

Cancer Chemopreventive Activity Mediated by Deguelin, a Naturally Occurring Rotenoid¹

George O. Udeani, Clarissa Gerhäuser,² Cathy F. Thomas, Richard C. Moon, Jerrome W. Kosmeder, A. Douglas Kinghorn, Robert M. Moriarty, and John M. Pezzuto³

Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy [C. G., A. D. K., J. M. P.] and Department of Pharmacy Practice [G. O. U.], College of Pharmacy, Department of Surgical Oncology [C. F. T., R. C. M., J. M. P.], College of Medicine, and Department of Chemistry [J. W. K., R. M. M.], College of Liberal Arts and Sciences, University of Illinois at Chicago, Chicago, Illinois 60612

ABSTRACT

Deguelin, a natural product isolated from *Mundulea sericea* (Leguminosae), was shown previously to mediate strong inhibition of 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ornithine decarboxylase (ODC) activity in cell culture and to reduce the formation of preneoplastic lesions when mouse mammary glands were exposed to 7,12-dimethylbenz(a)anthracene. As reported currently, deguelin was synthesized and evaluated for chemopreventive activity in the two-stage 7,12-dimethylbenz(a)anthracene/TPA skin carcinogenesis model with CD-1 mice and in the *N*-methylnitrosourea mammary carcinogenesis model with Sprague Dawley rats. In the mouse skin study, deguelin reduced tumor incidence from 60% in the control group to 10% in the group treated with a dose of 33 μ g, and multiplicity was reduced from 4.2 in the control group to 0.1 in the treatment group. When the dose was increased 10-fold to 330 μ g, no tumors were observed in the treatment group. These results correlated with the potential of deguelin to inhibit TPA-induced mouse epidermal ODC activity. When applied topically as a single dose in a time range of 2 h before to 2 h after TPA treatment, deguelin (384 μ g) reduced ODC induction by TPA (6.17 μ g) by more than 85%. Time course studies indicated that deguelin (33 μ g) inhibited TPA (1.17 μ g)-induced ODC activity by 70% without affecting the kinetics of induction over a period of 10 h. Complete inhibition of ODC induction was observed at a dose of 330 μ g of deguelin. In the rat mammary tumorigenesis study, intragastric administration of 2 or 4 mg of deguelin/kg of body weight daily, 5 days/week, reduced tumor multiplicity from 6.8 tumors/rat in the control group to 5.1 or 3.2 tumors/animal, respectively. At the 4 mg of deguelin/kg of body weight dose level, the tumor latency period was significantly increased. Tumor incidence, however, was unaffected. These data indicate that deguelin exhibits cancer chemopreventive effects in skin and mammary tumorigenesis models and that additional studies are warranted to characterize the cancer chemopreventive or chemotherapeutic potential of this substance more fully.

INTRODUCTION

Cancer remains the second leading cause of death in the United States, with an observed annual mortality rate increase that parallels the increasing incidence of the disease (1, 2). It seems that this trend will continue, and in the United States, cancer will be the leading cause of death by 2000. Prevention is a logical and obvious strategy to help alleviate this problem, and systemic cancer chemoprevention is an important prevention modality (3-5). The general principle of cancer chemoprevention involves the use of chemical or dietary agents to inhibit or delay the onset of neoplasia by blocking neoplastic

inception as well as reversing the progression of transformed cells before the appearance of malignant lesions. Retinoids, antihormones, antioxidants, biological response modifiers, nonsteroidal anti-inflammatory agents, trace elements, and ODC⁴ inhibitors are examples of chemopreventive agents that have been used successfully either in animal experimental carcinogenesis models or clinical trials (3-5). Of the large-scale clinical trials currently being conducted, preliminary results of a Phase III breast cancer chemopreventive study with fenretinide involving approximately 3000 subjects conducted by the European Institute of Oncology and the National Cancer Institute seem to be positive (6). Additional large-scale trials are being conducted with tamoxifen at the present time (6, 7).

The discovery and development of novel cancer chemopreventive compounds is central to the process of yielding agents that could either become available in clinics or for widespread public consumption. We have established a unique multidisciplinary and integrated approach for the discovery and development of chemopreventive agents from natural products (plant materials). The program includes: (a) global plant selection and procurement; (b) isolation and identification of active components; (c) *in vitro* bioassay studies of extracts and direction of fractionation; (d) *in vivo* evaluations in various carcinogenesis models to establish efficacy; and (e) chemical synthesis and structural modification of active leads (8, 9). Deguelin (Fig. 1) is one of four active rotenoids, derived from the African plant *Mundulea sericea*, that was discovered by this approach (10). This plant was selected for bioassay-directed fractionation after a crude extract was found to inhibit TPA-induced ODC activity with cultured mouse 308 cells and to inhibit DMBA-induced preneoplastic lesions with mouse mammary glands in organ culture. The mouse 308 cell test system was chosen to identify potential inhibitors of tumor promotion because ODC, a major enzyme that is essential in polyamine biosynthesis, is induced by treatment of 308 cells with TPA, and polyamines are overexpressed in neoplastic cells. Thus, compounds capable of interfering with ODC expression and subsequent polyamine levels are candidates for chemoprevention studies, and the mouse 308 cell culture system is suitable for use as a monitor in bioassay-directed fractionation studies.

As a result of this process, rotenoids were found to be highly active inhibitors of ODC induction, with deguelin demonstrating activity that was significantly greater than that observed with 13-*cis*-retinoic acid (10). Additional mechanistic studies revealed dose- and time-dependent inhibition of TPA-induced ODC mRNA expression, suggesting transcriptional modulation was an important aspect of rotenoid action. On the basis of these data, rotenoids were deemed candidates for development either as chemopreventive or chemotherapeutic agents, and we describe the results of studies in which the chemopreventive potential of deguelin was evaluated with two animal models. In the two-stage mouse skin model, carcinogenesis was initiated with DMBA, and TPA was used as a promoter. Also, because skin tumor-

Received 6/10/96; accepted 6/10/97.

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 Supported by National Cancer Institute Grant P01 CA48112. Presented in part at the 87th Annual Meeting of the American Association for Cancer Research, Washington, DC, April 20-24, 1996 (26).

² Present address: German Cancer Research Center, Division 0325, Toxicology and Cancer Risk Factors, Im Neuenheimer Feld 280, D 69120 Heidelberg, Germany.

³ To whom requests for reprints should be addressed, at the Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy (M/C 781), 833 South Wood Street, Chicago, Illinois 60612-7231.

⁴ The abbreviations used are: ODC, ornithine decarboxylase; DMBA, 7,12-dimethylbenz(a)anthracene; MNU, *N*-methylnitrosourea; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; i.g., intragastric.

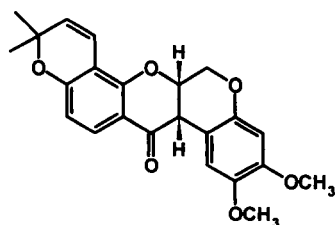


Fig. 1. Structure of deguelin.

igenesis in this model is clearly associated with a transient elevation of ODC activity after TPA application (11), it was of interest to establish whether a decrease in tumor formation correlated with deguelin-mediated inhibition of ODC induction. Lastly, the chemopreventive potential of deguelin was evaluated in a rat mammary tumor model in which carcinogenesis was induced by MNU treatment.

MATERIALS AND METHODS

Chemicals. DMBA was purchased from Sigma Chemical Co. (St. Louis, MO), and MNU was received from Ash Stevens (Detroit, MI). Deguelin was synthesized via a four-step process (97–98% purity), with commercially available rotenone as starting material (12). Deguelin was dissolved in corn oil (Sigma Chemical Co.) vehicle for i.g. administration on a weekly basis. TPA was obtained from Chemsyn Science Laboratories (Lenexa, KS). BSA used as a protein standard and all other chemicals were purchased from Sigma Chemical Co. Fisher Scientific high-performance liquid chromatography-grade acetone was used for the study. Deguelin was prepared weekly in spectrograde acetone for topical application.

Skin Carcinogenesis Study. CD-1 mice were received from Charles River Breeding Laboratories (Portage, MI) at 4–5 weeks of age. These animals were housed in a windowless room illuminated 14 h each day at $22 \pm 1^\circ\text{C}$. The basal diet was Prolab RMH 4020 rat/mouse chow (blox; Agway Inc., Syracuse, NY). After a quarantine period of 1 week, the hair on the dorsal surface of each mouse was removed by shaving. Mice showing regrowth of hair 3 days after shaving or nicks or cuts in the skin were removed from the study. Suitable animals were randomized by weight into 5 groups (20 animals/group), and ears were punched with individual numbers. The animals were initiated with DMBA (100 μg) 1 day after ear punching and promoted twice weekly for 15 weeks with TPA (1.17 μg). Deguelin (33 or 330 μg) or vehicle (acetone) application preceded each TPA application by 1 h. Each dose of deguelin was administered in 0.2 ml of acetone, and all animals were weighed and observed weekly for carcinoma and papilloma development. Fifteen weeks after DMBA administration, the animals were sacrificed by CO_2 asphyxiation, and necropsy was performed. All lesions from the deguelin-treated groups were removed and fixed in 10% buffered formalin for histopathological evaluation. Each lesion was coded according to its location on the animal. Statistical significance for tumor multiplicity between groups was determined by Armitage's test for trend in proportions. Statistical significance for tumor incidence was compared among the groups using Fisher's one-tailed test.

Mammary Carcinogenesis Study. Virgin female Sprague Dawley rats were received from Harlan Sprague Dawley (Indianapolis, IN) at 36 days of age. The animals were housed in a windowless room illuminated 14 h each day at $22 \pm 1^\circ\text{C}$. The basal diet was Prolab RMH 4020 rat/mouse chow (blox; Agway Inc.). After a quarantine period of 1 week, the animals were randomized by weight and placed into 5 groups (25 animals/group). Treatments with either i.g. deguelin (2 or 4 mg/kg of body weight) or vehicle, 5 days/week, were also started at this time. At 50 days of age, all animals received a single i.v. injection of MNU (50 mg/kg of body weight) in acidified saline (pH 5.0), as described previously (13). All animals were weighed weekly. The animals were palpated weekly for mammary tumors, starting at 3 weeks post-MNU administration, until termination of the study. The date of appearance and location of all tumors were recorded. One hundred and eighty days after carcinogen administration, the animals were sacrificed by CO_2 asphyxiation, and necropsy was performed. During necropsy, all mammary tumors and other grossly abnormal tissues were removed, verified, and recorded with regard to

location. Statistical significance tumor multiplicity between groups was determined by Armitage's test for trend in proportions. Statistical significance of tumor incidence was compared among the groups using a log-rank test.

Determination of Mouse Epidermal ODC Activity. Female CD-1 mice were received from Charles River Breeding Laboratories at 5–6 weeks of age and held in quarantine for 1 week. Animals were randomized by weight into groups of four, and the hair on the dorsal surface was removed by shaving before the application of deguelin and TPA (dissolved in 0.2 ml of spectrograde acetone). After the respective induction periods, mice were sacrificed by CO_2 narcosis followed by cervical dislocation. Skin samples were promptly removed, bisected, frozen in liquid N_2 , and kept at -85°C until analyzed. For the determination of ODC activity, one half of each skin sample was homogenized for 15 s in 2 ml of ice-cold homogenizing buffer containing 50 mM Tris buffer (pH 7.5), 0.1 mM pyridoxal phosphate, and 0.1 mM EDTA and centrifuged at 15,000 rpm for 30 min. In 24-well plates, 100 μl of the clear supernatants were mixed on ice with 115 μl of a reaction mixture containing 40 mM Tris buffer (pH 7.5), 8 mM DTT, 0.64 mM pyridoxal phosphate, and 0.8 mM EDTA. The reaction was initiated by the addition of 250 nCi of L -[1- ^{14}C]ornithine (56 mCi/mmol, 100 $\mu\text{Ci}/\text{ml}$; Moravex) in 1.94 mM L -ornithine (25 μl). After an incubation period of 1 h at 37°C , the reaction was stopped, and $^{14}\text{CO}_2$ release was measured as described previously (10). Protein content was determined according to Bradford (14) to calculate ODC specific activity (pmol of $^{14}\text{CO}_2/\text{mg}/\text{h}$).

RESULTS

Skin Carcinogenesis Study. In the skin carcinogenesis study, deguelin mediated a statistically significant reduction in tumor incidences at the two dose levels examined. The tumor incidences were reduced from 60% in the carcinogen control group to 10 and 0% at the two respective deguelin doses of 33 ($P < 0.05$) and 330 μg ($P < 0.05$) that were examined (Fig. 2A). Tumor multiplicity was reduced from 4.2 ± 1.4 to 0.1 ± 0.07 and 0 tumors, at doses of 33 and 330 μg ,

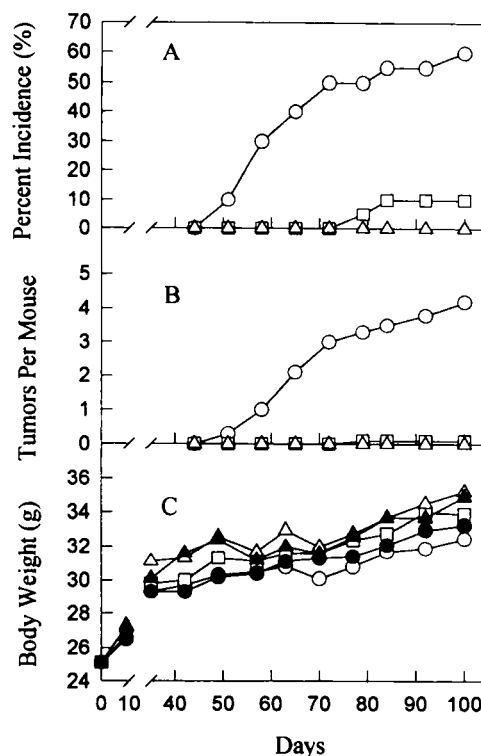


Fig. 2. The effect of deguelin on (A) the percentage of incidence of observable skin tumors, (B) the number of observable skin tumors/mouse, and (C) body weight (in grams) in female CD-1 mice receiving DMBA/TPA topically. The treatment groups were as follows: DMBA/TPA and deguelin vehicle (acetone), ○; DMBA/TPA and 33 μg of deguelin, □; DMBA/TPA and 330 μg of deguelin, △; vehicle/acetone and 330 μg of deguelin, ▲; and control, ●.

respectively (Fig. 2B). Survival was 100% for all groups, except groups 3 and 4, in which survival was 75 and 95%, respectively. Animal deaths that occurred in these two groups were accidental and were not related to deguelin. There were no significant differences in body weight among the animal groups (Fig. 2C).

Inhibition of Epidermal ODC Induction. As an initial attempt to assess a correlation between deguelin-mediated inhibition of skin tumorigenesis and a reduction of TPA-induced ODC activity, the influence of deguelin application time relative to treatment with TPA was examined. As anticipated, treatment of mice with 6.17 μg of TPA led to significant increases in ODC activity. In experiment 1 (Table 1), deguelin (384 μg) was applied either without TPA treatment or in a time interval spanning 2 h before to 2 h after TPA application. TPA application was defined as time 0. After an induction period of 5 h, the animals were killed, and ODC activity was determined. When deguelin was applied from 2 h before TPA to 40 min after TPA treatment, ODC induction was reduced by more than 90%. When deguelin was applied either 15 h before (experiment 2; Table 1) or 1 and 2 h posttreatment with TPA, activity was reduced by about 85% in comparison with that of the control.

Next, the effect of deguelin on the kinetics of ODC induction was determined using conditions corresponding to those used in the *in vivo* skin carcinogenesis model. Deguelin (33 μg) was applied 1 h before 1.17 μg of TPA, and ODC induction was determined over a period of 10 h. As illustrated in Fig. 3, deguelin reduced the maximum ODC induction observed after 5 h in the acetone-treated control group by 70% without affecting the kinetics of induction.

Finally, the potential of deguelin to inhibit TPA-induced ODC activity was measured in a dose-dependent manner. ODC activity was induced by treatment with 1.17 μg of TPA, various doses of deguelin were applied 1 h before treatment with TPA, and the experiment was terminated after 5 h. As summarized in Table 2, deguelin-mediated effects were clearly dose related, with significant inhibition of ODC induction in a treatment range of 1.65–330 μg . In good agreement with the results obtained in the two-stage mouse skin model, complete inhibition of TPA-induced ODC activity was observed at the highest concentration tested (330 μg), whereas 33 μg of deguelin reduced ODC induction by 75%.

Mammary Carcinogenesis Study. In the mammary carcinogenesis study, the first palpable tumors appeared at 56.3 ± 3.5 days in the control group. Treatment with deguelin at doses of 2 and 4 mg/kg of body weight significantly increased the latency period to 66.2 ± 6.2

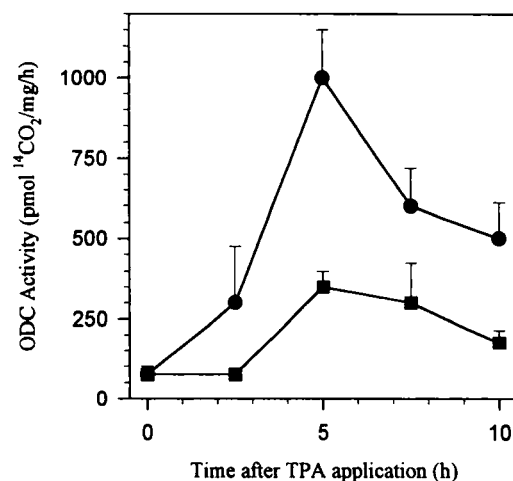


Fig. 3. Time course showing the inhibitory effect of deguelin on the induction of ODC activity in mouse skin after topical application of a single dose of TPA. Groups of mice were treated topically with either acetone (●; 0.2 ml) or deguelin (■; 33 μg in 0.2 ml of acetone) 1 h before treatment with TPA (1.17 μg in 0.2 ml of acetone). Treated mice were killed at the indicated times after TPA application (time 0), and ODC activity from total skin samples was determined as described in "Materials and Methods." Each value for ODC activity represents the mean \pm SD (bars) of separate duplicate determinations of enzyme activities from four mice.

and 85.8 ± 8.1 days, respectively. Tumor incidence was unaffected (Fig. 4A), but there was a significant reduction in tumor multiplicity from 6.8 ± 0.68 tumors/rat in the control group to 5.1 ± 0.52 and 3.2 ± 0.48 tumors/rat in the 2 and 4 mg/kg of body weight deguelin groups, respectively (Fig. 4B). These tumors were all classified as adenocarcinomas on gross examination. Survival in the vehicle and in the 2 and 4 mg/kg of body weight deguelin groups was 48, 40, and 64%, respectively. A dose-related lethargy and somnolence were observed in the deguelin-treated animals; otherwise, no toxicity was evident. There was no significant difference in body weight between the groups during the course of the study (Fig. 4C).

DISCUSSION

ODC inhibitors such as difluoromethylornithine have been shown to demonstrate chemopreventive activity (15). Using a model system with mouse epidermal 308 cells, we recently reported that deguelin inhibits TPA-induced ODC activity with an IC_{50} value of approxi-

Table 1 Influence of deguelin application time on TPA-induced ODC activity in mouse skin^a

Experiment	Treatment	TPA	Application time (Relative to TPA)	ODC activity (pmol ¹⁴ CO ₂ /mg/h)	% Inhibition ^b
1	Acetone	-	0	154 \pm 88	-
	Acetone	+	0	1615 \pm 391	-
	Deguelin	-	0	79 \pm 43	-
	Deguelin	+	-120 min	196 \pm 91 ^c	97
	Deguelin	+	-60 min	176 \pm 55 ^c	98
	Deguelin	+	-40 min	229 \pm 62 ^c	95
	Deguelin	+	-20 min	281 \pm 214 ^c	91
	Deguelin	+	0	138 \pm 27 ^c	101
	Deguelin	+	+20 min	204 \pm 98 ^c	97
	Deguelin	+	+40 min	285 \pm 59 ^c	91
	Deguelin	+	+60 min	378 \pm 135 ^c	85
	Deguelin	+	+120 min	379 \pm 138 ^c	85
	2	Acetone	-	0	36 \pm 7
Acetone		+	0	1292 \pm 346	-
Deguelin		+	-15 h	226 \pm 77 ^c	85

^a Female CD-1 mice ($n = 4$) were topically treated with deguelin (384 μg in 0.2 ml of acetone) at the time indicated relative to TPA application (6.17 μg in 0.2 ml of acetone; 0, simultaneous application). The animals were killed 5 h after TPA application, dorsal skin was removed, and ODC activity was determined (in duplicate) as described in "Materials and Methods."

^b After normalization for the untreated acetone control, the percentage of reduction of ODC activity was calculated in comparison with the TPA-treated acetone control.

^c Mean value statistically different from that of the control ($P < 0.0001$, Student's t test, $n = 4$).

Table 2 Dose-dependent inhibition of TPA-induced ODC activity in mouse skin^a

Treatment	TPA	Deguelin dose ($\mu\text{g}/\text{mouse}$)	ODC activity (pmol $^{14}\text{CO}_2/\text{mg}/\text{h}$)	% Inhibition ^b
Acetone	-	0	75 \pm 52	-
Acetone	+	0	1199 \pm 323	-
Deguelin	+	1.65	1148 \pm 174 ^c	5
Deguelin	+	8.25	958 \pm 375 ^c	21
Deguelin	+	33	328 \pm 113 ^c	76
Deguelin	+	165	139 \pm 63 ^c	94
Deguelin	+	330	65 \pm 37 ^c	101

^a Female CD-1 mice ($n = 4$) were topically treated with the indicated concentration of deguelin in (0.2 ml of acetone) 1 h before the induction of ODC by application of TPA (1.17 μg in 0.2 ml of acetone). The animals were killed 5 h after TPA treatment, dorsal skin was removed, and ODC activity was determined (in duplicate) as described in "Materials and Methods."

^b After normalization for the untreated acetone control, the percentage of reduction of ODC activity was calculated in comparison with the TPA-treated acetone control.

^c Mean value statistically different from that of the control ($P < 0.0001$, Student's t test, $n = 4$).

mately 10 ng/ml, whereas an IC_{50} value of approximately 0.18 $\mu\text{g}/\text{ml}$ was observed with the positive control compound, 13-*cis*-retinoic acid (10). This remarkably potent inhibition was mediated at a transcriptional level rather than direct inhibition of enzyme activity, as is the case with compounds such as difluoromethylornithine. As currently reported, consistent with the mouse 308 cell culture data, deguelin was highly effective in inhibiting TPA-induced mouse epidermal ODC activity. These data showed a strong correlation with the results obtained in the mouse two-stage skin carcinogenesis model. At a dose level of 33 μg , ODC induction was reduced by 75%, and tumor incidence decreased from 60 to 10%. Tumorigenesis as well as TPA-induced ODC activity was completely inhibited when the dosage was increased 10-fold to 330 μg . In addition, because the tumor incidence was reduced from 60% in the control group to 10% in the group treated with 33 μg of deguelin, it can be calculated by means of linear least squares analysis that a dosage of approximately 40 μg should be sufficient to completely inhibit tumorigenesis. Although this is a crude estimation, deguelin is certainly a very potent compound in this model system, and it is notable that no signs of overt toxicity were observed in these treatment groups.

As described previously, with a test concentration of 10 $\mu\text{g}/\text{ml}$, deguelin, tephrosin, 13 α -hydroxytephrosin, and 13 α -hydroxydeguelin inhibited the formation of DMBA-induced preneoplastic lesions with mouse mammary glands in organ culture. Deguelin demonstrated the highest activity, completely inhibiting the development of mammary lesions (10), and morphological examination of the glands revealed no signs of toxicity. These data, in conjunction with the unique mode of action and results obtained in the mouse skin study, led to an investigation of the chemopreventive potential of deguelin in the full-term MNU/rat mammary tumor model system. In this study, the first palpable tumors in the 2 mg/kg of body weight group appeared approximately 10 days after those in the carcinogen-treated control group, whereas tumors in rats receiving the 4 mg/kg of body weight dose appeared 30 days later. Overall survival was highest in the 4 mg/kg of body weight group. Thus, the 4 mg/kg of body weight dose seemed to be superior to the 2 mg/kg of body weight dose in terms of efficacy, but the 2-mg dose had a superior toxicity profile.

In chemoprevention settings, of course, the potential to mediate toxicity is of major concern. As illustrated in Fig. 4C, body weight was unaffected by treatment with deguelin. The major toxic response observed was neurological in nature and involved lethargy and somnolence only in the 4 mg/kg of body weight group. Although recovery was complete, this response profile discouraged additional dose escalation/efficacy studies with this agent. Nonetheless, using linear least squares analysis, it can be estimated that an oral dosage of approximately 12 mg/kg of body weight should completely inhibit

tumorigenesis. We are currently attempting to modify the structure of deguelin to achieve a higher chemopreventive index. However, as emphasized by Moon *et al.* (16), minor structural changes may enhance drug activity but invoke additional toxicity.

Another avenue worth exploring concerns combination chemopreventive therapy. Although still in its infancy, various reports have indicated efficacy with suboptimal doses of effective chemopreventive agents used in combination. For example, in a mammary carcinogenesis chemopreventive study, Rao *et al.* (17) demonstrated a reduction in tumor incidence from 64% in control animals to 8% with a combination of six chemopreventive agents (tamoxifen, tocopherol, retinyl acetate, aminoglutethimide, ergocryptine, and selenium). In other studies, Moon *et al.* (13), Ratko *et al.* (18), Thompson *et al.* (19), and Abou-Issa *et al.* (20) have shown the effects of combined retinoid and tamoxifen, difluoromethylornithine, or calcium glucarate to be additive or synergistic. On the other hand, as exemplified by a study conducted with antiestrogens, progesterone, and an aromatase inhibitor, aminoglutethimide, in the MNU-induced mammary carcinogenesis model, neither synergism nor additive effects were induced with any of the combinations tested, probably due to the fact that all of the agents used in the study mediated activity via a hormonally regulated sequence of events (21). Thus, as is the case with combination cancer chemotherapy, it is logical to construct combination regimens of cancer chemopreventive agents based on known mechanisms of action. Accordingly, it may be speculated that synergistic activity could be demonstrated by deguelin in combination with other agents that function independently of the ODC pathway, and suboptimal doses (as a single agent) may be suitable to enhance chemopreventive potential while reducing its toxicity. This approach warrants investigation in either mammary carcinogenesis or other tumor models.

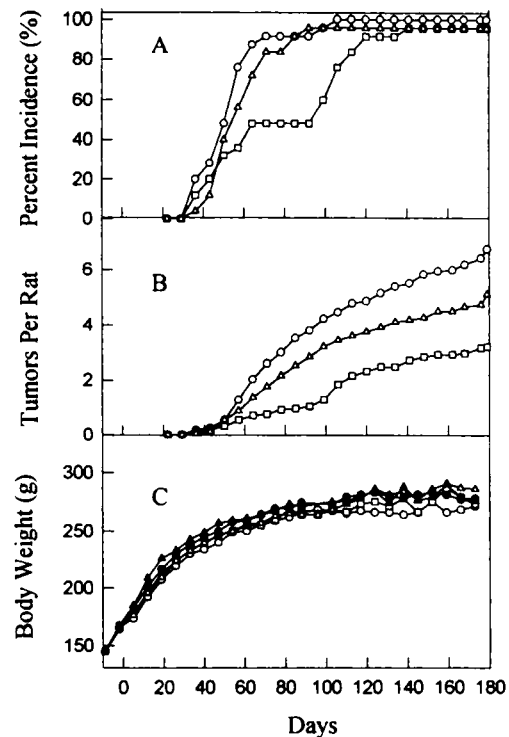


Fig. 4. The effect of i.g. administration of deguelin (5 days/week) on (A) the percentage of incidence of observed mammary tumors/rat, (B) the number of observed mammary tumors/rat, and (C) body weight (in grams) in female Sprague Dawley rats after a single dose of 50 mg/kg of body weight MNU administered i.v. The rat treatment groups were as follows: MNU and corn oil vehicle, \circ ; MNU and 2 mg/kg of body weight deguelin, Δ ; MNU and 4 mg/kg of body weight deguelin, \square ; saline and 4 mg/kg of body weight deguelin, \blacktriangle ; and saline and corn oil, \bullet .

As noted above, deguelin can mediate appreciable chemopreventive activity in the two model systems investigated, but efficacy may be somewhat limited by neurotoxicity. At the present time, it is not known if this neurotoxic response is species specific. Rotenoids have been shown to inhibit NADH dehydrogenase (22) and to inhibit microtubule assembly via binding to tubulin (23). In recent studies, we have found that antimetabolic compounds (podophyllotoxin, vinblastine, and colchicine) inhibit TPA-induced ODC activity with IC_{50} values in the nanogram/milliliter range, and NADH dehydrogenase inhibitors (asimicin and bullatacin) and most deguelin derivatives tested strongly inhibited TPA-induced ODC activity in mouse 308 cells and c-Myc-induced ODC activity in BALB/c MycER cells. Inhibition of c-Myc-induced ODC activity was subsequently found to correlate with intracellular ATP depletion via inhibition of NADH dehydrogenase (24, 25). These mechanistic findings are currently being exploited in studies designed to establish structure-activity relationships, again with the hope of developing an agent with a favorable therapeutic index. If inhibition of NADH dehydrogenase is integral to the chemopreventive mechanism, however, it is unlikely that a suitably innocuous structural derivative will be produced. On the other hand, it is reasonable to explore the use of deguelin as a cancer chemotherapeutic agent, because greater levels of toxicity may be tolerated. Chemotherapeutic studies are currently underway to explore this possibility, using athymic mice carrying human breast tumors.

ACKNOWLEDGMENTS

We thank Daniel Lantvit for expert technical assistance.

REFERENCES

- American Cancer Society. Cancer Facts and Figures 1997, pp. 1–32. Atlanta, GA, 1997.
- Lenhard, R. E. Cancer statistics: a measure of progress. *CA Cancer J. Clin.*, **46**: 3–4, 1996.
- Wattenberg, L. W. Chemoprevention of cancer by naturally occurring and synthetic compounds. *In*: L. Wattenberg, M. Lipkin, C. W. Boone, and G. J. Kelloff (eds.), *Cancer Chemoprevention*, pp. 19–39. Boca Raton, FL: CRC Press, Inc., 1992.
- Kelloff, G. J., Boone, C. W., Steele, V. E., Crowell, J. A., Lubet, R., and Sigman, C. C. Progress in cancer chemoprevention: perspectives on agent selection and short-term clinical intervention trials. *Cancer Res.*, **54** (Suppl.): 2015s–2024s, 1994.
- Kelloff, G. J., Crowell, J. A., Boone, C. W., Steele, V. E., Lubet, R. A., Greenwald, P., Alberts, D. S., Covey, J. M., Doody, L. A., Knapp, G. G., Nayfield, S., Parkinson, D. R., Prasad, V. K., Prorok, P. C., Sausville, E. A., and Sigman, C. C. Strategy and planning for chemopreventive drug development: clinical development plans. *J. Cell. Biochem.*, **20** (Suppl.): 55–299, 1994.
- Costa, A., Decensi, A., De Palo, G., and Veronesi, U. Breast cancer chemoprevention with retinoids and tamoxifen. *Proc. Am. Assoc. Cancer Res.*, **37**: 655–656, 1996.
- Jordan, V. C. (ed.). *Long-term Tamoxifen Treatment for Breast Cancer*, p. 320. Madison, WI: University of Wisconsin Press, 1994.
- Pezzuto, J. M. Cancer chemopreventive agents: from plant materials to clinical trials. *In*: A. D. Kinghorn and M. F. Balandrin (eds.), *Human Medical Agents from Plants*, pp. 205–217. ACS Symposium Series No. 534. Washington, DC: American Chemical Society Books, 1993.
- Pezzuto, J. M. Natural product cancer chemopreventive agents. *In*: J. T. Arnason, R. Mata, and J. T. Romeo (eds.), *Recent Advances in Phytochemistry: Phytochemistry of Medicinal Plants*, Vol. 29, pp. 19–45. New York: Plenum Publishing Corp., 1995.
- Gerhäuser, C., Mar, W., Lee, S. K., Suh, N., Luo, Y., Kosmeder, J. W., Luyengi, L., Fong, H. H. S., Kinghorn, A. D., Moriarty, R. M., Mehta, R. G., Constantinou, A., Moon, R. C., and Pezzuto, J. M. Rotenoids mediate potent cancer chemopreventive activity through transcriptional regulation of ornithine decarboxylase. *Nat. Med.*, **1**: 260–266, 1995.
- Gilmour, S. K., Verma, A. K., Madara, T., and O'Brien, T. G. Regulation of ornithine decarboxylase gene expression in mouse epidermis and epidermal tumors during two-state tumorigenesis. *Cancer Res.*, **47**: 1221–1225, 1987.
- Anzeveno, P. B. Rotenoid interconversion: synthesis of deguelin from rotenone. *J. Org. Chem.*, **44**: 2578–2580, 1979.
- Moon, R. C., Kelloff, G. J., Detrisac, C. J., Steele, V. E., and Sigman, C. C. Chemoprevention of MNU-induced mammary tumors in the mature rat by 4-HPR and tamoxifen. *Anticancer Res.*, **12**: 1147–1154, 1992.
- Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**: 248–254, 1976.
- Meyskens, F. L. Development of difluoromethylornithine as a chemoprevention agent for the management of colon cancer. *J. Cell. Biochem.*, **22** (Suppl.): 126–131, 1995.
- Moon, R. C., Mehta, R. G., and Rao, K. V. N. Retinoids and cancer in experimental animals. *In*: M. B. Sporn, A. B. Roberts, and D. S. Goodman (eds.), *The Retinoids: Biology, Chemistry and Medicine*, pp. 573–595. New York: Raven Press, Ltd., 1994.
- Rao, A. R., Hussain, S. P., Jannu, L. N., Kumari, M. V. R., and Aradhana, . Modulatory influences of tamoxifen, tocopherol, retinyl acetate, aminoglutethimide, ergocryptine, and selenium on DMBA-induced initiation of mammary carcinogenesis in rats. *Indian J. Exp. Biol.*, **28**: 409–416, 1990.
- Ratko, T. A., Detrisac, C. J., Dinger, N. M., Thomas, C. F., Kelloff, G. J., and Moon, R. C. Chemopreventive efficacy of combined retinoid and tamoxifen treatment following surgical excision of a primary mammary cancer in female rats. *Cancer Res.*, **49**: 4472–4476, 1989.
- Thompson, H. J., and Roman, A. M. Effects of D, L-2-difluoromethyl-ornithine and endocrine manipulation on their induction of mammary carcinogenesis by MNU. *Carcinogenesis (Lond.)*, **7**: 2003–2006, 1986.
- Abou-Issa, H., Koolemans-Beynen, A., Minton, J. P., and Webb, T. E. Synergistic interaction between 13-*cis*-retinoic acid and glucarate: activity against rat mammary tumor induction and MCF-7 cells. *Biochem. Biophys. Res. Commun.*, **163**: 1364–1369, 1989.
- Moon, R. C., Steele, V. E., Kelloff, G. J., Thomas, C. F., Detrisac, C. J., Mehta, R. G., and Lubet, R. A. Chemoprevention of MNU-induced mammary tumorigenesis by hormone response modifiers: toremifene, RU 16117, tamoxifen, aminoglutethimide, and progesterone. *Anticancer Res.*, **14**: 889–893, 1994.
- Burgos, J., and Redfeam, E. R. The inhibition of mitochondrial reduced NAD⁺ oxidation by rotenoids. *Biochim. Biophys. Acta*, **110**: 475–483, 1965.
- Marshall, L. E., and Himes, R. H. Rotenone inhibition of tubulin self-assembly. *Biochim. Biophys. Acta*, **543**: 590–594, 1978.
- Gerhäuser, C., Kosmeder, J., Lee, S. K., Moriarty, R. M., and Pezzuto, J. M. Mechanistic investigation of rotenoid-mediated cancer chemopreventive activity. *Proc. Am. Assoc. Cancer Res.*, **37**: 279, 1996.
- Gerhäuser, C., Lee, S. K., Kosmeder, J. W., Moriarty, R. M., Hamel, E., Mehta, R. G., Moon, R. C., and Pezzuto, J. M. Regulation of ornithine decarboxylase induction by deguelin, a natural product cancer chemopreventive agent. *Cancer Res.*, **57**: 3429–3435, 1997.
- Udeani, G. O., Thomas, C. F., Kosmeder, J., Moriarty, R. M., Pezzuto, J. M., and Moon, R. C. Deguelin: a natural product for chemoprevention. *Proc. Am. Assoc. Cancer Res.*, **37**: 275, 1996.