[CANCER RESEARCH 59, 521–524, February 1, 1999]

Advances in Brief

Chemoprevention of Rat Prostate Carcinogenesis by 9-*cis*-Retinoic Acid[1]

David L. McCormick,[2] K. V. N. Rao, Vernon E. Steele, Ronald A. Lubet, Gary J. Kelloff, and Maarten C. Bosland

Experimental Toxicology and Carcinogenesis Division, IIT Research Institute, Chicago, Illinois 60616 [D. L. M., K. V. N. R.]; Chemoprevention Branch, National Cancer Institute, Bethesda, Maryland 20892 [V. E. S., R. A. L., G. J. K.]; and Departments of Environmental Medicine and Urology, New York University School of Medicine, New York, New York 10016 [M. C. B.]

Abstract

A chemoprevention study was conducted to evaluate the activity of 9-*cis*-retinoic acid (9-*cis*-RA) as an inhibitor of prostate carcinogenesis in male Wistar-Unilever (HsdCpb:Wu) rats. After pretreatment with a sequential regimen of cyproterone acetate (50 mg/kg/day for 21 days) and testosterone propionate (100 mg/kg/day for 3 days), groups of 40 rats received a single i.v. injection of *N*-methyl-*N*-nitrosourea (MNU; 30 mg/kg body weight). Beginning 2 weeks after carcinogen administration, rats received chronic exposure to testosterone administered in s.c. implanted silastic capsules. The study was terminated at 13 months after MNU administration, and prostate cancer incidence was determined by histopathological evaluation of step sections of accessory sex glands. Continuous dietary administration of 9-*cis*-RA at 100 mg/kg diet or 50 mg/kg diet beginning 1 week before MNU administration reduced cancer incidence in the dorsolateral + anterior prostate from 65% in dietary controls to 18 and 20%, respectively ($P < 0.001$ for both comparisons). Similarly, these dose levels of 9-*cis*-RA reduced the incidence of cancer in all accessory sex glands from 79% in dietary controls to 48 and 33% ($P < 0.01$ for both comparisons), respectively. Chronic dietary administration of 9-*cis*-RA induced no gross or organ-specific toxicity in any animal and did not suppress group mean body weight gain. The potent anticarcinogenic activity of 9-*cis*-RA in the rat prostate, when considered with its apparent lack of toxicity in rodents, suggests that this and other ligands for the retinoid X receptor merit consideration for evaluation in clinical prostate cancer chemoprevention trials.

Introduction

Vitamin A and its natural and synthetic analogues (retinoids) have a broad range of chemopreventive activity in experimental models for human cancer (1, 2). Many retinoids confer protection against cancer induction in animal model systems; however, individual members of this class demonstrate significant differences in anticarcinogenic efficacy, in pharmacokinetic profiles, and in patterns of systemic and organ-specific toxicity. The chemopreventive activity of many natural vitamin A compounds and synthetic retinoids is associated with toxicity that precludes their consideration for chronic, high-dose administration to humans (1). As a result, efforts continue to identify retinoids that possess both significant anticarcinogenic activity and a toxicity profile that is appropriate for long-term administration.

9-*cis*-RA[3] is a natural metabolite of RA in which the all-*trans* configuration of the polyene side chain is replaced with a *cis* configuration at the 9 position. 9-*cis*-RA serves as a high affinity ligand for a class of nuclear retinoid receptors (RXRs) to which all-*trans*-RA does not bind (3, 4). 9-*cis*-RA also binds to nuclear RARs. Because it binds to RARs and RXRs with similar affinity, the compound is classified as a pan-agonist. On the basis of its receptor binding characteristics, 9-*cis*-RA has been proposed to be a critical mediator of retinoid action at the molecular level (3, 4). The high affinity binding of 9-*cis*-RA to the RXR, as well as its ability to bind to the RAR, may underlie its mechanism of action as a chemopreventive agent.

9-*cis*-RA has demonstrated antiproliferative and/or differentiating activity in *in vitro* models of prostate cancer (5), breast cancer (6, 7), leukemia and lymphoma (7, 8), lung cancer (9), and head and neck cancer (10), among other malignancies; the compound is ~40-fold more potent than all-*trans* RA in transfection assays (3). *In vivo*, 9-*cis*-RA has significant anticarcinogenic activity in the rat mammary gland (11, 12) and in the rat colon (13). In view of the chemopreventive activity of 9-*cis*-RA in *in vivo* models for mammary and colon cancer (11–13), its antiproliferative activity in a broad range of neoplastic cells *in vitro* (5–10), and its reduced level of human toxicity in comparison to its all-*trans* isomer (14), 9-*cis*-RA merits further evaluation for chemopreventive efficacy in animal model systems. The present report summarizes the results of a study in which the activity of 9-*cis*-RA as an inhibitor of hormone-dependent prostate carcinogenesis in rats was evaluated.

Materials and Methods

Animals and Animal Husbandry. Before initiation, the study protocol was reviewed and approved by the IIT Research Institute Animal Care and Use Committee. All aspects of the study involving animal care, use, and welfare were performed in compliance with U.S. Department of Agriculture regulations and the NIH Guide for the Care and Use of Laboratory Animals.

Male Wistar-Unilever (HsdCpb:Wu) rats (7–8 weeks of age at the time of receipt) were purchased from virus-free colonies maintained at Harlan/Sprague Dawley (Indianapolis, IN). After a 1-week quarantine, rats were assorted into experimental groups (Table 1) using a randomization process designed to ensure comparable initial body weights in all groups. Rats were housed in pairs on hardwood bedding in polycarbonate cages in a temperature-controlled room maintained on a 12-h light/dark cycle. At all times during the study, rats were permitted free access to Teklad 4% fat rat/mouse chow (Teklad Test Diets, Madison, WI) with or without added 9-*cis*-RA. City of Chicago drinking water was supplied by an automatic watering system. Twice weekly, all rats were transferred to clean cages with fresh food and bedding. Animals were observed a minimum of once daily to monitor their general health status and received a weekly clinical examination and body weight measurement. Beginning 6 months after carcinogen administration, this clinical examination included palpation to identify gross lesions in the accessory sex gland area.

Pretreatment. After release from quarantine, all rats received daily oral (gavage) administration of 50 mg of cyproterone acetate (Berlex Laboratories, Wayne, NJ) in sesame oil per kg body weight for 21 consecutive days. One day after the final dose of cyproterone acetate, rats received daily s.c. injections of 100 mg of testosterone propionate (Sigma Chemical Co., St. Louis, MO) in sesame oil per kg body weight for 3 days. This sequence of anti-androgen followed by androgen results in maximal stimulation of prostatic epithelial proliferation at ~60 h after the first dose of testosterone.

Carcinogen Administration and Androgen Promotion. Sixty hours after the first dose of testosterone propionate, rats in designated groups received a

Received 10/12/98; accepted 12/14/98.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Research supported by contract N01-CN-35566-02 from the National Cancer Institute, Department of Health and Human Services.

[2] To whom requests for reprints should be addressed, at IIT Research Institute, 10 West 35th Street, Chicago, Illinois 60616.

[3] The abbreviations used are: RA, retinoic acid; RXR, retinoid X receptor; RAR, retinoic acid receptor; MNU, *N*-methyl-*N*-nitrosourea; 4-HPR, *N*-(4-hydroxyphenyl)-all-*trans*-retinamide.

single i.v. injection of 30 mg of MNU (Ash-Stevens, Detroit, MI) per kg body weight. MNU was administered in sterile saline (pH 5.0). Two weeks after carcinogen administration, all MNU-treated rats received s.c. implants of two silastic tubes (Dow-Corning, Midland, MI) containing 40 mg of crystalline testosterone (Sigma). Silastic tubes containing testosterone were replaced at 6-month intervals throughout the study.

Chemopreventive Agent Administration. Dietary administration of 9-*cis*-RA was begun 1 week before administration of MNU and was continued until termination of the study at 13 months after carcinogen. 9-*cis*-RA was administered at 100 mg/kg diet and 50 mg/kg diet; dose levels of 9-*cis*-RA were selected on the basis of the results of a preliminary subchronic toxicity/diet tolerance study (data not shown). To maintain its stability under ambient animal room conditions, 9-*cis*-RA was mixed into experimental diets using a vehicle containing (per kg diet): 12.5 g of ethanol, 0.6 g of Tenox, and 36.9 g of corn oil (15); dietary controls received basal diet supplemented with vehicle only. The concentration of 9-*cis*-RA in replicate samples of formulated test diets was measured at 10 time points throughout the study; mean concentrations of 9-*cis*-RA in formulated diets were 97.5 and 97.7% of the target concentrations at the high and low doses, respectively.

Necropsy and Histology. Intercurrent deaths were necropsied immediately upon discovery; moribund animals and animals surviving until the terminal necropsy were euthanized by CO_2 asphyxiation and were necropsied immediately after death. At necropsy, all gross lesions were excised, and the accessory sex glands and urinary bladder were carefully removed *en bloc* from each rat and fixed in 10% neutral buffered formalin. After fixation, accessory sex glands were dissected as described previously (15) and embedded in paraffin; six step sections each were prepared at intervals of 200 μm from the dorsolateral prostate and from the anterior prostate + seminal vesicle. In addition, a single section was prepared from the ventral prostate of each animal. Tissues were stained with H&E for histopathological evaluation.

Histopathological Evaluation. The approach used to classify accessory sex gland tumors in the rat has been described in detail (15, 16). Briefly, accessory sex gland tumors were categorized on the basis of: (*a*) degree of malignancy; (*b*) site of origin; and (*c*) size of lesion. Proliferative epithelial lesions were classified as adenocarcinoma, carcinoma *in situ*, adenoma, or atypical hyperplasia using established criteria (16). Simultaneous with the histopathological classification of malignancy, the site of each lesion was identified. For small lesions (hyperplasia, carcinoma *in situ*, and small carcinomas), it was possible to define whether the site of origin was in the dorsolateral prostate, anterior prostate, ventral prostate, or seminal vesicle. The precise origin of large carcinomas demonstrating invasive growth patterns could often not be defined. In such cases, tumors were scored for location in either the anterior prostate/seminal vesicle region (if clearly confined to this tissue complex) or to the dorsolateral prostate region, in which instance the lesion could have originated from any of the accessory sex gland structures except the ventral prostate (dorsolateral prostate, anterior prostate, and/or seminal vesicle). The ventral prostate was never involved in large carcinomas and did not develop smaller proliferative lesions. During histopathological evaluations, accessory sex gland lesions were also classified as macroscopic ($\geq$4 mm in diameter) or microscopic ($<$4 mm in diameter) using criteria described previously (15).

Statistical Analyses. Body weight data were compared by ANOVA. Survival curves were compared using life table analysis and the logrank test (17). Comparisons of prostate cancer incidence were made using Fisher's Exact test (two-sided). The number of rats evaluated from each group (effective number of rats) excludes animals that did not survive for a minimum of 10 months after carcinogen and excludes animals whose tissues were lost to evaluation for reasons of cannibalism or autolysis. Chemopreventive activity was defined as a statistically significant ($P < 0.05$) reduction in cancer incidence (all cancers or cancers in a specific organ site) in a group treated with 9-*cis*-RA in comparison to the appropriate dietary control.

Results

Sequential administration of antiandrogen (cyproterone acetate), androgen (testosterone propionate), chemical carcinogen (MNU), and androgen (testosterone) induced a high incidence of prostate cancer in male Wistar-Unilever rats. Both the incidence of accessory sex gland tumors observed and the specificity of cancer induction for the dorsolateral and anterior prostate are similar to previous results from our laboratory using this model system (15). In the dietary control group, cancer incidence in all accessory sex glands combined was 79%; 65% of the animals in this group demonstrated cancers that were clearly confined to the dorsolateral and/or anterior prostate, whereas only 3% demonstrated seminal vesicle cancers that were not accompanied by invasive malignant lesions in either the dorsolateral or anterior prostate. The size and growth patterns of lesions in remaining cancer-bearing rats precluded definitive determination of their site of origin.

Dietary administration of both dose levels of 9-*cis*-RA conferred significant protection against prostate cancer induction by MNU + hormones; the chemopreventive activities of the high (100 mg/kg diet) and low (50 mg/kg diet) dose levels of 9-*cis*-RA were approximately equal (Table 1). Analysis of cancers that were confined to the prostate gland provided the most striking evidence of chemopreventive efficacy; the incidence of invasive cancers confined to the dorsolateral and anterior prostate was reduced from 65% in dietary controls to 18 and 20% in groups fed the high- and low-dose levels of 9-*cis*-RA, respectively ($P < 0.001$ for both comparisons). When carcinomas *in situ* are included

Table 1 *Influence of 9-cis-RA on prostate carcinogenesis in Wistar-Unilever rats*

Group	1	2	3
Chemopreventive agent	Control	9-*cis*-RA	9-*cis*-RA
Chemopreventive agent dose level (mg/kg diet)	0	50	100
Effective number of animals	34	40	38
		No. (%) of rats with lesion	
All accessory sex glands combined (dorsolateral and anterior prostate plus seminal vesicle)			
Adenocarcinoma or carcinosarcoma (± carcinoma *in situ*)	27 (79)	13 (33)[a]	17 (48)[a]
Carcinoma *in situ* only	3 (9)	5 (13)	8 (21)
Dorsolateral plus anterior prostate (clearly confined to these glands; ± seminal vesicle lesions)			
Adenocarcinoma (± carcinoma *in situ*)	22 (65)	8 (20)[b]	7 (18)[b]
Carcinoma *in situ* only	2 (6)	5 (13)	6 (16)
Dorsolateral prostate region (originating from dorsolateral or anterior prostate or seminal vesicle)			
Adenocarcinoma or carcinosarcoma	3 (9)	4 (11)	2 (5)
Sarcoma	1 (3)	0 (0)	0 (0)
Anterior prostate/seminal vesicle region (originating from anterior prostate or seminal vesicle)			
Adenocarcinoma	4 (12)	2 (5)	4 (11)
Seminal vesicle only (clearly confined to this gland; ± carcinoma *in situ* in dorsolateral/anterior prostate)			
Adenocarcinoma	1 (3)	3 (8)	4 (11)
Carcinoma *in situ* only	2 (6)	0 (0)	4 (11)
Sarcoma	0	3 (8)	0

[a] $P < 0.01$ *versus* group 1.
[b] $P < 0.001$ *versus* group 1.

in the analysis, the high and low doses of the retinoid reduced the incidence of malignancy in the dorsolateral and anterior prostate from 71% in dietary controls to 34 and 33% ($P < 0.01$). The total incidence of accessory sex gland cancers was also reduced by dietary administration of the retinoid; the combined incidence of invasive cancer in all accessory sex glands was 48 and 33% in groups fed 9-*cis*-RA, as compared with 79% in the dietary control group ($P < 0.01$ for both groups *versus* control).

The dose levels of 9-*cis*-RA that suppressed prostate cancer induction induced no toxicity that was identifiable by clinical observation, alterations in body weight gain, or gross pathology. Survival curves were similar in all MNU-treated groups, and no toxicity that could be ascribed to the retinoid was identified in any animal at any point in the study. Gross pathology in tissues other than the accessory sex glands was unremarkable in animals exposed to 9-*cis*-RA and was comparable with that seen in dietary controls. Mean body weights in MNU-treated groups fed 9-*cis*-RA were greater than or equal to those of dietary controls at essentially all points during the 13-month period of exposure (Fig. 1); mean terminal body weights in groups fed 9-*cis*-RA were 100 and 106% of dietary control.

Discussion

The prostate presents an ideal target for human cancer chemoprevention; prostate cancer occurs in high incidence in Western male populations (18), has a predictable natural history in terms of age of onset, and generally presents with an indolent clinical course that offers a relatively long time period for interventions directed at disease prevention or stabilization. The long natural history of prostate cancer development suggests that strategies designed to inhibit tumor promotion or progression may be effective when initiated in either middle-aged or elderly men.

Because carcinoma of the prostate occurs primarily in elderly men, any delay in the time course of neoplastic development achieved through pharmacological, hormonal, and/or nutritional intervention could result in a substantial reduction in cancer incidence. Furthermore, even a modest reduction in the slope of the tumor latency curve could delay the onset of prostate cancer in individuals at risk for the disease until far later in life. On this basis, development of effective drugs for prevention of prostate cancer appears likely to result not only in a reduction in disease incidence and associated morbidity but also in a significant decrease in cancer mortality.

The results of the present study demonstrate that 9-*cis*-RA, the endogenous ligand for RXR, has significant potency as an inhibitor of cancer induction in the rat prostate. Administration of 9-*cis*-RA at 50 and 100 mg/kg diet resulted in a significant suppression of prostate carcinogenesis, without suppression of body weight gain, induction of gross hepatic pathology, or other common indicators of retinoid toxicity. On this basis, and in consideration of its chemopreventive efficacy in rat models for breast (11, 12) and colon cancer (13), 9-*cis*-RA is clearly a leading candidate retinoid for evaluation in clinical trials for cancer prevention in several organ sites.

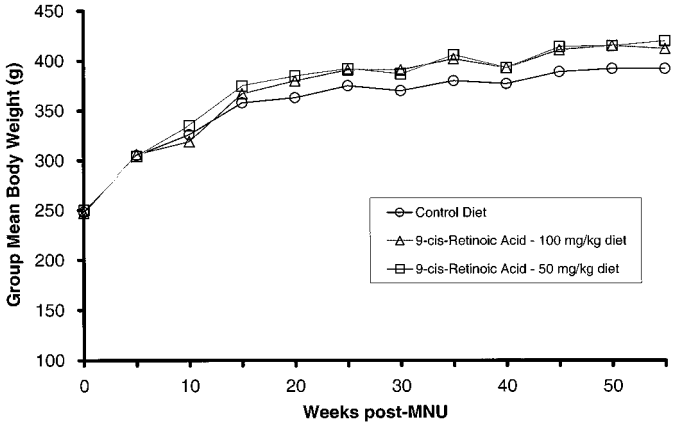

Fig. 1. Body weight curves for carcinogen-treated Wistar-Unilever rats fed control diet or control diet supplemented with 9-*cis*-RA. ○, control diet; □, 50 mg of 9-*cis*-RA/kg diet; △, 100 mg of 9-*cis*-RA/kg diet.

An unexpected result from the present study was that the 100 and 50 mg/kg diet dose levels of 9-*cis*-RA conferred essentially equal protection against prostate cancer induction. It is unclear whether this lack of differential activity is based on agent pharmacodynamics (*e.g.*, saturation of a drug target by the lower dose of 9-*cis*-RA), agent bioavailability or pharmacokinetics (through saturation of absorption or metabolism pathways by the lower dose of 9-*cis*-RA), or other mechanism(s). However, the essentially flat dose-response relationship for chemopreventive efficacy suggests the possibility that even lower doses of 9-*cis*-RA (<50 mg/kg diet) may retain much or all of its chemopreventive activity. Such a situation would yield an increased "chemopreventive ratio" for 9-*cis*-RA, defined as the ratio between the minimal dose where effective chemoprevention is achieved and the dose at which limiting toxicity is encountered. When considered with the well-known patterns of toxicity associated with long-term administration of other retinoids, an increased "chemopreventive ratio" has clear implications for possible clinical application of 9-*cis*-RA for cancer chemoprevention.

An important consideration in the selection of agents for clinical chemoprevention trials is their toxicity in preclinical model systems. In this regard, the toxicity profile of 9-*cis*-RA differs considerably from those of most natural vitamin A compounds and from first and second generation synthetic retinoids. In the present study, administration of 9-*cis*-RA to rats did not induce characteristic patterns of "retinoid-like" toxicity; unlike natural retinyl esters and many synthetic retinoids, progressive suppression of rodent body weight gain was not induced by administration of 9-*cis*-RA over extended periods, and no evidence of gross hepatic pathology was observed.

In toxicity and chemoprevention studies conducted with numerous retinoids over the past 20 years, we have found that suppression of body weight gain is a highly sensitive indicator of retinoid toxicity. In long-term studies, delayed suppression of body weight gain may occur in response to retinoid dose levels where no acute or subacute effects were seen; furthermore, this body weight suppression often occurs in the absence of other clinical indications of toxicity. The results of the present study demonstrated no body weight alterations or other indications of toxicity in rats receiving chronic dietary exposure to effective chemopreventive dose levels of 9-*cis*-RA. The relative lack of chronic toxicity of 9-*cis*-RA is in general agreement with data suggesting a low level of toxicity associated with subchronic administration of targretin, an RXR-specific ligand (19, 20). The fact that 9-*cis*-RA is well tolerated by animals receiving long-term exposure suggests the potential suitability of this retinoid for chronic administration protocols. In consideration of the apparent reversibility of retinoid chemopreventive activity (21, 22), chronic administration regimens are likely to be required to sustain protection against cancer induction.

Of particular interest is the differential activity of 9-*cis*-RA and 4-HPR as chemopreventive agents in the Wistar-Unilever rat prostate cancer model system. In the present study, 9-*cis*-RA demonstrated potent chemopreventive activity without toxicity; by contrast, we have previously reported that chronic dietary administration of 4-HPR is without chemopreventive activity in this model system (15). Whether the differential chemopreventive efficacy of 9-*cis*-RA and 4-HPR in the prostate reflects differences in retinoid pharmacokinetics or me-

tabolism (23, 24), tissue distribution, or in the intrinsic anticarcinogenic activity and mechanism(s) of action of the parent compound or metabolite(s) merits further study.

Through its binding to RXR, 9-*cis*-RA may indirectly influence a wide range of functions, the activity of which is regulated by other nuclear receptors. RXR forms heterodimers with nuclear receptors for thyroid hormone (25), vitamin D (26), peroxisome proliferators (27), and a number of "orphan" receptors (28). As part of a heterodimer, RXRs activate the transcription of several permissive partners, including peroxisome proliferator activated receptors (27), LXR (29), and OR1 (30). Through this mechanism, pharmacological levels of 9-*cis*-RA could influence a relatively large number of nuclear receptors and thereby modulate a wide range of downstream processes over which receptor-ligand interactions provide regulatory control.

A logical hypothesis based on the existing data for retinoids and prostate cancer chemoprevention in the Wistar-Unilever rat is that binding to the RXR receptor is essential to retinoid activity. This hypothesis would suggest that specific ligands for the RXR receptor may have significant activity as inhibitors of carcinogenesis in the prostate, whereas retinoids whose binding is limited to RAR may be inactive. Additional evaluations of the chemopreventive efficacy of pure RXR agonists, pure RAR agonists, and other RAR/RXR panagonists in the rat prostate may identify the most sensitive molecular targets for retinoid action in this organ and thereby provide a mechanistic basis for the design of novel anticarcinogenic drugs.

Acknowledgments

We thank Teresa Bowman-Gram, Lawrence Dooley, Gloria Gardner Smith, Joanne Schmoll, and Larry Richards for excellent technical assistance.

References

1. McCormick, D. L., and Moon, R. C. Vitamin A deficiency and cancer. *In:* J. C. Bauernfeind (ed.), Vitamin A Deficiency and Its Control, pp. 245–284. New York: Academic Press, 1986.
2. Lotan, R. Retinoids in cancer chemoprevention. FASEB J., *10:* 1031–1039, 1996.
3. Heyman, R. A., Mangelsdorf, D. J., Dyck, J. A., Stein, R. B., Eichele, G., Evans, R. M., and Thaller, C. 9-*cis*-Retinoic acid is a high affinity ligand for the retinoid X receptor. Cell, *68:* 397–406, 1992.
4. Levin, A. A., Sturzenbecker, L. J., Kazmer, S., Bosakowski, T., Huselton, C., Allenby, G., Speck, J., Kratzeisen, C., Rosenberger, M., Lovey, A., and Grippo, J. F. 9-*cis* Retinoic acid stereoisomer binds and activates the nuclear receptor RXRα. Nature (Lond.), *355:* 359–361, 1992.
5. Blutt, S. E., Allegretto, E. A., Pike, J. W., and Weigel, N. L. 1,25-Dihydroxyvitamin D3 and 9-*cis*-retinoic acid act synergistically to inhibit the growth of LNCaP prostate cells and cause accumulation of cells in G1. Endocrinology, *138:* 1491–1497, 1997.
6. Rubin, M., Fenig, E., Rosenauer, A., Menendez-Botet, C., Achkar, C., Bentel, J. M., Yahalom, J., Mendelsohn, J., and Miller, W. H., Jr. 9-*cis*-Retinoic acid inhibits growth of breast cancer cells and down-regulates estrogen receptor RNA and protein. Cancer Res., *54:* 6549–6556, 1994.
7. Gottardis, M. M., Lamph, W. W., Shalinsky, D. R., Wellstein, A., and Heyman, R. A. The efficacy of 9-*cis*-retinoic acid in experimental models of cancer. Breast Cancer Res. Treat., *38:* 85–96, 1996.
8. Lutzky, J., Vujicic, M., Yamanishi, D. T., and Bhalla, K. Antiproliferative effects of all-*trans*-retinoic acid (tRA) and 9-*cis*-retinoic acid (9-cisRA) on human lymphoid cell lines. Proc. Am. Assoc. Cancer Res., *34:* 292, 1993.
9. Guzey, M., Demirpence, E., Criss, W., and DeLuca, H. F. Effects of retinoic acid (all-*trans* and 9-*cis*) on tumor progression in small-cell lung carcinoma. Biochem. Biophys. Res. Commun., *242:* 369–375, 1998.
10. Giannini, F., Maestro, R., Vukosavljevic, T., Pomponi, F., and Boiocchi, M. All-*trans*, 13-*cis*, and 9-*cis* retinoic acids induce a fully reversible growth inhibition in HNSCC cell lines: implications for *in vivo* retinoic acid use. Int. J. Cancer, *70:* 194–200, 1997.
11. Anzano, M. A., Byers, S. W., Smith, J. M., Peer, C. W., Mullen, L. T., Brown, C. C., Roberts, A. B., and Sporn, M. B. Prevention of breast cancer in the rat with 9-*cis*-retinoic acid as a single agent and in combination with tamoxifen. Cancer Res., *54:* 4614–4617, 1994.
12. Anzano, M. A., Peer, C. W., Smith, J. M., Mullen, L. T., Shrader, M. W., Logsdon, D. L., Driver, C. L., Brown, C. C., Roberts, A. B., and Sporn, M. B. Chemoprevention of mammary carcinogenesis in the rat: combined use of raloxifene and 9-*cis*-retinoic acid. J. Natl. Cancer Inst., *88:* 123–125, 1996.
13. Zheng, Y., Kramer, P. M., Olson, G., Lubet, R. A., Steele, V. E., Kelloff, G. J., and Pereira, M. A. Prevention by retinoids of azoxymethane-induced tumors and aberrant crypt foci and their modulation of cell proliferation in the colon of rats. Carcinogenesis (Lond.), *18:* 2119–2125, 1997.
14. Rizvi, N. A., Marshall, J. L., Ness, E., Yoe, J., Gill, G. M., Truglia, J. A., Loewen, G. R., Jaunakais, D., Ulm, E. H., and Hawkins, M. J. Phase I study of 9-*cis*-retinoic acid (ALRT1057 capsules) in adults with advanced cancer. Clin. Cancer Res., *4:* 1437–1442, 1998.
15. McCormick, D. L., Rao, K. V. N., Dooley, L., Steele, V. E., Lubet, R. A., Kelloff, G. J., and Bosland, M. C. Influence of *N*-methyl-*N*-nitrosourea, testosterone, and *N*-(4-hydroxyphenyl)-all-*trans*-retinamide on prostate cancer induction in Wistar-Unilever rats. Cancer Res., *58:* 3282–3288, 1998.
16. Bosland, M. C. Age-related lesions in the male accessory sex glands and penis of the rat. *In:* U. Mohr, D. L. Dungworth, and C. C. Capen (eds.), Monographs on Pathobiology of the Aging Rat, Vol. 1, pp. 443–467. Washington, DC: ILSI Press, 1992.
17. Peto, R., Pike, M. C., Armitage, P., Breslow, N. E., Cox, D. R., Howard, S. V., Mantel, N., McPherson, K., Peto, J., and Smith, P. G. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br. J. Cancer, *35:* 1–39, 1977.
18. Jones, G. W. Prostate cancer: magnitude of the problem. American College of Surgeons database and epidemiologic study. Cancer (Phila.),*71:* 887–890, 1993.
19. Gottardis, M. M., Bischoff, E. D., Shirley, M. A., Wagoner, M. A., Lamph, W. W., and Heyman, R. A. Chemoprevention of mammary carcinoma by LGD1069 (Targretin): an RXR-selective ligand. Cancer Res., *56:* 5566–5570, 1996.
20. Bischoff, E. D., Gottardis, M. M., Moon, T. E., Heyman, R. A., and Lamph, W. W. Beyond tamoxifen: the retinoid X receptor-selective ligand LGD1069 (targretin) causes complete regression of mammary carcinoma. Cancer Res., *58:* 479–484, 1998.
21. McCormick, D. L., Burns, F. J., and Albert, R. E. Inhibition of rat mammary carcinogenesis by short dietary exposure to retinyl acetate. Cancer Res., *40:* 1140–1143, 1980.
22. McCormick, D. L., Burns, F. J., and Albert, R. E. Inhibition of benzo(*a*)pyrene-induced mammary carcinogenesis by retinyl acetate. J. Natl. Cancer Inst., *66:* 559–564, 1981.
23. Achkar, C. C., Bentel, J. M., Boylan, J. F., Scher, H. I., Gudas, L. J., and Miller, W. H., Jr. Differences in the pharmacokinetic properties of orally administered all-*trans*-retinoic acid and 9-*cis*-retinoic acid in the plasma of nude mice. Drug. Metab. Dispos., *22:* 451–458, 1994.
24. Howell, S. R., Shirley, M. A., and Ulm, E. H. Effects of retinoid pretreatment of rats on hepatic microsomal metabolism and cytochromes P450. Correlation between retinoic acid receptor/retinoid X receptor selectivity and effects on metabolic enzymes. Drug Metab. Dispos., *26:* 234–239, 1998.
25. Cooney, A., Leng, X., Tsai, S. Y., O'Malley, B. W., and Tsai, M. J. Multiple mechanisms of chicken ovalbumin upstream promoter transcription factor-dependent repression of activation by the vitamin D, thyroid hormone, and retinoic acid receptors. J. Biol. Chem., *268:* 4152–4160, 1993.
26. Carlberg, C., and Saurat, J. H. Vitamin D-retinoid association: molecular basis and clinical applications. J. Invest. Dermatol. Symp. Proc., *1:* 82–87, 1996.
27. Gearing, K. L., Gottlicher, M., Teboul, M., Widmark, E., and Gustafsson, J. A. Interaction of the peroxisome proliferator-activated receptor and the retinoid X receptor. Proc. Natl. Acad. Sci. USA, *90:* 1440–1444, 1993.
28. Mangelsdorf, D. J., and Evans, R. M. The RXR heterodimers and orphan receptors. Cell, *83:* 841–850, 1995.
29. Janowski, B. A., Willey, P. J., Devi, T. R., Falck, J. R., and Mangelsdorf, D. R. An oxysterol signalling pathway mediated by the nuclear receptor LXRα. Nature (Lond.), *383:* 728–731, 1996.
30. Wiebel, F. F., and Gustafsson, J. A. Heterodimeric interaction between retinoid X receptor α and orphan nuclear receptor OR1 reveals dimerization induced activation as a novel mechanism of nuclear receptor activation. Mol. Cell. Biol., *17:* 3977–3986, 1997.

Chemoprevention of Rat Prostate Carcinogenesis by 9-*cis*-Retinoic Acid

David L. McCormick, K. V. N. Rao, Vernon E. Steele, et al.

Cancer Res 1999;59:521-524.