Molecular Nutrition & Food Research
www.mnf-journal.com

Effects of a Grapevine Shoot Extract Containing Resveratrol and Resveratrol Oligomers on Intestinal Adenoma Development in Mice: In Vitro and In Vivo Studies

Michael T. Empl, Hong Cai, Shan Wang, Johannes Junginger, Tina Kostka, Marion Hewicker-Trautwein, Karen Brown, Andreas J. Gescher, and Pablo Steinberg*

Scope: Evidence suggests that the dietary consumption of plant extracts containing polyphenols might help prevent the onset of cancers of the gastrointestinal tract. In the present study, the chemopreventive and antiproliferative efficacy of a grapevine shoot extract (Vineatrol®30) containing resveratrol and resveratrol oligomers is investigated in vivo and in vitro.
Methods and results: The in vivo study is performed using *Apc^{Min}* mice on a high-fat diet, which represents a model of human adenomatous polyposis, while the potential of the extract as well as some of its isolated constituents to inhibit intestinal adenoma cell proliferation in vitro is investigated using APC10.1 cells derived from an *Apc^{Min}* mouse. Vineatrol®30 at a low (2.3 mg kg^{-1} diet) or high dose (476 mg kg^{-1} diet) reduces the adenoma number in male and adenoma volume in female animals. Furthermore, Vineatrol®30 as well as resveratrol and two resveratrol tetramers compromise the expansion of APC10.1 cells by reducing cell number, inducing cell cycle arrest, cellular senescence, and apoptosis. However, except for the extract, none of the isolated resveratrol oligomers is more efficacious than resveratrol in these cells.
Conclusion: Vineatrol®30 may merit further investigation as a potential dietary gastrointestinal cancer chemopreventive agent in humans.

1. Introduction

The feasibility of preventing the onset of gastrointestinal tumors pharmacologically is a topic of considerable research activity, especially in the light of the high incidence of these malignancies (≈1.4 million new cases worldwide in 2012[1]). Over the past 20 years, encouraging evidence has emerged which suggests that the daily consumption of anti-inflammatory drugs such as aspirin may be an effective strategy for the chemoprevention of human gastrointestinal tract neoplasms (reviewed in [2]). As aspirin, even at low doses, exerts a number of adverse effects,[3] the search for safer, well-tolerated strategies that match or even surpass the chemopreventive efficacy of this drug continues. Since it has long been known that colorectal cancer development can be influenced by diet and foodstuffs,[4,5] modifying dietary habits, or consuming foodstuffs with putative health-promoting properties could constitute a rational strategy to prevent this and other (chronic) diseases.[6,7] Recently, resveratrol, which occurs in low concentrations in grapes and red wine,[8] was shown to interfere with the development of adenomas in *Apc^{Min}* mice on a high-fat diet at a low dose equivalent to that contained in a couple of glasses of red wine in humans.[9] *Apc^{Min}* mice, which harbor a mutation in the *Apc* gene,[10] are a model of human adenomatous polyposis and frequently used "to study the effect of dietary agents on colorectal cancer".[11]

There is tentative evidence to suggest that extracts of grapevine shoots such as the commercially available grapevine shoot extract Vineatrol®30, which contains resveratrol and a series of resveratrol oligomers, exert beneficial effects on human health.[12–19] Most notably, Vineatrol®30 has been shown to reduce the number of malignantly transformed foci in BALB/c 3T3 mouse fibroblasts treated with the tumor initiator 3-methylcholanthrene and the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate.[20] This finding suggests that this extract may be capable of interfering with the process of carcinogenesis.

The aim of the present study was twofold: First, we wished to explore whether Vineatrol®30 affects adenoma development in

Dr. M. T. Empl, Dr. S. Wang[+], T. Kostka, Prof. P. Steinberg[++]
Institute for Food Toxicology
University of Veterinary Medicine Hannover
Hannover, Germany
E-mail: michael.empl@tiho-hannover.de
Dr. H. Cai, Prof. K. Brown, Prof. A. J. Gescher
Department of Cancer Studies
Leicester Royal Infirmary
University of Leicester
Leicester, United Kingdom
Dr. J. Junginger, Prof. M. Hewicker-Trautwein
Department of Pathology
University of Veterinary Medicine Hannover
Hannover, Germany

[+]Current address: College of Animal Science and Veterinary Medicine, Henan Institute of Science and Technology, Xinxiang, P.R. China
[++]Current address: Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Karlsruhe, Germany

DOI: 10.1002/mnfr.201700450

Table 1. Experimental groups, number of animals, and diets used in the present study.

Group	No. of female animals	No. of male animals	Diet (abbreviation)[a)]	Fat content [%]	Vineatrol®30 content [mg kg^{-1} diet]
1	11	10	AIN-93G (**NF**)	7.1 ("normal")	0
2	7	7	modified AIN-93G (**HF**)	35 ("high-fat")	0
3	10	9	modified AIN-93G (**LD**)	35 ("high-fat")	2.3
4	6	9	modified AIN-93G (**HD**)	35 ("high-fat")	476

a) All diets were supplied by Purina TestDiet® (Richmond, VA, USA).

the *Apc*^{Min} mouse model in a fashion commensurate with that observed for resveratrol.[9] Second, the hypothesis was tested that the grapevine shoot extract or its isolated constituents r2-viniferin and hopeaphenol can rival resveratrol in terms of ability to compromise adenoma cell viability and proliferation in vitro, and if potential effects of these constituents can explain any efficacy of Vineatrol®30 observed in vivo. To that end, APC1.10 cells, originally derived from *Apc*^{Min} mice,[21] were exposed to the extract, r2-viniferin, hopeaphenol, or resveratrol, and the effects of these agents on cell number, cell cycle distribution, and propensity of cells to undergo apoptosis were investigated. Overall, the study was conceived to determine whether Vineatrol®30 should be taken into account when developing new strategies for the chemoprevention of gastrointestinal malignancies.

2. Experimental Section

2.1. Chemicals and Reagents

Vineatrol®30 was kindly provided by the German distributor Breko (Bremen, Germany) in conjunction with the manufacturer Actichem (Montauban, France). The latter also provided hopeaphenol (≥95% purity). r2-Viniferin (≥ 95% purity) was isolated and purified by the group of Prof P. Winterhalter (Institute of Food Chemistry, Technical University of Braunschweig, Germany), while resveratrol and nocodazole (both ≥99% purity) were purchased from Sigma–Aldrich (Schnelldorf, Germany). All test substances were dissolved in DMSO. The composition of the Vineatrol®30 stock used in the present study has previously been published[18,22] and comprises a total of 37.1% resveratrol and resveratrol oligomers (7.7% *trans*-resveratrol, 0.6% *trans*-piceatannol, 3.4% ampelopsin A, 14.6% ε-viniferin, 2.4% iso-*trans*-ε-viniferin, 2.5% miyabenol C, 2.5% r-viniferin, 1.6% r2-viniferin, and 1.8% hopeaphenol). The composition of the remaining 62.9% of the extract remain presently unknown.[18]

2.2. Assessment of the Effect of Vineatrol®30 In Vivo

The potential chemopreventive efficacy of Vineatrol®30 was investigated using the *Apc*^{Min} mouse model. The animal breeding as well as the experimental design and procedure of this exploratory study were based on previous work investigating the effects of resveratrol in *Apc*^{Min} mice.[9,23] The whole experiment was strictly carried out under the Animals (Scientific Procedures) Act 1986 with project license 80/2167 granted to Leicester University by the United Kingdom Home Office. Briefly, weaning offspring mice of both sexes were randomly allocated at 4 weeks of age to one of four study groups. Then, after a short adaptation phase, each group received a different test diet (control normal fat content [NF], control high fat content [HF], high fat content + 2.3 mg Vineatrol®30 kg^{-1} diet [LD], high fat content + 476 mg Vineatrol®30 kg^{-1} diet [HD]) as described in **Table 1**. Since resveratrol has been shown to exert potent inhibitory activity in *Apc*^{Min} mice fed a high-fat diet,[9] the effects of the grapevine shoot extract were studied under similar conditions. The rationale for choosing these extract doses was to approximately emulate the amounts of resveratrol used in a previous study.[9] Moreover, it was based on the fact that Vineatrol®30 roughly consists of 30% resveratrol and resveratrol-related species and that the resveratrol doses used in the above-mentioned prior study were a dietary relevant 0.7 mg kg^{-1} diet and a 200-fold higher dose (translating to 2.3 and 476 mg Vineatrol®30 kg^{-1} diet, respectively). After 9–10 weeks of receiving the different diets, the animals were euthanized by cardiac puncture and exsanguination under terminal anaesthesia with isoflurane (4 vol%). The number and size of adenomas was determined and other samples and parameters were taken/recorded as previously published.[9,23] In addition, haematoxylin and eosin-stained sections of gut samples of selected female and male mice from each group were subjected to histopathological analysis.

2.3. Cell Culture

APC10.1 cells[21] were a kind gift from Prof C. De Giovanni as well as Dr L. Landuzzi (both Cancer Research Section, University of Bologna, Bologna, Italy) and were cultivated under standard cell culture conditions (37 °C, 95% rel. humidity, 5% CO_2) using DMEM (Biochrom, Berlin, Germany) supplemented with fetal bovine serum (20%; Biochrom) and L-glutamine (2 mM; Biochrom).

2.4. Assessment of Cytotoxicity

Cytotoxicity (i.e., changes in cell number/mass) was assessed by means of the sulforhodamine B (SRB) assay, which was performed as previously described, with slight variations.[24] Briefly, 1000 APC10.1 cells were seeded in 96-well plates and treated once with the solvent (0.1% DMSO) or the test compounds for 48, 72, and 120 h. In order to mimic the animal's daily Vineatrol®30 consumption during the in vivo experiment, the APC10.1 cells were also treated with the above-mentioned compounds on a daily basis for 48, 72, and 120 h. Finally, the absorption was recorded at

492 nm using a plate reader (Infinite® 200; Tecan, Crailsheim, Germany).

2.5. Cell Cycle Analysis

The cell cycle analysis was mostly performed as described beforehand.[24] Cells (0.5×10^6) were seeded on 10 cm dishes and treated with the different test substances for 24, 48, and 72 h. After each incubation time point, APC10.1 cells were collected, fixed in ethanol (70%) and stored at 4 °C until analysis. Samples to be analyzed were subsequently incubated with ribonuclease A (50 μg mL^{-1}) and propidium iodide (50 μg mL^{-1}), before data were collected (20 000 events per sample) on an Accuri C6 flow cytometer (BD Biosciences, Heidelberg, Germany). Finally, the analysis of the cell cycle distribution of resveratrol-, Vineatrol® 30-, or resveratrol oligomer-treated APC10.1 cells was performed using FlowJo (version 7.6.5; FlowJo, Ashland, OR, USA).

2.6. Assessment of Apoptosis, Necrosis, and Cellular Senescence

The putative induction of apoptosis and necrosis was investigated as previously reported[25] using commercially available kits (apoptosis: "Caspase-Glo® 3/7 Assay"; necrosis/direct cytotoxicity: "CytoTox-ONE™ Homogeneous Membrane Integrity Assay") from Promega (Mannheim, Germany). In short, 5000 (for the apoptosis assay) or 20,000 APC10.1 cells (for the necrosis assay) were seeded in 96-well plates and treated with the solvent (0.1% DMSO) as well as the test substances for 48 (apoptosis) or 6 h (necrosis). Then, either luminescence (apoptosis) or fluorescence (necrosis; excitation/emission wavelengths: 560/590 nm) was recorded by means of an Infinite® 200 (Tecan) plate reader. The possible induction of cellular senescence was assessed by using the "96-Well Cellular Senescence Assay Kit" (Cell Biolabs, San Diego, USA) according to the manufacturer's instructions. Briefly, APC10.1 cells were plated at a density of 1000 cells per well in a 96-well plate and treated on a daily basis with either the solvent control (0.1% DMSO) or the test compounds in duplicate for five consecutive days. Following this, the cells were lysed for 1 h at 4 °C using the kit-supplied lysis buffer supplemented with 1 mM PMSF, and the samples stored at –80 °C. Afterwards, the samples, presumably containing the so-called senescence-associated ß-galactosidase (SA β-gal[26]), were incubated with a substrate for that enzyme for 3 h and fluorescence quantified using the above-mentioned plate reader (excitation/emission wavelengths: 360/465 nm). The protein content of each individual well was recorded in parallel using the "Pierce™ BCA Protein Assay Kit" (Thermo Fisher Scientific, Schwerte, Germany) and its fluorescence normalized to its protein content. Finally, the SA β-gal activity of the normalized samples was expressed as fold change in relation to the solvent control.

2.7. Statistical Analysis

All data were statistically examined using Prism (version 6.04; GraphPad, La Jolla, CA, USA), with details being indicated in the legend of each figure. The normality of the larger in vivo data set was investigated by means of the D'Agostino & Pearson omnibus test, the Shapiro–Wilk test, and the Kolmogorov–Smirnov test, with the Shapiro–Wilk test being decisive when $n \geq 7$. The significance level (α) was constantly set to 0.05 for all analyses.

3. Results

3.1. Effects of Vineatrol® 30 on Intestinal Adenomagenesis in *Apc^{Min}* Mice

As depicted in **Figure 1**, the HF diet alone increased both the adenoma number and the adenoma volume in the small intestine of mice two- to threefold, regardless of the gender, when compared to mice on the NF diet. Unlike in animals which received the HF diet only, intervention with Vineatrol® 30 decreased small intestinal adenoma development in a gender-dependent manner (Figure 1). In male mice, the LD and HD diets decreased the number of adenomas significantly by ≈46 and 53%, respectively, but failed to affect total adenoma volume (Figure 1). In contrast, both dose regimes diminished the total adenoma volume significantly by ≈55% without affecting adenoma number in female mice (Figure 1). The histopathological analysis verified the nodular lesions in the intestine of selected animals as being intestinal adenomas according to current guidelines.[27] While the macroscopically determined parameters (i.e., adenoma number and volume) markedly differ between groups, microscopical assessment of gut samples did not show significant differences between study groups regarding cellular pleomorphism, atypia, dysplasia, nuclear morphology, or growth pattern. Though Vineatrol® 30 had no significant effect on the adenoma number or volume in the colon (Supporting Information Figure S1) as well as on the body weight of mice in comparison to those which received the HF diet only (Supporting Information Figure S2), animals which received the HF diet with or without the extract presented with diminished hematocrit values and increased weights of spleen and liver (Supporting Information Figure S3). While the differences in liver weight were gender-independent, those with respect to hematocrit and spleen weight were significant only in female mice (Supporting Information Figure S3).

3.2. Effects of Vineatrol® 30 and Selected Constituents on Intestinal Adenoma Cells In Vitro

In the light of its activity in *Apc^{Min}* mice in vivo, we explored potential direct effects of Vineatrol® 30 and three of its constituents (resveratrol, r2-viniferin, and hopeaphenol) on intestinal adenoma cells in vitro in terms of cell growth, cell cycle distribution, apoptogenicity, and direct cytotoxicity (necrosis). APC10.1 cells were exposed to the agents on a repeated daily administration schedule, and cell numbers were assessed after 120 h (**Table 2**). Agents decreased cell mass in a concentration-dependent manner, and the IC_{50} value for resveratrol under these conditions was 3.73 μg mL^{-1} (Table 2). The equivalent values for Vineatrol® 30 were approximately fourfold, and those for r2-viniferin and hopeaphenol approximately eightfold higher (Table 2). In molar

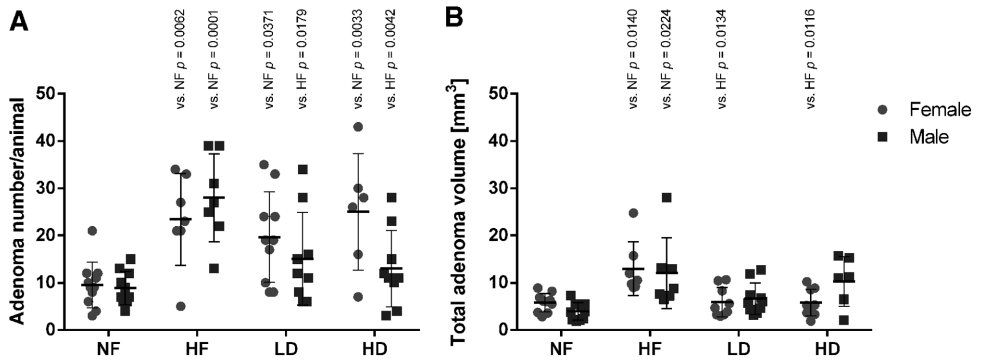

Figure 1. Effect of a normal-fat (**NF**) and a high-fat (**HF**) diet as well as a low (**LD**; 0.23 mg kg^{-1} diet) and high dose (**HD**; 476 mg kg^{-1} diet) of Vineatrol® 30 on the adenoma number (A) and the total adenoma volume (B) in the small intestine of female and male *Apc^{Min}* mice. Shown is the value of each animal as well as the mean ± SD. The animals received the grapevine shoot extract for 9–10 weeks. The data were either analyzed using a two-way ANOVA followed by Tukey's post-hoc test (A: all groups compared with each other for each gender separately) or a Kruskal–Wallis test followed by Dunn's post-hoc test (B: all groups compared with each other for each gender separately). The *p*-values indicate statistical significance in comparison to the group mentioned above each plot. The adenoma volume of one male mouse in the NF group (B) was 76.6 mm^3, which was removed from the figure for visibility reasons. Consequently, the mean and SD depicted are slightly altered, although this animal was included in the actual statistical analysis.

Table 2. Absolute IC_{50} values determined in APC10.1 cells using the SRB assay after daily administration of the test compounds for 120 h.

Test compound	IC_{50} [μg mL^{-1} (μM)]	95% CI [μg mL^{-1} (μM)]
Resveratrol	**3.73** (16.19)	**2.90–4.79** (12.56–20.87)
Vineatrol® 30	**15.00**	**11.87–18.95**
r2-Viniferin	**31.24** (34.44)	**23.35–41.78** (25.75–46.07)
Hopeaphenol	**29.42** (32.44)	**26.21–33.02** (28.90–36.41)

terms, this translates into approximately twofold higher IC_{50} values for r2-viniferin and hopeaphenol when compared to resveratrol (Table 2). When agents were added to the cellular incubates only once rather than on a repeated dose schedule as well as for lower incubation times, inhibition tended to be markedly weaker, except in the case of resveratrol (Supporting Information Figure S4 and Supporting Information Table S1).

Vineatrol® 30, resveratrol, and hopeaphenol affected the cell cycle distribution of APC10.1 cells in a fashion which was vaguely similar between agents (**Figure 2**). Resveratrol at 50 and 100 μM caused a significant accumulation of cells in the sub-G_1 fraction after 24, 48, and 72 h as well as a slight arrest in the G_1 phase after 24 h, while resveratrol at 10 μM significantly arrested the cells in the S phase after 24 and 72 h (Figure 2A1–A3). Vineatrol® 30 at 23 μg mL^{-1} caused a significant accumulation of the cells in the S phase after 24 and 72 h, thereby mimicking the effect of resveratrol at 10 μM (Figure 2B1–B3). Hopeaphenol at 50 μM also induced an S phase arrest after 72 h, whereas at 100 μM it caused a significant accumulation of cells in the sub-G_1 fraction after 24, 48, and 72 h as well as a G_1 arrest after 24 h (Figure 2C1–C3). In contrast, r2-viniferin hardly altered cell cycle distribution; it only induced a slight G_1 arrest at 50 μM after 24 and 48 h (Supporting Information Figure S5).

While Vineatrol® 30 and resveratrol at concentrations in the 10^{-5}–10^{-4} M range failed to increase the activity of the caspases 3 and 7 in the cellular incubate, treatment with 40 μM hopeaphenol led to a significant increase of caspase activity (Supporting Information Figure S6). In contrast, the activity of the SA β-gal was only significantly increased after incubation of the APC10.1 cells with 10 μM resveratrol, whereas none of the agents under study exerted direct cytotoxic/necrotic effects, as reflected by a lack of increase in LDH activity in the cell culture supernatant (Supporting Information Figure S6).

4. Discussion

The results presented herein show for the first time that a grapevine shoot extract can interfere with adenoma development in *Apc^{Min}* mice fed a high-fat diet. However, the efficacy of Vineatrol® 30 at 2.3 mg kg^{-1} diet containing ≈0.18 mg resveratrol kg^{-1} diet, failed to show the intriguing nonlinear dose-effect relationship recently described for resveratrol at 0.7 mg kg^{-1} diet.[9] Instead, the two doses of Vineatrol® 30 administered in the present study were similarly efficacious, a dose-effect scenario which suggests the possibility that the grapevine shoot extract may interfere with adenomatogenesis in *Apc^{Min}* mice at doses even below 2.3 mg kg^{-1} diet. The discrepancy in dose-dependency of efficacy between Vineatrol® 30 and pure resveratrol[9] indicates that the mechanisms by which adenoma growth is compromised differ between the two interventions. This conclusion is further supported by the dichotomy in efficacy exerted by the extract between male and female mice. In the former, it only affected adenoma number, while in the latter it only reduced the adenoma volume, and this gender difference was not seen with resveratrol.[9] In addition, resveratrol clearly reduced the proliferative index of intestinal adenomas,[9] while results regarding expression of Ki-67, reflecting the cellular proliferation rate, were inconclusive in the present work (data not shown). An apparent gender difference was also observed regarding the hematocrit and spleen weight. That being said, a diminished hematocrit and an increased spleen as well as liver weight are expected outcomes in *Apc^{Min}* mice fed a high-fat diet.[28–30] There are a few studies describing gender-dependent effects on adenoma development in mice carrying a mutation in the *APC* gene, which are, for example, related to factors such as physical exercise or genotype.[31–33] Nevertheless, the specific reason for the observation that

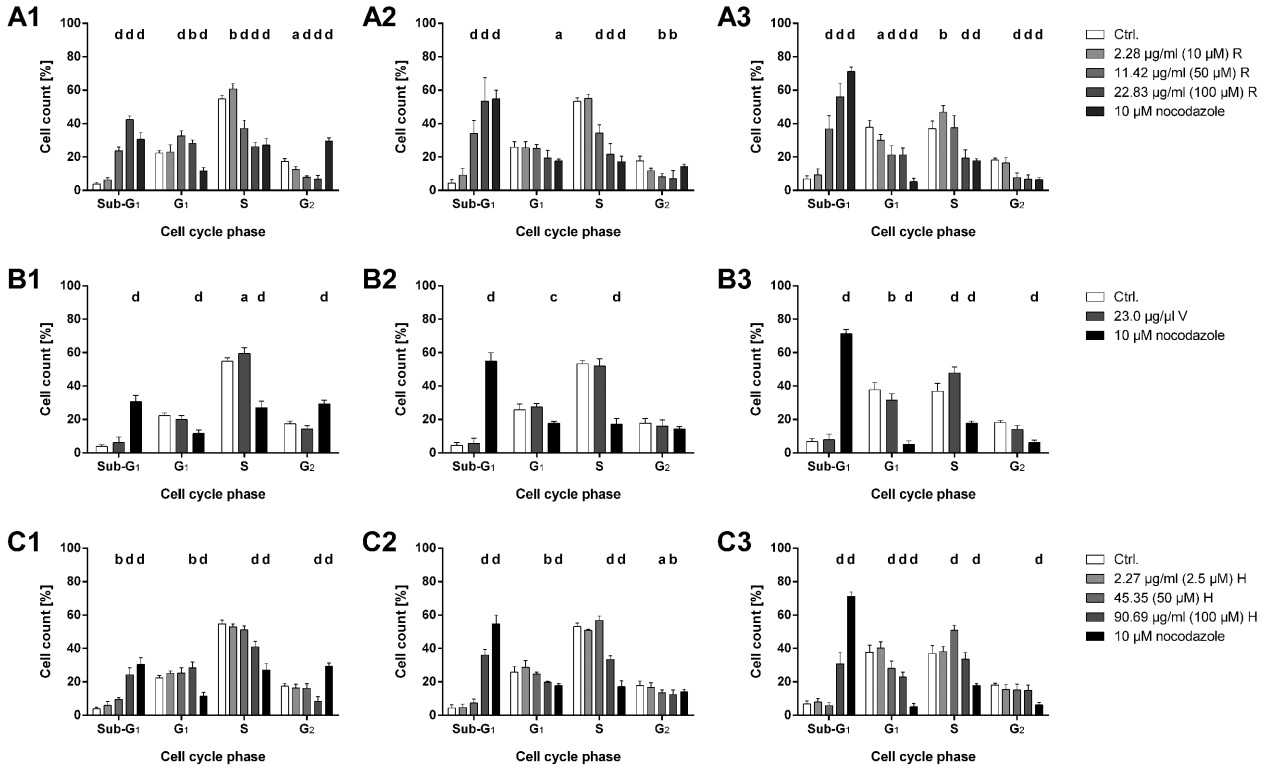

Figure 2. Effect of resveratrol (**R**; **A**), Vineatrol®30 (**V**; **B**), and hopeaphenol (**H**; **C**) on the cell cycle distribution of APC10.1 cells after 24 (A1, B1, and C1), 48 (A2, B2, and C2), and 72 h (A3, B3, and C3) of incubation. Shown is the mean ± SD of five independent experiments per experimental group. The data of the solvent (ctrl.; 0.1% DMSO) and positive controls (10 μM nocodazole) are identical for each test substance. The data were analyzed using a two-way ANOVA followed by Dunnett's post-hoc test (each test substance concentration was tested against the corresponding solvent control [0.1% DMSO] for each cell cycle phase and time point separately); a, $p \leq 0.05$; b, $p \leq 0.01$; c, $p \leq 0.001$; and d, $p \leq 0.0001$.

Vineatrol®30 is more effective in male animals remains elusive. A hypothetical mechanism could involve the reduction of testosterone production or body levels by Vineatrol®30 constituents, as the elimination of this androgen in castrated *Apc*$^{Pirc/+}$ rats and azoxymethane-treated C57BL/6J mice has been shown to suppress adenomagenesis in the colon.[34] In addition, testosterone is being discussed as a possible colorectal cancer-promoting factor in humans.[35] However, as is often the case, data on the impact of resveratrol or other stilbenoid polyphenols on testosterone production or levels are scarce and quite divergent. On the one hand, resveratrol as well as some of its analogs inhibit testosterone production in primary rat Leydig cells[36] or TRAP rats,[37] while, on the other hand, it enhances serum testosterone levels in Sprague–Dawley rats.[38] Moreover, several other non-stilbenoid polyphenols or phenolic extracts have been identified as inhibitors of testosterone production in vitro[39,40] and in vivo.[41,42]

To gain preliminary information as to which of the constituents of Vineatrol®30 may contribute to its activity in the *Apc*^{Min} mouse model, the consequences of exposure to the extract or constituents for cell growth, cell cycle distribution, apoptogenesis, and induction of cellular senescence were investigated in APC10.1 cells. The concentrations of hopeaphenol, r2-viniferin, or resveratrol required to alter cell behavior (i.e., the induction of a cell cycle arrest, apoptosis, or cellular senescence) were in the 10^{-5}–10^{-4} M range. These are considerably higher than the concentrations at which these agents are likely to occur at the adenoma target site after administration of a dose of 2.3 mg Vineatrol®30 kg^{-1} diet, which comprises ≈0.04 mg r2-viniferin and hopeaphenol as well as 0.18 mg resveratrol. Analytical chemical studies on levels of Vineatrol®30 constituents in murine organs after oral administration of the extract have thus far not been performed. However, the assumption that the administration of a low dose of Vineatrol®30 most probably will lead to vanishingly small concentrations of its constituents in the gastrointestinal tract is speculatively illustrated by an earlier study in which *Apc*^{Min} mice received resveratrol; after a daily dose of 2000 mg kg^{-1} diet, levels of parent compound recovered from gut tissue were ≈36 ± 48 nmol g^{-1} tissue.[43] At a dose which is about 1/1000 of this, gastrointestinal levels of Vineatrol®30 constituents should not exceed 100 pmol g^{-1} tissue (translating into 10^{-7} M in concentration terms). It is therefore doubtful whether the effects exerted by the extract in the murine gastrointestinal tract in vivo can be adequately modeled in the conventional short-term cell culture paradigm. Nevertheless, cautious interpretation of the results obtained in vitro militates against the notion that resveratrol oligomers, exemplified by hopeaphenol and r2-viniferin, exert direct effects on APC10.1 cell development which are dramatically different to or stronger than those elicited by resveratrol. Additional preliminary experiments using ε-viniferin (data not shown), another resveratrol oligomer present in Vineatrol®30,

are consistent with this inference although one cannot exclude the possibility that grapevine shoot constituents other than those investigated in the present study may have a superior effect. Also, it appears that the cytotoxic efficacy of r2-viniferin and hopeaphenol is dependent on the cellular model used. For example, in canine glioblastoma cells, the IC_{50} values of both tetramers were approximately ten times lower than those of resveratrol.[18] The difference in susceptibility to the above-mentioned compounds may be related to differences in cellular uptake or to other unknown factors. Evidence supporting the former idea has recently been acquired in Caco-2 cells, which are, like APC10.1 cells, of intestinal origin; while resveratrol passed a tight cellular monolayer, the resveratrol oligomers ε-viniferin and hopeaphenol did not.[44] Additionally, the highest concentration of Vineatrol®30 used in the present study, containing ≈7.7 μM resveratrol,[18] inhibited the growth of the APC10.1 cells by ≈66%, while resveratrol at 10 μM reduced cell numbers by only ≈52% after daily administration for 120 h. This significant difference (23.0 μg mL^{-1} Vineatrol®30 versus 10 μM resveratrol; $p = 0.0123$, calculated using an unpaired *t*-test with Welch's correction) implies that the resveratrol content of the extract is probably not solely responsible for the observed inhibition of cell proliferation, a finding observed before in other tumor cell types.[18,45,46]

In conclusion, the inhibitory effect of the grapevine shoot extract Vineatrol®30 on adenoma development observed in *Apc^{Min}* mice suggests that this extract may merit further investigation aimed at the exploration of its potential as an adenoma growth-retarding intervention in humans. The resveratrol tetramers hopeaphenol and r2-viniferin may contribute to the overall efficacy of Vineatrol®30, although it is unlikely that their contribution is superior to that of resveratrol, at least in the cellular model used in the present study.

Abbreviations

HD, high fat content diet + 476 mg Vineatrol®30 kg^{-1}; HF, control high fat content diet; LD, high fat content diet + 2.3 mg Vineatrol®30 kg^{-1}; NF, control normal fat content diet; SRB, sulforhodamine B

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgments

M.T.E. conceived, designed, and performed (SA β-gal activity assay) the in vitro experiments, helped in the design and performance of the in vivo animal experiment, analyzed all the data, and wrote the manuscript. H.C. conceived and performed the in vivo animal experiment, while J.J. and M.H.T. conceived and performed the histopathological analysis. S.W. and T.K. performed the remaining in vitro experiments. K.B. and A.J.G. conceived and designed the in vivo animal experiment and the latter contributed to the writing of the manuscript. P.S. oversaw all parts of the study. All authors critically revised the manuscript draft and approved the final submitted version. The authors wish to thank Julia Hausmann and Jutta Barras-Akhnoukh for the performance of the caspase 3/7 assay, Laura Menke for help in the performance of the SA β-gal activity assay as well as Prof H.-J. Schuberth (Immunology Unit, University of Veterinary Medicine Hannover, Germany) for letting us use the flow cytometer. In addition, the authors would also like to thank Prof C. De Giovanni as well as Dr L. Landuzzi (both Cancer Research Section, University of Bologna, Bologna, Italy) for the provision of the APC10.1 cells. The authors also gratefully acknowledge the funding support of the German Federal Ministry of Education and Research (grant no. 0315373C to P.S.), Cancer Research UK (grant no. C325/A13101 to K.B.), and the China Scholarship Council (grant no. 201206170045 to S.W.).

Conflict of Interest

The authors declare no competing financial and personal interests.

Keywords

Apc^{Min} mouse, chemoprevention, grapevine shoot extract, resveratrol, resveratrol oligomers

Received: May 24, 2017
Revised: October 10, 2017

[1] M. Ervik, F. Lam, J. Ferlay, L. Mery, I. Soerjomataram, F. Bray, Cancer Today. *Lyon, France: International Agency for Research on Cancer. Cancer Today* 2016. Available from: http://gco.iarc.fr/today; (accessed June 2016).
[2] D. A. Drew, Y. Cao, A. T. Chan, *Nat. Rev. Cancer* **2016**, *16*, 173.
[3] E. H. Awtry, J. Loscalzo, *Circulation* **2000**, *101*, 1206.
[4] Z. Chen, P. P. Wang, J. Woodrow, Y. Zhu, B. Roebothan, J. R. McLaughlin, P. S. Parfrey, *Nutr. J.* **2015**, *14*, 8.
[5] P. Czadek, *Pol. Ann. Med.* **2016**, *23*, 75.
[6] I. Lenoir-Wijnkoop, P. J. Jones, R. Uauy, L. Segal, J. Milner, *Br. J. Nutr.* **2013**, *109*, 1.
[7] J. Vanamala, *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 2573.
[8] V. Neveu, J. Perez-Jimenez, F. Vos, V. Crespy, L. du Chaffaut, L. Mennen, C. Knox, R. Eisner, J. Cruz, D. Wishart, A. Scalbert, *Database (Oxford)* **2010**, *2010*, bap024.
[9] H. Cai, E. Scott, A. Kholghi, C. Andreadi, A. Rufini, A. Karmokar, R. G. Britton, E. Horner-Glister, P. Greaves, D. Jawad, M. James, L. Howells, T. Ognibene, M. Malfatti, C. Goldring, N. Kitteringham, J. Walsh, M. Viskaduraki, K. West, A. Miller, D. Hemingway, W. P. Steward, A. J. Gescher, K. Brown, *Sci. Transl. Med.* **2015**, *7*, 298ra117.
[10] L. K. Su, K. W. Kinzler, B. Vogelstein, A. C. Preisinger, A. R. Moser, C. Luongo, K. A. Gould, W. F. Dove, *Science* **1992**, *256*, 668.
[11] D. E. Corpet, F. Pierre, *Cancer Epidemiol. Biomarkers Prev.* **2003**, *12*, 391.
[12] Y. K. Gupta, S. Briyal, *Eur. Neuropsychopharmacol.* **2006**, *16*, 85.
[13] S. Briyal, U. Sharma, N. R. Jagannathan, Y. K. Gupta, *Methods Find. Exp. Clin. Pharmacol.* **2009**, *31*, 505.
[14] C. Müller, K. Ullmann, A. Wilkens, P. Winterhalter, S. Toyokuni, P. Steinberg, *Biosci. Biotechnol. Biochem.* **2009**, *73*, 1831.
[15] L. Brizuela, A. Dayon, N. Doumerc, I. Ader, M. Golzio, J. C. Izard, Y. Hara, B. Malavaud, O. Cuvillier, *FASEB J.* **2010**, *24*, 3882.
[16] C. Romain, S. Gaillet, J. Carillon, J. Vide, J. Ramos, J. C. Izard, J. P. Cristol, J. M. Rouanet, *J. Agric. Food Chem.* **2012**, *60*, 11029.
[17] N. Chaher, K. Arraki, E. Dillinseger, H. Temsamani, S. Bernillon, E. Pedrot, J. C. Delaunay, J. M. Merillon, J. P. Monti, J. C. Izard, D. Atmani, T. Richard, *J. Sci. Food Agric.* **2014**, *94*, 951.
[18] M. T. Empl, S. Macke, P. Winterhalter, C. Puff, S. Lapp, G. Stoica, W. Baumgartner, P. Steinberg, *Vet. Comp. Oncol.* **2014**, *12*, 149.

[19] C. Romain, L. Bresciani, S. Gaillet, C. Feillet-Coudray, L. Calani, B. Bonafos, J. Vidé, N. Rugani, J. Ramos, D. Del Rio, J. P. Cristol, J. M. Rouanet, *Mol. Nutr. Food Res.* **2014**, *58*, 1212.
[20] C. Müller, K. Ullmann, P. Steinberg, *J. Med. Food* **2011**, *14*, 34.
[21] C. De Giovanni, L. Landuzzi, G. Nicoletti, A. Astolfi, S. Croci, M. Micaroni, P. Nanni, P. L. Lollini, *Int. J. Cancer* **2004**, *109*, 200.
[22] L. M. Bode, D. Bunzel, M. Huch, G. S. Cho, D. Ruhland, M. Bunzel, A. Bub, C. M. Franz, S. E. Kulling, *Am. J. Clin. Nutr.* **2013**, *97*, 295.
[23] S. Perkins, R. D. Verschoyle, K. Hill, I. Parveen, M. D. Threadgill, R. A. Sharma, M. L. Williams, W. P. Steward, A. J. Gescher, *Cancer Epidemiol. Biomarkers Prev.* **2002**, *11*, 535.
[24] S. Wang, I. Willenberg, M. Krohn, T. Hecker, S. Meckelmann, C. Li, Y. Pan, N. H. Schebb, P. Steinberg, M. T. Empl, *PLoS One* **2017**, *12*, e0170502.
[25] M. T. Empl, M. Albers, S. Wang, P. Steinberg, *Phytother. Res.* **2015**, *29*, 1640.
[26] C. Abbadie, O. Pluquet, A. Pourtier, *Cell Mol. Life Sci.* **2017**, *74*, 4471.
[27] T. Nolte, P. Brander-Weber, C. Dangler, U. Deschl, M. R. Elwell, P. Greaves, R. Hailey, M. W. Leach, A. R. Pandiri, A. Rogers, C. C. Shackelford, A. Spencer, T. Tanaka, J. M. Ward, *J. Toxicol. Pathol.* **2016**, *29*, 1S.
[28] A. R. Moser, H. C. Pitot, W. F. Dove, *Science* **1990**, *247*, 322.
[29] S. You, M. Ohmori, M. M. Pena, B. Nassri, J. Quiton, Z. A. Al-Assad, L. Liu, P. A. Wood, S. H. Berger, Z. Liu, M. D. Wyatt, R. L. Price, F. G. Berger, W. J. Hrushesky, *Int. J. Exp. Pathol.* **2006**, *87*, 227.
[30] M. Bose, J. D. Lambert, J. Ju, K. R. Reuhl, S. A. Shapses, C. S. Yang, *J. Nutr.* **2008**, *138*, 1677.
[31] K. A. Mehl, J. M. Davis, J. M. Clements, F. G. Berger, M. M. Pena, J. A. Carson, *J. Appl. Physiol.* **2005**, *98*, 2219.
[32] D. Trani, B. H. Moon, B. Kallakury, D. P. Hartmann, K. Datta, A. J. Fornace, *Int. J. Radiat. Oncol. Biol. Phys.* **2013**, *85*, 223.
[33] V. I. Leo, S. H. Tan, H. Bergmann, P. Y. Cheah, M. H. Chew, K. H. Lim, J. Ruland, P. T. Reilly, *Cancer Immunol. Res.* **2015**, *3*, 721.
[34] J. M. Amos-Landgraf, J. Heijmans, M. C. Wielenga, E. Dunkin, K. J. Krentz, L. Clipson, A. G. Ederveen, P. G. Groothuis, S. Mosselman, V. Muncan, D. W. Hommes, A. Shedlovsky, W. F. Dove, G. R. van den Brink, *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 16514.
[35] M. H. Roshan, A. Tambo, N. P. Pace, *EPMA J.* **2016**, *7*, 22.
[36] K. Svechnikov, C. Spatafora, I. Svechnikova, C. Tringali, O. Soder, *J. Appl. Toxicol.* **2009**, *29*, 673.
[37] A. Seeni, S. Takahashi, K. Takeshita, M. Tang, S. Sugiura, S. Y. Sato, T. Shirai, *Asian Pac. J. Cancer Prev.* **2008**, *9*, 7.
[38] M. E. Juan, E. Gonzalez-Pons, T. Munuera, J. Ballester, J. E. Rodriguez-Gil, J. M. Planas, *J. Nutr.* **2005**, *135*, 757.
[39] A. Donaldson, S. B. Sufi, S. L. Jeffcoate, *Contraception* **1985**, *31*, 165.
[40] M. S. Figueiroa, J. S. Cesar Vieira, D. S. Leite, R. C. Filho, F. Ferreira, P. S. Gouveia, D. P. Udrisar, M. I. Wanderley, *Asian J. Androl.* **2009**, *11*, 362.
[41] M. Akdogan, M. Ozguner, A. Kocak, M. Oncu, E. Cicek, *Urology* **2004**, *64*, 394.
[42] M. R. Panjeh-Shahin, F. Dehghani, T. Talaei-Khozani, Z. Panahi, *Arch. Iranian Med.* **2005**, *8*, 211.
[43] S. Sale, R. G. Tunstall, K. C. Ruparelia, G. A. Potter, W. P. Steward, A. J. Gescher, *Int. J. Cancer* **2005**, *115*, 194.
[44] I. Willenberg, M. Michael, J. Wonik, L. C. Bartel, M. T. Empl, N. H. Schebb, *Food Chem*. **2015**, *167*, 245.
[45] D. Colin, A. Lancon, D. Delmas, G. Lizard, J. Abrossinow, E. Kahn, B. Jannin, N. Latruffe, *Biochimie* **2008**, *90*, 1674.
[46] A. K. Marel, G. Lizard, J. C. Izard, N. Latruffe, D. Delmas, *Mol. Nutr. Food Res.* **2008**, *52*, 538.