

# E-7869 (*R*-Flurbiprofen) Inhibits Progression of Prostate Cancer in the TRAMP Mouse<sup>1</sup>

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## ABSTRACT

E-7869 (*R*-flurbiprofen) is a single enantiomer of a racemic nonsteroidal anti-inflammatory drug. E-7869 does not inhibit either cyclooxygenase-1 or cyclooxygenase-2. We used the transgenic adenocarcinoma mouse prostate (TRAMP) mouse, a prostate cancer model, to evaluate the effect of this drug on prostate cancer progression. Sixty 12-week-old male TRAMP mice were placed randomly into five groups. The animals were treated by daily oral gavage with vehicle (1% carboxymethylcellulose) or E-7869 for 18-weeks. During the course of the study, two diets were used. Three groups (vehicle, 15-mg/kg, and 20-mg/kg drug treatments) received a Teklad diet containing 2.4% saturated fat [a high saturated fat (HSF) diet], and two groups (vehicle and 20 mg/kg drug treatment) received an AIN-93G diet containing 1.05% saturated fat [a low saturated fat (LSF) diet]. At necropsy, the urogenital system and periaortic lymph nodes were removed and weighed. The prostate lobes, seminal vesicles, lungs, and periaortic lymph nodes were preserved and sectioned for histological evaluation. The lung and periaortic lymph nodes were graded as to the presence (+) or absence (–) of metastasis; the urogenital tissues were graded on a 1–6 scale for degree of neoplasia/carcinoma. For both diets, the urogenital wet weights and lymph node wet weights in the 20-mg/kg treatment groups were significantly lower as compared to vehicle control groups. In addition, treatment with 20 mg/kg E-7869 in the LSF diet group resulted in a significantly lower primary tumor incidence ( $P < 0.05$ ) and reduced incidence of metastasis. In this treatment group, the reduced incidence of metastasis was not statistically significant because the LSF diet itself resulted in a remarkably lower incidence of metastasis in the vehicle control group (10% LSF versus 40% HSF). Treatment with 20 mg/kg E-7869 on the HSF diet resulted in a significantly lower incidence of metastasis ( $P < 0.05$ ) and a reduction in the primary tumor incidence. These results suggest that E-7869 is a promising chemopreventive and treatment for human prostate cancer.

## INTRODUCTION

Prostate cancer is the second leading cause of cancer death among males in the United States. In 1998, an estimated 185,000 men were diagnosed with prostate cancer, and >39,000 men died of the disease (1). Although survival rates are good for prostate cancer that is diagnosed early, the treatments for advanced disease are limited to hormone ablation techniques and palliative care. Hormone ablation techniques (orchiectomy and antiandrogen treatments) generally allow only temporary remission of the disease. It usually recurs within 1–3 years of treatment, with the recurrent tumors no longer requiring

androgens for growth or survival (2). Therefore, new methods of treatment and prevention are needed, which will inhibit the progression of the disease to this hormone refractory state.

Studies in our laboratory have shown the efficacy of E-7869 (*R*-flurbiprofen) in reducing the number and size of adenomas in the C57BL/6J-APC<sup>Min/+</sup> mouse, a model of familial adenomatous polyposis in man (3). E-7869 is a single enantiomer of the commercial racemic mixture of flurbiprofen (Ansaid). Although E-7869 is an analogue of the aryl-propionic class of nonsteroidal anti-inflammatory drugs, it does not significantly inhibit COX<sup>3</sup>-1 or COX-2. The inhibition of COX-1 and COX-2 enzymes resides only in the *S*-enantiomer, as demonstrated by its potent inhibition of prostaglandin synthesis (4). However, it is possible that E-7869 may be involved in the down-regulation of COX-2 mRNA transcripts. In a recent study (5), racemic flurbiprofen was shown to decrease COX-2 mRNA expression in PC-3 cells, even in the presence of PGE<sub>2</sub>. However, it is unclear whether one or both isomers were responsible for the reduction of the COX-2 mRNA in that study.

The inhibition of prostaglandin production may play an important role in slowing or inhibiting carcinogenesis. Prostaglandins have been shown to increase colony and cell number in numerous cancer cell lines (5, 6). Several papers have demonstrated an increase in PGE<sub>2</sub> levels in colorectal carcinoma tumors compared with surrounding normal tissue (7, 8). Prostaglandins have also been shown to have a role in programmed cell death or apoptosis. Researchers have demonstrated that PGE<sub>2</sub> can inhibit apoptosis and up-regulate bcl-2 expression in colon cancer cell lines (6).

Apoptosis is a valuable physiological restraint against uncontrolled growth and differentiation. Prostate cancer, as with many cancers, is known to be a disease where the regulation of apoptosis is inhibited, allowing unchecked cellular proliferation (9). In advanced hormone-refractory prostate cancer, although the rate of proliferation is low, the rate of apoptosis is also substantially reduced, allowing progression and growth of the cancer (10). The presence of apoptotic bodies has also been shown to correlate with a better prognosis for certain grades of prostate cancer (11). Therefore, identification of agents that induce apoptosis may provide an effective treatment for advanced prostate cancer.

E-7869 has been shown to significantly enhance the apoptotic index in the distal colon of rats treated with azoxymethane.<sup>4</sup> Therefore, given the suggestion that E-7869 might act as an apoptotic agent and its putative effects on COX-2 mRNA down-regulation, we extended our studies to another epithelial adenocarcinoma model. This model, the TRAMP mouse, is an ideal model for the pharmacological evaluation of drugs to treat prostate cancer in humans. The model exhibits many similarities to human prostate cancer, including epithelial origin, progression to adenocarcinoma, and metastasis. The transgene

Received 10/4/99; accepted 2/18/00.

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<sup>1</sup> Supported by Adventist Health Systems Loma Linda (to W. J. W., D. D. L., E. D. M., D. D. Q., and J. D. M.), National Cancer Institute Grant CA58204 Specialized Program of Research Excellence [S.P.O.R.E.], and CapCure (to R. J. B. and N. M. G.). A preliminary report of these results was presented at the meeting for the Prevention and Cure of Prostate Cancer (AACR; Indian Wells, CA), December 1998 and at the annual meeting of the AACR (Philadelphia, PA), April 1999.

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<sup>3</sup> The abbreviation used are: COX, cyclooxygenase; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; TRAMP, transgenic adenocarcinoma mouse prostate; PIN, prostatic intraepithelial neoplasia; CMC, carboxymethylcellulose; LSF, low saturated fat; HSF, high saturated fat; PPAR, peroxisome proliferator-activated receptor.

<sup>4</sup> G. Young, personal communication.

construct in these mice is a rat probasin-SV40 large T antigen introduced into the C57BL/6 mouse (12).

This study investigates the efficacy of E-7869 against prostate cancer in the TRAMP model. The drug was given early in the history of the disease, and treatment continued for 18 weeks. Efficacy was evaluated by measurements of primary tumor growth and differentiation as well as inhibition of metastases. We found that E-7869 reduced the progression of prostate cancer in these mice.

## MATERIALS AND METHODS

**Animals.** C57BL/6J-TRAMP heterozygous male mice were bred in the Loma Linda Medical Center Vivarium. Each mouse was genotyped by PCR for the rat probasin-SV40 large T antigen construct before enrollment in the study. Housing and care of all animals was in accordance with the guidelines established by the University's Animal Research Committee. The animals were housed in groups of five in wood chip bedded cages in a room having controlled temperature (68°F–72°F), light (12-h light-dark cycle), and humidity (45–55%). The animals were allowed free access to water and food during the experiment.

**Chemicals.** E-7869 (*R*-flurbiprofen; >99.8% enantiomeric excess) was purchased from Wyckoff Inc. (South Haven, MI). All other chemicals were reagent grade or better.

**Drug Preparation.** The E-7869 was suspended in 1% CMC by homogenization with heating (60°C for 15 min) in a tissue homogenizer. One % CMC was used as the vehicle control.

**Groups and Treatment.** Sixty 12-week-old TRAMP mice were randomized into control or treatment groups using the Monte Carlo method. The mice in the HSF groups were fed Teklad mouse/rat chow, which contains 4.2% total fat and 2.4% saturated fat. The mice in the LSF groups were fed Dyets AIN 93-G chow, which contains 7% total fat and 1.05% saturated fat. The animals in all groups were treated by oral gavage with either 1% CMC (vehicle control), 15 mg/kg E-7869 (LSF only), or 20 mg/kg E-7869 once daily between 8 a.m. and 11 a.m.. Each animal was weighed weekly, and the administered dose was adjusted accordingly. The animals were treated for 18 weeks. Necropsy was performed at 30 weeks of age.

**Necropsy and Histology.** At the time of necropsy, each mouse was anesthetized with an i.p. injection of 50 mg/kg ketamine and 10 mg/kg xylazine. A laparotomy was performed to expose all major organs, and the animal was exsanguinated by cardiac puncture. The hematocrit was measured, and the remaining blood (~500–700  $\mu$ l) was placed in a 1.5-ml microcentrifuge tube. Plasma was obtained by centrifugation (13,000  $\times$  *g* for 5 min) and stored at –20°C for drug analyses. All major organs were inspected for frank toxicity or evidence of metastases. Necropsy notes were collected on all animals. These notes included quantitative and qualitative descriptions of the prostate, lymph nodes, and any tissues showing any visible abnormality. Photographs were made for documentation of the animals and their urogenital systems *in situ*. A PCR assay, as described below, was performed on any animals that did not display any obvious disease. Four animals that were originally included in the study were removed after this PCR assay revealed that they were wild-type mice.

The urogenital system (seminal vesicles, prostate lobes, and bladder) was removed and placed in a Petri dish containing PBS (137 mM NaCl, 3 mM KCl, 4 mM Na<sub>2</sub>HPO<sub>4</sub>, and 1.5 mM KH<sub>2</sub>PO<sub>4</sub>) to prevent desiccation. The bladder was emptied, and the tissues were gently blotted to remove excess PBS; then the entire urogenital system was weighed. Under a dissecting microscope, the different lobes of each prostate were separated and placed in labeled biopsy cassettes. Periaortic lymph nodes were identified (lower lumbar region of aorta, distal to femoral artery bifurcation) and removed, then placed in Petri dishes containing PBS. The lymph nodes were gently blotted to remove excess PBS and then weighed. The lymph nodes were then placed on biopsy foam pads inside biopsy cassettes.

The following specimens were collected for histology: periaortic lymph nodes; lung tissue; seminal vesicles; and ventral, dorsolateral, and anterior prostate. Any tissues containing visible metastases or other abnormalities were also collected for histological evaluation. All tissue samples were placed in 4:1 water:Zn-buffered formalin for 12–24 h, after which the formalin solution was replaced with 70% ethanol (v/v). The tissue samples were dehydrated and

embedded in paraffin. Sections (5  $\mu$ m) were cut from paraffin-embedded tissues and mounted on ProbeOn-Plus slides (Fisher Scientific) as described by Greenberg *et al.* (13). Histological sections were analyzed with a standard H&E stain. The sections were evaluated by a pathologist and blinded to the treatment groups for the incidence and degree of pathology within the tissue samples. The urogenital tissues were graded on a 1–6 scale: noncancerous lesions were graded 1, 2, or 3 indicating normal tissue, low PIN, and high PIN, respectively. Grades 4, 5, or 6 indicated well-, moderately, and poorly differentiated cancerous lesions. The periaortic lymph node and lung tissues were graded as to the presence (+) or absence (–) of metastases.

**Analysis of Drug Levels.** Plasma samples were taken 2–4 h ( $C_{max}$ ) after the final drug dose for the determination of plasma drug levels and inversion of *R*- to *S*-flurbiprofen. The extraction and analysis procedures were previously described (3).

**PCR Analysis.** The PCR method for identification of TRAMP animals derived from our breeding colony was adapted from Greenberg *et al.* (14). Our TRAMP mice were an inbred strain obtained by crossing against C57BL/6J mice. Animals were genotyped by allele-specific PCR using 5 mm of tail tissue. A DNA isolation kit (Pharmacia Biotech) was used for DNA extraction, and the DNA pellet was dissolved in a hydration solution supplied with the DNA isolation kit. A PCR premix solution containing *Taq* polymerase, dNTPs, sense and antisense gene primers, and MgCl<sub>2</sub> was added to template DNA in a total volume of 50  $\mu$ l. The PCR products were separated after electrophoresis in a 1.8% agarose gel. The gel was stained with ethidium bromide, viewed by UV light, and photographed with a direct screen camera.

**Statistical Analysis.** The central tendency and variability were determined using descriptive statistics for the different data variables. The normality of data were determined through graphical representation and the Shapiro-Wilk test, with  $P < 0.05$  representing a nonnormal data set. Based on the normality of data, an ANOVA or Kruskal-Wallis test was used for statistical analysis. Dunnett's test and the nonparametric Dunnett's test were used for multiple comparisons of the data, with a significance level set at  $\alpha = 0.05$ . Statistical analysis of histological specimens used Fisher's exact test to determine significance ( $P < 0.05$ ). Analyses were conducted using Analyze-it for Microsoft Excel 95/97 (Analyze-it Software Ltd., Leeds, United Kingdom) and Microsoft Excel (Microsoft Corp., Seattle, WA).

## RESULTS

**Prostate and Lymph Node Wet Weights.** To determine the activity of E-7869 in the TRAMP mouse, gross biological indices (wet weights) were used to assess tumorigenicity. Daily administration of either 15 or 20 mg/kg of E-7869 for 18 weeks significantly reduced the wet weights of the urogenital system on either diet (Table 1). The urogenital and lymph node weights were highly variable in both vehicle control groups (LSF and HSF) and in the lower (15 mg/kg) E-7869 dose (Fig. 1). By contrast, both 20-mg/kg E-7869 groups had much less variability and significantly lower tissue wet weights. For animals treated with 20 mg/kg E-7869 on the LSF diet, the range of urogenital weights was ~1.0 g, whereas the range was ~4 g for the vehicle control animals on the same diet. Similarly, animals treated with 20 mg/kg E-7869 on the HSF diet also displayed a range of urogenital weights of ~1.0 g, whereas the vehicle controls ranged ~3.5 g (Fig. 1). The lymph node weights displayed even greater differences between weight ranges for the 20-mg/kg-treated animals and the vehicle control animals on both diets.

The 20-mg/kg treatment, with either diet, effectively reduced the mean urogenital and lymph node wet weights by ~55–60% compared with their respective controls (Table 1). The animals in the 15-mg/kg E-7869 group given the HSF diet demonstrated a similar significant reduction (48%) with average urogenital weights of 0.90 g *versus* 1.74 g in the vehicle control animals (Table 1). Conversely, 15-mg/kg E-7869 treatment in the group fed the HSF diet failed to show a significant reduction in the lymph node wet weights. The mean lymph node weight for 15-mg/kg-treated animals was 7.9 mg, whereas for the corresponding vehicle control animals, it was 5.3 mg (Table 1).

Table 1 Effect of E-7869 treatment on urogenital and periaortic wet weights in TRAMP mice<sup>a</sup>

No. of animals <sup>b</sup>	HSF			LSF	
	Vehicle control I 10	15 mg/kg E-7869 8	20 mg/kg E-7869 8	Vehicle control II 10	20 mg/kg E-7869 7
Urogenital wet weight (g)					
Mean (median) ± SE	1.74 (1.32) ± 1.07	0.90 (0.71) ± 0.64	0.72 (0.74) ± 0.27	2.07 (1.76) ± 1.16	0.94 (1.09) ± 0.34
(% reduction vs. control) <sup>c</sup>		(48) <sup>d</sup>	(58) <sup>d</sup>		(54) <sup>d</sup>
Lymph node wet weights (mg)					
Mean (median) ± SE	5.3 (4.7) ± 4.0	7.9 (2.4) ± 16.0	2.2 (2.2) ± 0.8	6.8 (4.6) ± 7.2	3.1 (3.5) ± 1.1
(% reduction vs. control) <sup>c</sup>		(-49)	(59) <sup>e</sup>		(54) <sup>d</sup>

<sup>a</sup> Animals were sacrificed; the urogenital [four lobes of prostate, seminal vesicles, and (emptied) bladder] and periaortic lymph node LSF wet weight measured.

<sup>b</sup> The original number of animals was 60 (12 animals/group). The following 17 animals were excluded: 11 animals died before completing 18 weeks of treatment (our protocol called for 90% completion of the 18-week treatment for inclusion into any analyses). 2 animals had unusual progression of disease (hepatic and/or submandibular tumors with no prostatic tumors). 4 animals were wild-type animals after retest by PCR.

<sup>c</sup> Percent reductions were calculated using the following formula: [(Mean<sub>VC</sub> - Mean<sub>Treatment</sub>)/(Mean<sub>VC</sub>) × 100.

<sup>d</sup> P < 0.01 versus corresponding vehicle control.

<sup>e</sup> P < 0.05 versus corresponding vehicle control.

**Hematocrit and Body Weight.** To assess the toxicity of the drug treatment in the TRAMP mouse, we measured hematocrits at necropsy and body weight changes throughout treatment. There was a significant hematocrit reduction in all treatment groups versus their respective vehicle controls (Table 2). The drug did not appear to have an effect on body weight on either diet. However, the animals on the LSF diet showed a greater overall weight gain than mice fed the HSF diet. All animals receiving the HSF diet (treated or untreated) had an average of a 2% weight gain, whereas the LSF group had a >10-fold greater increase in their average weight over the course of the study (Table 2).

**Incidence of Primary Tumors and Metastases.** We examined the incidence of carcinoma in prostatic tissues, and metastases to lymph nodes and lung tissue. Carcinoma in the prostatic tissues was reduced in all groups treated with E-7869 compared with their respective controls. In the HSF diet group, the 20-mg/kg dose had a substantially lower tumor incidence compared to the vehicle controls. In the vehicle control group, 19 of 40 tissues examined (48%) had evidence of

carcinoma in the prostate, whereas only 8 of 28 tissues examined (29%) in the 20-mg/kg E-7869 treatment group had evidence of carcinoma (Table 3).

In the LSF diet group, the 20-mg/kg treatment group showed a significant (P < 0.05) reduction in the prostate tumor incidence. The prostate tissue from the vehicle control animals in the LSF diet group had tumors in 14 of 24 samples examined (58%), compared to 6 of 26 samples examined (21%) for the 20-mg/kg treatment group receiving the same diet.

Compared with vehicle controls, the incidence of metastases was significantly reduced in the 20-mg/kg treatment group given the HSF diet. The metastasis incidence for the vehicle controls fed the HSF diet was 8 of 20 tissue samples (40%), whereas only 1 of 16 samples (6%) from animals treated with 20 mg/kg E-7869 developed any evidence of metastasis (Table 3). Animals treated with 15 mg/kg E-7869 failed to show any reduction in metastases in the HSF diet group.

The 20-mg/kg E-7869 treatment also gave a reduction in the incidence in metastases in the LSF diet group. Within this group, 12

Fig. 1. Variability and range of urogenital and lymph node wet weights from TRAMP mice after 18 weeks of treatment with 15- and 20-mg/kg E-7869. Animals were fed either a HSF or LSF diet for the 18 weeks of treatment. A, lymph node wet weights; B, urogenital wet weights. Right-hand bracket, the range of the data.

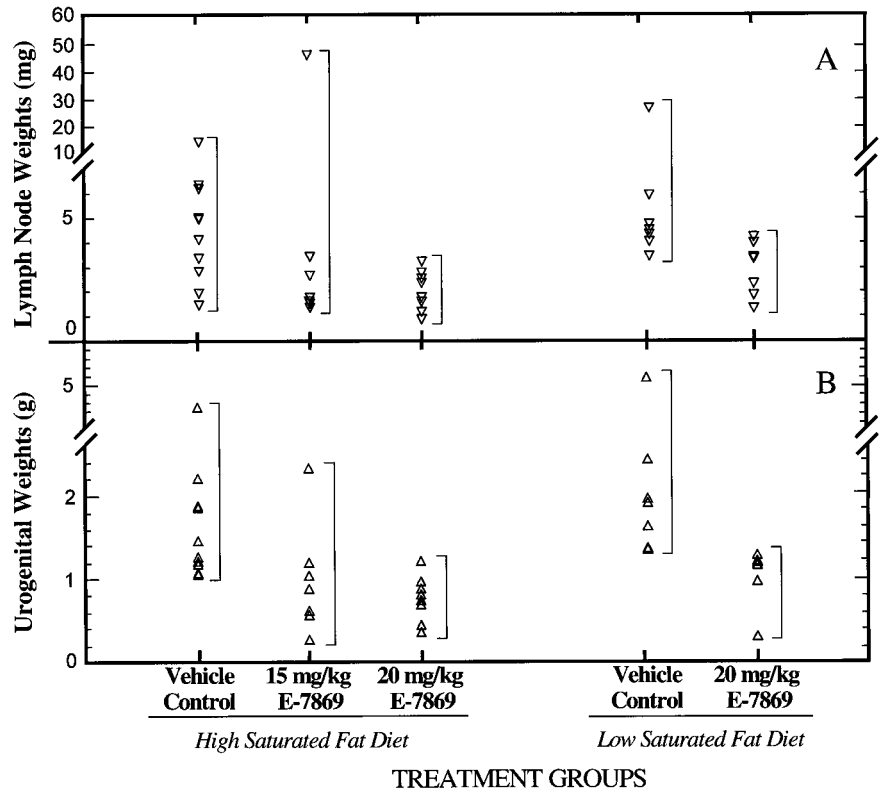




Table 2 Effect of E-7869 treatment on hematocrit and percent changes in body weights in TRAMP mice<sup>a</sup>

Diet fat and dose (mg/kg/day)	No. of animals	Hematocrit (%) (Mean ± SD)	% changes in body weights <sup>b</sup> (Mean ± SD)
<b>HSF</b>			
VC	10	37.2 ± 3.8	0.1 ± 11.7
15 E-7869	8	31.0 ± 6.9 <sup>c</sup>	0.5 ± 13.7
20 E-7869	8	30.5 ± 3.7 <sup>c</sup>	3.4 ± 6.3
<b>LSF</b>			
VC	10	40.1 ± 1.6	27.0 ± 23.2
20 E-7869	7	30.7 ± 4.2 <sup>d</sup>	14.5 ± 15.7

<sup>a</sup> Blood was drawn at the time of sacrifice at completion of 18 weeks of treatment; body weight changes were calculated from week 0 and week 18 of treatment; urogenital weight was subtracted from final body weight measurement before percent body weight calculation.

<sup>b</sup> Determination of the percent change in body weight used the following formula: [(final body weight – initial body weight)/initial body weight] × 100.

<sup>c</sup> *P* < 0.05 versus corresponding vehicle control.

<sup>d</sup> *P* < 0.01 versus corresponding vehicle control.

tissues were examined for incidence of metastases, and none had any evidence of metastatic disease. However, in the vehicle control group, only 2 of 20 tissues examined (10%) had any evidence of metastases.

The lower incidence of carcinoma in the prostate lobes and metastatic sites for animals treated with 20 mg/kg E-7869 suggests an overall reduction in the total cancer prevalence. Two of 6 animals (33%) in the 20-mg/kg E-7869 (LSF) treatment group had no histologically demonstrable cancer (Table 3). Similarly, in the 20-mg/kg E-7869 (HSF) treatment group, 1 of 7 animals had no detectable cancer. All vehicle control animals in this study had histological evidence of carcinoma. This data suggests that treatment with E-7869 had a profound ability to inhibit the progression of cancer in these animals.

## DISCUSSION

The efficacy of E-7869 in the treatment of the prostate and accessory glands in TRAMP mice clearly implies that this drug is a prospective treatment against human prostate cancer. In the TRAMP model, PIN develops at about 12 weeks, the time when treatment with E-7869 was initiated. Therefore, it is reasonable to assume that the mice had developed low-to-high-grade PIN at the start of treatment. The activity of E-7869 can be attributed to its ability to inhibit the progression of these precancerous lesions to carcinoma. The 20-mg/kg dose treatment groups demonstrated a reduction in the incidence of carcinoma in the prostatic tissue on either diet. This reduction reached statistical significance only in the animals fed the LSF diet.

Any therapy that could prevent or control metastatic prostate cancer would have immediate clinical importance. Unfortunately, once metastatic prostate cancer has developed, virtually all men develop androgen-independent disease. Once the disease has become hormone refractory, most treatment is palliative; the median life span of these

patients is <12 months (15). A virtue of the TRAMP model is its similarity to human disease in the progression to metastatic prostate cancer. The ability of E-7869 to reduce the incidence of metastatic disease in this model suggests that it may be able to control metastatic prostate cancer in man.

The ability of E-7869 to prevent metastases appears to be dose-related. A 10-mg/kg dose of E-7869 showed minimal activity in the TRAMP mouse model (data not shown). The 15-mg/kg dose group appears to have marginal activity in preventing metastasis suggested by the rise in incidence (Table 3). The 20-mg/kg dose group, on either diet, had a combined 1 of 28 (3.5%) tissues demonstrating any evidence of metastatic disease. This dose-effect relationship suggests that a higher dose of E-7869 might prove even more effective.

In TRAMP mice, ~15% of *R*-flurbiprofen is converted to *S*-flurbiprofen at 2–4 h after administration (data not shown); this result is consistent with earlier studies in Min mice (3). It is now recognized that the inhibition of COX-1 results in alterations in the regulation of mucosal blood flow and mucous and bicarbonate secretion; these changes can lead to gastrointestinal ulceration and bleeding (16, 17). The decrease in the hematocrits in all treated groups (Table 2) indicates that some R-to-S inversion was probably causing gastrointestinal ulceration. Although the TRAMP animals were not inspected for ulcers, previous studies in our laboratory have shown clearly that the *S*-enantiomer is ulcerogenic in the mouse (3, 18). The inversion of E-7869 makes this drug somewhat toxic in mice and limits our ability to increase the dose. This limitation prevents us from determining whether the dose-response relationship can be sustained at higher doses. Fortunately, this limitation is unlikely to be a problem in humans because the R-to-S inversion rate is substantially lower (<1%) in man (19). Thus, there may be a larger therapeutic window for E-7869 in human trials for the treatment of prostate or other cancers.

In animal colon cancer models, the COX-2 pathway has been used as a therapeutic target for many selective inhibitors. Several potent COX-2 inhibitors have shown a decrease in the degree of malignancy in animal models (20, 21). *S*-flurbiprofen is a potent inhibitor of the COX-1 and COX-2 enzymes, whereas *R*-flurbiprofen (E-7869) has a 150- and 600-fold lower affinity for the COX-1 and 2 enzymes, respectively (4). As seen with other COX-2 inhibitors (22), *S*-flurbiprofen has some anticancer effect in the Min mouse model. The adenoma inhibition is not, however, solely the result of COX-2 inhibition by the *S*-enantiomer. *R*-flurbiprofen was far more effective than one would expect simply from the activity of *S*-flurbiprofen arising from the 10–15% inversion in the Min mouse (3). As previously stated, it is unclear if *R*-flurbiprofen alone or both enantiomers contribute to the antineoplastic effects demonstrated in our animal studies. *R*-flurbiprofen (E-7869) may down-regulate COX-2 mRNA through effects on other factors indirectly associated with COX-2

Table 3 Incidence of primary tumor and metastases after 18 weeks of treatment with 15 mg/kg or 20 mg/kg E-7869 in TRAMP mice

No. of animals	HSF			LSF	
	Vehicle control I 10	15 mg/kg E-7869 8	20 mg/kg E-7869 8	Vehicle control II 10	20 mg/kg E-7869 7
Primary tumor incidence <sup>a,b</sup>	19/40 (48)	14/32 (44)	8/28 (29)	14/24 (58)	6/28 <sup>c</sup> (21)
Metastasis incidence <sup>a,c</sup>	8/20 (40)	5/12 (42)	1/16 <sup>c</sup> (6)	2/20 (20)	0/12 (0)
Animals with nondetectable cancer <sup>a,d</sup>	0/10	0/6	1/7	0/6	2/6

<sup>a</sup> No. (%) of tissue per group with evidence of carcinoma.

<sup>b</sup> Tissues assessed for carcinoma (grade 4): dorsolateral, ventral, anterior prostate, and seminal vesicles (four tissues examined/animal). If any tissue was lost during dissection, that animal was removed from this calculation.

<sup>c</sup> Tissues assessed for metastases (grade + or –): lymph nodes and lung (two tissues examined/animal). If any tissue was lost during dissection, that animal was removed from this calculation.

<sup>d</sup> Detectable cancer is based on the histological analysis of all six tissues examined (dorsolateral, ventral, anterior prostate and seminal vesicles, lymph nodes, and lung). If any tissue was lost during dissection, that animal was excluded from this analysis.

<sup>e</sup> *P* < 0.05 versus corresponding vehicle control.

transcription. The *R*-enantiomer of ibuprofen, another arylpropionic acid, has been shown to inhibit the activation of nuclear factor  $\kappa$ B (23). The PPAR has been shown to negatively regulate COX-2 activity without affecting the COX-1 enzyme (24). Ibuprofen has also been shown to bind to PPAR $\alpha$  and PPAR $\gamma$  (25). The activation of PPAR $\gamma$  by certain nonsteroidal anti-inflammatory drugs has also been shown to decrease the malignancy of prostate and breast cancer *in vivo* (26, 27).

The effect of dietary fat in the modulation of prostate tumor growth has been documented in numerous studies (28–30). Most of the studies were concerned with high dietary fat intake, such as the traditional Western diet, which derives ~40% of energy from total fat. In this study, the AIN-93G diet yields 16.7% of its energy from fat, whereas the Teklad diet yields 9.7% of its energy from fat. Although the Teklad diet derives less of its energy from fat, it contains a larger amount of saturated fat, 2.4% *versus* 1.05% in the AIN-93G diet. With these same diets, we previously have shown that the LSF diet was associated with greater effects of *R*-flurbiprofen in the APC<sup>Min/+</sup> mouse; mice on the HSF developed more adenomas (3). In prostate cancer, saturated fat has been shown to be associated with advanced prostate cancer risk (31). Although there is a difference in the saturated fat composition between these two diets, it did not seem to influence the wet weights of the urogenital tissues or lymph nodes (Table 1). All E-7869 treatment groups, except the 15-mg/kg group, demonstrated significant reductions in lymph node wet weights when compared to their respective controls. The use of tissue wet weights as a measurement of tumorigenicity may lack the resolution necessary to discern small changes in dietary fat composition. Therefore, a finer scale of observation would be necessary to distinguish any differences in tumorigenicity relative to the fat content in the diet.

In the studies reported here, the histological analysis demonstrated a remarkable difference in the metastatic effect between the diets. In the HSF group, the animals receiving vehicle control had primary tumors in 19 of 40 tissues (48%) and metastases in 8 of 20 tissues (40%; Table 3). The vehicle control animals in the LSF diet group also had a high prostate tumor incidence (58%), but they had a dramatically lower incidence of metastasis (10%). In the absence of any other treatment, the difference in the metastatic incidence between the HSF group (40%) *versus* the LSF group (10%), although not statistically significant, is quite remarkable. The reduced metastatic potential seen in vehicle control animals on the LSF diet prevents the 20-mg/kg E-7869 group from demonstrating significance, although no metastases were found in any of the treated animals (Table 3). These data suggest that metastatic potential or invasiveness of the cancer was considerably diminished due to a difference in the saturated fat content of the diet.

All animals fed the LSF diet gained significantly more weight than the animals fed the HSF diet (Table 2). Considering only the vehicle controls, to exclude drug-associated events, the possible explanation for the disparity in the weight gain appears to have two components. Because the low saturated diet actually contains twice the amount of total fat as does the higher saturated fat diet (7% *versus* 4.2%), differences in body weights between control animals given the two diets could be due simply to the overall total fat content. However, given the reduced metastatic potential of this model when animals received the LSF diet (Table 3), these animals may also be generally less compromised by disease than those animals that received the HSF diet.

In conclusion, we have evidence that E-7869 (*R*-flurbiprofen), the non-COX-inhibiting enantiomer of flurbiprofen, can significantly reduce urogenital and periaortic lymph node wet weights in the TRAMP mouse model. A diet lower in saturated fat was associated with a decreased incidence of metastasis in the TRAMP mouse. Further-

more, in the model, daily oral dosing with E-7869 reduces the incidence of cancer in the prostate and in metastatic sites.

## ACKNOWLEDGMENTS

We thank Yoshimitsu Mineyama for his work on PCR analysis of the TRAMP animals and animal husbandry; and Karina Gibson for high-performance liquid chromatography analysis of drug levels.

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