0 cc syringe.

Statistical analysis

A student *t* test was used to determine significance of difference between tumor burdens in vehicle control mice and mice treated with HE as a single agent or in combination with 5-FU. Survival data are presented in days as mean ± SE.

RESULTS

Mice receiving varying doses of HE, 5-FU or combination experienced significant tumor growth inhibition as compared to controls. Mean % tumor growth inhibition obtained for HE was 65%; 5-FU, 55%; and HE + 5-FU, 82% relative to vehicle control. The additive effect of HE + 5-FU combination resulted in 69% ILS as compared to 25% ILS for HE and 40% ILS for 5-FU when tested alone at each of their respective lowest doses (Table 1). Treatment was started when tumors had a mean volume of 80-90 mm^3. Five female BALB/c mice were allocated to each group. Tumor volume at the start of treatment (d 0) and on d 18 after tumor implantation is shown. MST and ILS (%) were calculated for each group. MST was calculated from the period between tumor implantation and the day of death. ILS (%) was calculated using MST for each drug-treated mouse as follows:

$$\text{ILS (\%)} = \frac{[\text{MST of drug-treated mouse} - \text{MST of vehicle control}]}{\text{MST of vehicle control}} \times 100$$

This experiment was conducted twice with similar results.

DISCUSSION

HE is a novel and unique nonsteroidal antiinflammatory drug (NSAID) because it does not inhibit cyclooxygenase (COX) activity[6]. The mechanism of action that defines NSAIDs as a class is their ability to inhibit COX activity[9,10]. Several studies have established that numerous NSAIDs such as sulindac, aspirin, celecoxib, piroxicam and ibuprofen inhibit or prevent colorectal neoplasia in rodents and humans because of their ability to inhibit COX activity[9-11]. HE has been shown in this report to be effective at reducing tumor burden in mice with colorectal cancer and it is thus similar to sulindac sulfide, a metabolite of sulindac sulfoxide, an NSAID which has also be shown to be effective against murine and human colorectal cancer without inhibitory effect on COX activity[10,12]. The various mechanisms by which HE inhibits tumor growth include its ability to arrest cell cycle at G_2-M phase by interference with actin assembly, inhibit angiogenesis, vascular endothelial growth factor (VEGF)-induced cell proliferation and endothelial cell migration[5]. All of these mechanisms have been shown to contribute to the treatment of colon cancer[10-14]. Conversely, 5-FU is known to trigger apoptosis by depleting thymidine, partially through inhibition of thymidine synthase and partially through direct incorporation into RNA and DNA[15,16]. Because HE lacks alkylating properties[6], toxicity[5], and uses several other aforementioned mechanisms, it is therefore consistent that the combination of low doses of HE with low doses of 5-FU enhances the anti-tumor responses of 5-FU.

Interestingly, consumption of the dried leaf powder of *Hypoestes rosea* (the parent plant of HE), as a dietary supplement, resulted in the elimination of existing intestinal polyps in human subjects (Nchekwube, unpublished results).

Collectively, these results indicate that HE may be a promising chemotherapeutic agent either alone or in combination with 5-FU against colorectal cancer.

COMMENTS

Background

Plants and their products have been used for medicinal purposes for thousands of years. Drugs from natural bio-resources have often been discovered on the basis of ethno-botanical information provided by herbalists living in regions of the world rich in bio-resources. Hypoestoxide is an investigational new drug isolated from the plant *Hypoestes rosea* (Acanthaceae) which is indigenous to the rain forest

regions of Nigeria. The natives have long used the *H rosea* leaf extracts in folk medicine to treat various ailments. In this article, old world and new world medicine are brought together.

Research frontiers

The findings in this article relate well to the present state of the field in regard to the use of natural products as pharmaceutical agents. Artemisinin is an example of a natural product isolated from the Chinese plant, *Artemisia annua*. Currently, Artemisinin is used for the treatment of malaria. Interestingly, Hypoestoxide has also been found to possess anti-malarial activity. Curcumin, a polyphenolic antioxidant from a dietary spice is in clinical development for anti-cancer and anti-inflammatory activities. Vincristine is another example of a natural product (isolated from the rose periwinkle flower) which is used as a standard anti-cancer agent.

Innovations and breakthroughs

The findings are significant and novel because Hypoestoxide is an investigational new drug for which new indications are being sought. This is the first report on both the chemotherapeutic effect of Hypoestoxide on colon cancer and its additive effect with a standard chemotherapeutic agent, 5-Fluorouracil.

Applications

Future applications of the findings in this article will be in the areas of single and combination drug therapies for colorectal cancer. The findings also lend support to the use of NSAIDs such as Hypoestoxide, as anti-cancer agents. NSAIDs have long been associated with tumor chemoprevention and inhibition of the growth of established tumors.

Terminology

NSAIDs are anti-inflammatory drugs that are not steroids. Anti-angiogenesis is the process by which a drug inhibits the growth of an established tumor by shutting off the blood supply to the tumor. CT26 is an N-nitroso-N-methylurethane-(NNMU)-induced, undifferenciated colon carcinoma cell line. It was cloned to generate the cell line CT26.WT (ATCC CRL-2638).

Peer review

The paper is well written. Using the simple and reliable methods, the authors studied the effect of hypoestoxide (HE) on mouse colon adenocarcinoma, and give us a definite conclusion: HE is an effective chemotherapeutic agent for colorectal cancer in mice and that HE may be used alone or in combination with 5-FU. The conclusion will help us to deeply understand HE's value in the treatment of colorectal cancer.

REFERENCES

1 **Cubas R**, Li M, Chen C, Yao Q. Colorectal cancer: new advances in immunotherapy. *Cancer Biol Ther* 2007; **6**: 11-17

2 **Norwood AA**, Tucci M, Benghuzzi H. A comparison of 5-fluorouracil and natural chemotherapeutic agents, EGCG and thymoquinone, delivered by sustained drug delivery on colon cancer cells. *Biomed Sci Instrum* 2007; **43**: 272-277

3 **Braun AH**, Achterrath W, Wilke H, Vanhoefer U, Harstrick A, Preusser P. New systemic frontline treatment for metastatic colorectal carcinoma. *Cancer* 2004; **100**: 1558-1577

4 **Wilke HJ**, Van Cutsem E. Current treatments and future perspectives in colorectal and gastric cancer. *Ann Oncol* 2003; **14** Suppl 2: ii49-ii55

5 **Ojo-Amaize EA**, Nchekwube EJ, Cottam HB, Bai R, Verdier-Pinard P, Kakkanaiah VN, Varner JA, Leoni L, Okogun JI, Adesomoju AA, Oyemade OA, Hamel E. Hypoestoxide, a natural nonmutagenic diterpenoid with antiangiogenic and antitumor activity: possible mechanisms of action. *Cancer Res* 2002; **62**: 4007-4014

6 **Ojo-Amaize EA**, Kapahi P, Kakkanaiah VN, Takahashi T, Shalom-Barak T, Cottam HB, Adesomoju AA, Nchekwube EJ, Oyemade OA, Karin M, Okogun JI. Hypoestoxide, a novel anti-inflammatory natural diterpene, inhibits the activity of IkappaB kinase. *Cell Immunol* 2001; **209**: 149-157

7 **Ojo-Amaize EA**, Nchekwube EJ, Cottam HB, Oyemade OA, Adesomoju AA, Okogun JI. Plasmodium berghei: antiparasitic effects of orally administered hypoestoxide in mice. *Exp Parasitol* 2007; **117**:218-221

8 **Adesomoju AA**, Okogun JI, Cava MP, Carroll PJ. Hypoestoxide, a new diterpene from Hypoestes rosea (Acanthaceae). *Heterocycles* 1983; **20**: 2125-2128

9 **Vane JR**, Flower RJ, Botting RM. History of aspirin and its mechanism of action. *Stroke* 1990; **21**: IV12-IV23

10 **Thun MJ**, Henley SJ, Patrono C. Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. *J Natl Cancer Inst* 2002; **94**: 252-266

11 **Sandler RS**. Aspirin and other nonsteroidal anti-inflammatory agents in the prevention of colorectal cancer. *Important Adv Oncol* 1996; 123-137

12 **Williams CS**, Goldman AP, Sheng H, Morrow JD, DuBois RN. Sulindac sulfide, but not sulindac sulfone, inhibits colorectal cancer growth. *Neoplasia* 1999; **1**: 170-176

13 **Yokoi K**, Thaker PH, Yazici S, Rebhun RR, Nam DH, He J, Kim SJ, Abbruzzese JL, Hamilton SR, Fidler IJ. Dual inhibition of epidermal growth factor receptor and vascular endothelial growth factor receptor phosphorylation by AEE788 reduces growth and metastasis of human colon carcinoma in an orthotopic nude mouse model. *Cancer Res* 2005; **65**: 3716-3725

14 **Flis S**, Soltysiak-Pawluczuk D, Jedrych A, Jastrzebski Z, Remiszewska M, Splawinski J. Antiangiogenic effect of sulindac sulfide could be secondary to induction of apoptosis and cell cycle arrest. *Anticancer Res* 2006; **26**: 3033-3041

15 **Wada Y**, Yoshida K, Suzuki T, Mizuiri H, Konishi K, Ukon K, Tanabe K, Sakata Y, Fukushima M. Synergistic effects of docetaxel and S-1 by modulating the expression of metabolic enzymes of 5-fluorouracil in human gastric cancer cell lines. *Int J Cancer* 2006; **119**: 783-791

16 **Allegra CJ**. New therapeutic strategies for patients with gastrointestinal malignancies using biochemical modulation of fluorouracil. *JAMA* 1995; **273**: 236-239

S- Editor Zhu LH **L- Editor** Li M **E- Editor** Li JL