Prevention of mouse lung tumors by budesonide and its modulation of biomarkers

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Chemopreventive drugs have the potential to decrease the morbidity and mortality of lung cancer. The development of these drugs could be expedited by the application of surrogate end-point biomarkers that demonstrate chemopreventive efficacy. In this study, the ability of budesonide to prevent lung tumors in mice was characterized further and its effects on biomarkers were determined. Lung tumors were induced in female strain A mice by vinyl carbamate (16 mg/kg) administered once weekly for 2 consecutive weeks. Four weeks later the mice started to receive 0.6, 1.2 or 2.4 mg/kg budesonide continually in the diet until killed at week 20. Budesonide caused a dose-dependent decrease in the multiplicity of lung tumors of 25, 58 and 82%, respectively. Budesonide (2.4 mg/kg diet) administered starting at weeks 4, 10 or 16, decreased tumor multiplicity by 82, 66 and 30% at week 20. Administering 2.4 mg/kg budesonide at weeks 4–20 or 20–35 and killing the mice at week 35 did not significantly decrease the yield of tumors, although both treatment regimens did decrease the size of the tumors and the progression of adenomas to carcinomas. Thus, budesonide delayed the appearance of lung tumors and decreased their growth and progression to carcinomas. To determine the effect of limited exposure to budesonide on biomarkers, it was administered for only 25, 58 and 82%, respectively. Budesonide (2.4 mg/kg diet) administered starting at weeks 4, 10 or 16, decreased tumor multiplicity by 82, 66 and 30% at week 20. Administering 2.4 mg/kg budesonide at weeks 4–20 or 20–35 and killing the mice at week 35 did not significantly decrease the yield of tumors, although both treatment regimens did decrease the size of the tumors and the progression of adenomas to carcinomas. Thus, budesonide delayed the appearance of lung tumors and decreased their growth and progression to carcinomas. To determine the effect of limited exposure to budesonide on biomarkers, it was administered for only 7 days prior to death at week 35. Budesonide decreased the proliferating cell nuclear antigen labeling in lung adenomas, carcinomas, parenchyma and bronchial airways by 87.6, 59.0, 41.1 and 25.4%, respectively. Budesonide treatment also increased the protein level of the p21 and p27 genes and increased the mRNA level of p21. Thus, short-term treatment with budesonide modulated biological and molecular end-points in lung tumors that might be developed further as biomarkers for its clinical chemopreventive efficacy in the lung.

Introduction

Chemoprevention is recognized as a viable means to reduce cancer deaths in humans. As lung cancer is the most common cause of cancer-related deaths in both men and women in the US, agents that might prevent it are of particular importance. Budesonide, a glucocorticoid drug, has been shown to prevent lung tumors, primarily adenomas induced by benzo[a]pyrene (B[a]P) in strain A mice (1,2). It was effective in preventing lung tumors when administered up to 5 weeks after B[a]P treatment suggesting that budesonide decreased tumor yield by slowing the growth and progression of B[a]P-initiated cells to cancer. Should the budesonide mode of action be to delay the occurrence of lung tumors and their progression to cancers, then the longer the duration of treatment with the drug the greater the likelihood that cancer would be prevented. The studies reported here further characterized the effect on the prevention of lung tumors in mice and explored how late intervention might delay the progression to cancer.

Surrogate end-point biomarkers for chemoprevention are currently being developed for use in animal studies and in clinical trials (3–6). These biomarkers are biological and molecular end-points that are quantitatively modulated by chemopreventive agents and as such could indicate the efficacy of the agents. Conceivably, surrogate end-point biomarkers could be examined in the lung in morphologically normal-appearing epithelium as well as in pre-cancerous and frankly neoplastic lesions. Biomarkers do not have to be directly related to tumor development. However, biomarkers directly related to the development of lung cancer would be preferred as they could give information regarding the mechanism of the chemopreventive agents. One proposed mechanism for chemopreventive agents involves decreased cell proliferation and associated alteration in the expression of the mRNA and protein of genes involved in the regulation and process of cell proliferation (7–12). P21Cip1/Waf1/Slp1 (p21) and p27Kip1 (p27) are tumor suppressor genes and cyclin-dependent kinase inhibitors that inhibit the progression of the cell cycle and play critical roles in the pathogenesis of cancer (13–15). Agents that increase the expression of these genes are likely to result in decreased cell proliferation and may be effective chemopreventive agents. In this report, we demonstrate the ability of budesonide to decrease cell proliferation and to increase the expression of the mRNA and protein of the p21 and p27 tumor suppressor genes.

Material and methods

Chemicals and reagents

Vinyl carbamate (purity >99%) was purchased from Toronto Research Chemicals (North York, Ontario, Canada) and budesonide was from Sigma Chemical (St Louis, MO). AIN-76A diet (casein 20%, DL-methionine 0.3%, cornstarch 15%, sucrose 50%, corn oil 5%, cellulose 5%, AIN mineral mixture 3.5%, AIN vitamin mixture 1.0% and choline bitartrate 0.2%) was obtained from Dyets (Bethlehem, PA).

Animals

Female strain A mice (5–6 weeks old) were purchased from Jackson Laboratories (Bar Harbor, ME). The mice were housed in the AAALAC-accredited laboratory animal facility of the Medical College of Ohio and were maintained in compliance with all applicable animal welfare criteria, guidelines and regulations. They were housed in polycarbonate solid-bottom, shoebox-type cages (height 13 cm, width 18 cm, length 28 cm) with Anderson Bed-

Abbreviations: B[a]P, benzo[a]pyrene; PCNA, proliferating cell nuclear antigen.
The mice were then killed at week 20 (groups 1–4). Other mice received budesonide (2.4 mg/kg diet) starting at 10 or 16 weeks and were also killed at week 20 (groups 5 and 6). Some mice in treatment groups 1 and 4 that, respectively, received control diet or budesonide starting at week 4 were killed at week 16. This was done to determine whether there would be sufficient lung tumors at week 20 for the evaluation of the effect of budesonide on tumor yield and of the effect of starting budesonide treatment at week 20 on tumor progression. From the results of the killing at week 16, it was determined that there would be a sufficient yield of tumors to proceed with the planned killing at week 20 and with starting budesonide treatment of the mice in treatment group 8 at week 20. Thus, the effect of starting or stopping treatment after the occurrence of tumors at week 20 was determined by administering 2.4 mg/kg budesonide in the diet at either weeks 4–20 or 20–35 and the mice were killed at week 35 (groups 7 and 8). Mice that received the control AIN-76A diet or 2.4 mg/kg budesonide at weeks 4–8 were killed at week 20. In addition, groups 9–11 were killed at week 35 (groups 7 and 8). Mice that received the control AIN-76A diet or 2.4 mg/kg budesonide at weeks 4–20 were also killed at week 20 (groups 5 and 6). Some mice in treatment groups 1 and 4 that, respectively, received control diet or budesonide starting at week 4 were killed at week 16. This was done to determine whether there would be sufficient lung tumors at week 20 for the evaluation of the effect of budesonide on tumor yield and of the effect of starting budesonide treatment at week 20 on tumor progression. From the results of the killing at week 16, it was determined that there would be a sufficient yield of tumors to proceed with the planned killing at week 20 and with starting budesonide treatment of the mice in treatment group 8 at week 20. Thus, the effect of starting or stopping treatment after the occurrence of tumors at week 20 was determined by administering 2.4 mg/kg budesonide in the diet at either weeks 4–20 or 20–35 and the mice were killed at week 35 (groups 7 and 8). Mice that received the control AIN-76A diet or 2.4 mg/kg budesonide at weeks 4–35 were also killed at week 35 (groups 1 and 4). Finally, four mice were administered 2.4 mg/kg budesonide for only 7 days prior to being killed (group 9).

The mice were killed by carbon dioxide asphyxiation. The lungs were harvested and evaluated for tumors with the aid of a microscope. The right lobes of the lungs were then fixed overnight in 10% neutral-buffered formalin, transferred to 70% alcohol, and embedded in paraffin for subsequent sectioning for histologic evaluation and proliferating cell nuclear antigen (PCNA) staining. The left lobes of the lungs were frozen in liquid nitrogen, and stored at −70°C for determination of protein and mRNA levels of the p21 and p27 genes.

Histopathological classification of lung adenomas and adenocarcinomas

The classification of lung tumors was based upon morphologic characteristics and includes solid adenomas, papillary adenomas and adenocarcinomas, respectively (16–19). Morphological features of solid adenomas were similar to those of type II cells including an alveolar growth pattern of cells having cuboidal shape, an oval to round nucleus and a vacuolated ‘foamy’ cytoplasm characteristic of surfactant. Papillary adenomas displayed growth patterns with characteristic finger-like papillary structures composed of cells having a columnar shape and pleomorphic nucleus. Adenocarcinomas contained very large cells with varying degrees of differentiation and had increased nuclear/cytoplasmic ratio and nuclear pleomorphism.

Western blot analyses for protein expression of p21 and p27 genes

Lung tumors and normal lung tissue were homogenized in a buffer containing 20 mM Tris–HCl (pH 7.5), 10 mM EDTA (pH 7.5), 1 mM EGTA (pH 8.0), 10 mM β-mercaptoethanol, 1 mM phenylmethyl-sulfonyl fluoride, 0.02% leupeptin, 0.04% trypsin inhibitor, 0.25 M sucrose and 0.1% Triton X-100, sonicated and centrifuged at 12 000 g for 10 min.

Protein concentration in the supernatant was determined using the Bio-Rad Protein Assay (Bio-Rad, Richmond, CA). The supernatant (40 mg protein) was electrophoresed on 15% SDS–PAGE mini-gels under reducing conditions and blotted electrophoretically to Immobilon-P membranes (Millipore, Bedford, MA). Marker molecular weight standards (Santa Cruz Biotechnology, Santa Cruz, CA) were included with each gel as protein molecular size markers. The membranes were incubated in a 5% milk/Tris-buffered saline + Tween 20 (TBST) blocking solution (pH 7.6) for 1 h at room temperature and then probed with mouse monoclonal antibodies for p21 and p27 at room temperature for 1 h. To confirm the results, primary antibodies for p21 and p27 from two different companies (Santa Cruz Biotechnology and Rockland, Gilbertsville, PA) were used. The membranes were washed with TBST (pH 7.6) and incubated with 1:1000 dilution of horseradish peroxidase (HRP)-conjugated secondary antibody IgG for 1 h. The membranes were washed again in TBST (pH 7.6) and target protein bands were visualized by treating the membranes with enhanced-chromiluminescence western blotting detection reagents (Amersham, Arlington Heights, IL) and exposing them to Kodak autoradiograph films. The optical density for the proteins of p21 and p27 genes was measured with the Scion Image Analysis System (Scion, Frederick, MD).

Analysis for the mRNA expression of the p21 and p27 genes

The expression of the mRNAs for the p21 and p27 was evaluated by reverse transcription–polymerase chain reaction (RT–PCR). Total RNA was extracted from lung tumors using TRIzol Reagent (Gibco BRL/Life Technologies, Gaithersburg, MD) and treated with DNase I at 3–4 °C, which makes it possible to co-amplify with p21 and p27 genes, respectively. Then, the total RNA was reverse transcribed into cDNA using 15 U AMV (avian myeloblastosis virus) reverse transcriptase in 20 µl of reaction mixture containing 0.5 µg oligo(dT)15 primer, 5 mM MgCl2, 1 U/µl recombinant RNASin ribonuclease inhibitor, 1× reverse transcription buffer (50 mM KCl, 0.1% Triton X-100 and 10 mM Tris–HCl pH 9.0), and 1 U/µl of each deoxynucleoside triphosphate. The reaction was at 42°C for 40 min, followed by 99°C for 5 min and 4°C for 5 min. The cDNA samples were diluted 5-fold with nuclease-free water and used for PCR amplification.

The cDNA for the p21 and p27 genes were co-amplified with the cDNA of the housekeeping gene, β-actin. The reason to choose β-actin as the housekeeping gene is its wide range of optimal annealing temperature, from 52 to 66°C, which makes it possible to co-amplify with p21 and p27 genes, respectively. The reverse transcription incubation (50 µl) contained 10 ng/µl first strand cDNA, 100 µM each deoxynucleoside triphosphate, 1 mM MgCl2, 1.25 U Taq DNA polymerase, and 20 pmol of primers for the p21, p27 and β-actin genes. Primer sequences for p21 gene (GenBank accession number: BC002043) were upstream: 5′-AAT CTT GGT GAT GTC CC-3′ (61–80 bp) and downstream: 5′-AAA GTT CCA CCT TCG G-3′ (185–204 bp). For p27 gene (GenBank accession number: NM000875) the upstream primer sequence was 5′-GAG GGC AGA AGC TCG CGG C-3′ (428–448 bp) and the downstream primer sequence was 5′-CTG CAT ACT GCT CCG ACA ACC-3′ (645–665 bp). For β-actin gene (GenBank accession number X03765) the upstream primer sequence was 5′-GGT GGC CGC TCT AGG CAA CAA-3′ (25–45 bp) and the downstream primer sequence was 5′-CTC TTT GTG GTC ACAG CAT GCC TAC-3′ (541–564 bp).
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Fig. 1. Dose–response of the effect of budesonide on the yield of lung tumors. Female A/J mice received two doses of 16 mg/kg vinyl carbamate and were then administered budesonide at 0, 0.6, 1.2 or 2.4 mg/kg diet at weeks 4–20. The mice were killed at week 20. The results are means ± SE for treatment groups of 16 mice each and the asterisks indicate statistical significance with a P-value of <0.05.

The incubation underwent 32 cycles of 94°C for 45 s, 53°C for p21 or 65°C for p27 for 45 s, and 72°C for 60 s, followed by 72°C for 10 min. The PCR products were electrophoresed in 1% agarose gel containing 0.1 µg/ml ethidium bromide in 0.5× TBE buffer. After electrophoresis, the gels were photographed under UV-irradiation and the optical densities for the mRNA for the p21, p27 and β-actin genes were measured with the Scion Image Analysis System (Scion). The optical densities of the mRNA for the p21 and p27 genes were standardized using the density of the β-actin gene.

Statistical analysis
The results were analyzed for statistical significance either by a t-test or when comparing three or more experimental groups by an ANOVA followed by the Tukey test. Statistical significance was indicated by a P-value <0.05.

Results
Dose–response and effect of delaying the treatment with budesonide
Budesonide when administered from week 4 after the last dose of vinyl carbamate until death at week 20 demonstrated a dose-related prevention of lung tumors (Figure 1). Thus, the 0.6, 1.2 and 2.4 mg/kg diet doses of budesonide resulted in 25.1, 57.9 and 82.2% reduction in lung tumors, respectively. Budesonide did not alter the body weight gain of the mice. When 2.4 mg/kg budesonide administration was begun 4, 10 or 16 weeks after administering the vinyl carbamate, its ability to prevent lung tumors was diminished, decreasing tumor multiplicity by 82, 66 and 30%, respectively (Figure 2).

Effect of stopping or starting the treatment with budesonide at week 20 on the progression of adenomas
In previous studies at week 20 post-vinyl carbamate exposure, the preponderance of the tumors were adenomas (19). Thus, should treatment with budesonide be started at this time, one could determine its effect on the progression of adenomas to carcinomas. Furthermore, stopping treatment with budesonide at week 20 could test whether suppressed tumors would grow out. Thus, budesonide was administered to the mice at weeks 4–35, 4–20 or 20–35 and the mice killed at week 35. The experiment was terminated at week 35 because the mice appeared to be losing weight (Figure 3) and because numerous large tumors were found in three mice fed the control diet. The presence of large tumors indicated that there were a sufficient number of carcinomas in the control group in order to determine the effect of budesonide on the progression to cancer.

The effect of either stopping or starting budesonide treatment at week 20 on the multiplicity of lung tumors is presented in Figure 4. At week 20, there were 12.6 tumors/mouse and almost all were adenomas. Treatment with budesonide from week 4 greatly reduced the multiplicity of lung tumors at weeks 16 and 20. Continuing treatment until week 35 also resulted in a reduced multiplicity of lung tumors, although the reduction was not as great as at the earlier times. Thus, continual budesonide treatment
Fig. 4. Effect on lung tumor multiplicity of starting or stopping treatment with budesonide at week 20. Female A/J mice received two doses of 16 mg/kg vinyl carbamate and were then administered budesonide at 2.4 mg/kg diet starting at either weeks 4 or 20 and stopping at weeks 20 or 35. The results are expressed as mean ± SE for the number of animals listed in Table I. The asterisks indicate statistical significance with a P-value of <0.05.

Fig. 5. Effect of budesonide on the size of the lung tumors: week 35. The lung tumors from the mice killed at week 35 in Figure 4 were classified according to size as 1 mm or >1 mm. The results are expressed as mean ± SE and the asterisks indicate statistical significance with a P-value of <0.05.

administered the control diet, 95.5% of the tumors were >1 mm in diameter, while in mice administered the three treatment schedules for budesonide only 51.0–73.3% of the tumors were >1 mm. Furthermore, when the larger tumors of >3 mm were compared separately, 6.04% of the tumors in mice administered the control diet were of this size, while all three treatment regimens of budesonide significantly decreased the yield of these large tumors. The percentage of tumors >3 mm was only 1.75, 2.28 and 0.61% in mice administered budesonide at weeks 4–20, 20–35 and 35, respectively.

Histopathological evaluation of the tumors indicated that budesonide treatment also delayed the progression to carcinomas (Figure 6). Mice administered the control diet had a greater percentage of carcinomas (49.7%) than mice administered the three treatment schedules of budesonide (20.9–33.0%). In fact, the longer the treatment with budesonide the smaller the percentage of carcinomas (20.9%). Most of the adenomas were solid alveolar tumors (82%) with the remaining adenomas having a papillary growth pattern. Budesonide did not significantly alter the ratio of solid/papillary adenomas.

Effect of budesonide on potential biomarkers in lung tumors
Seven days prior to death, mice were administered budesonide (2.4 mg/kg diet) in order to determine its ability to modulate biomarkers. One potential biomarker evaluated was the ability of budesonide to alter cell proliferation both in lung tumors and in histologically normal-appearing airways and parenchyma of the lung as measured by the PCNA-labeling index. The PCNA-labeling index was significantly greater in carcinomas than in adenomas, being 15.1 ± 2.1 and 5.93 ± 0.70, respectively, so that they were evaluated separately (Figure 7). In the bronchial airways, the PCNA-labeling index was 4.73 ± 0.37 that was only significantly less than the carcinomas. Administering 2.4 mg budesonide/kg diet for only the last 7 days of the study decreased the PCNA labeling in the parenchyma, airways, adenomas and carcinomas (Figure 7). PCNA labeling was decreased by a greater percentage in adenomas (87.6%) and carcinomas (59.0%) than in parenchyma (41.1%) and airways (25.4%). Tumor morphology was not altered by the 7 days of treatment with budesonide just prior to death.
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Fig. 7. Effect of 7 days of treatment with budesonide on PCNA labeling. Female A/J mice received two doses of 16 mg/kg vinyl carbamate and 34 weeks later were administered budesonide at 2.4 mg/kg diet for 7 days prior to death at week 35. The PCNA-labeling index was determined for the airways, adenomas and carcinomas as the percentage of cells that were PCNA-positive and in the normal-appearing parenchyma as the total number of PCNA-positive cells in the tissue section. The asterisks indicate significant difference from mice not administered budesonide, P-value <0.05. A total of 75 adenomas and 27 carcinomas were evaluated among all groups to determine the PCNA index whereas 25 high-power fields for both conducting airways and uninvolved lung parenchyma were used from each of 4 slides/treatment group.

Another potential biomarker for chemopreventive agents is the ability to increase the relatively low level of the protein of tumor suppressor genes in tumors. The ability of limited treatment with budesonide (7 days) to increase the protein level of the tumor suppressor genes, p21 and p27 was determined in the lung tumors (Figure 8). The protein level of both tumor suppressor genes was greatly decreased by ~80% in lung tumors relative to normal lung tissue. Budesonide increased the protein levels of the p21 and p27 genes by ~3-fold. Thus, after budesonide treatment the protein level of both genes in lung tumors was no longer different from normal lung tissue. Budesonide could increase the protein levels of the p21 and p27 genes by increasing the transcription of their mRNA. Thus, the effect of budesonide on the mRNA expression of the two genes was determined (Figure 9). Of the two genes, only the mRNA level of the p21 gene but not the p27 gene was increased by budesonide. This implies that budesonide increased the protein level of the p21 gene by increasing transcription of its mRNA while it increased the protein level of the p27 gene by a mechanism independent of its transcription.

Discussion

Budesonide has been reported by others to prevent B[a]P-induced lung tumors in strain A mice (1,2). In the study reported here, budesonide caused a dose-dependent prevention of vinyl carbamate-induced lung tumors with the lowest concentration evaluated, 0.6 mg/kg diet, causing a 25% reduction in tumor multiplicity. There was also a temporal-dependency in the efficacy of budesonide to reduce tumor multiplicity. Budesonide was effective in decreasing the lung tumor multiplicity when administered starting 4, 10 or 16 weeks after administration of vinyl carbamate. The shorter the delay or the longer the duration of administering budesonide the greater the efficacy in preventing lung tumors, primarily adenomas at the 20 week time point. A 35 week death was examined in order to determine the effect of treatment with budesonide of longer duration and on a carcinoma end-point. Although continuous treatment with budesonide at weeks 4–35 did result in a decreased yield of lung tumors at week 35, the percent reduction in tumor yield was much less than that at week 20. Thus, at week 20, budesonide caused an 82% reduction in tumor yield while at week 35 the reduction was only 20.9%. This would suggest that budesonide delayed the appearance of lung tumors. When, the mice were treated with budesonide at weeks 4–20 and then maintained on control diet until week 35, tumor multiplicity that was reduced at week 20 was no longer significantly reduced at week 35 due to the occurrence of tumors.

The ability of budesonide to delay the appearance of tumors suggests that it would also decrease the size, growth rate and progression of the tumors. This is, in fact, what was found. Budesonide administered starting at week 4 not only delayed
the longer the treatment with budesonide the greater the delay in the occurrence of cancer and that late treatment with budesonide after the occurrence of pre-cancerous lesions could be useful in delaying the occurrence of cancer.

The development of biomarkers has the potential to greatly shorten the duration of clinical trials, if they can indicate potential clinical efficacy and can be modulated by a short duration of treatment with a chemopreventive agent. Thus, the potential efficacy of chemopreventive agents might be determined in less time and with fewer patients. Although this is not a substitute for Phase III cancer incidence trials, it should allow one to determine if agents are promising. Furthermore, biomarkers do not have to be related to the mechanism of chemoprevention, mechanism-related biomarkers have the potential advantage of demonstrating the mechanism of an agent. As budesonide appeared to prevent lung tumors by decreasing their growth and delaying their occurrence it would appear that decreased cell proliferation is involved in its mechanism. We found a higher level of cell proliferation in carcinomas than in adenomas or bronchial airways. Short-term budesonide treatment decreased the PCNA-labeling index in adenomas (88%) and carcinomas (59%). Lung tumors were more sensitive than the parenchyma and airways to budesonide reduction in PCNA labeling. However, budesonide did decrease the PCNA labeling in these normal-appearing tissues indicating that one might be able to assess a biological response to budesonide in normal tissue. Thus, normal-appearing epithelial tissue obtained during bronchoscopy might be used for demonstrating the ability of budesonide to decrease cell proliferation in the human lung.

A molecular alteration in tumors having the potential of being developed as a biomarker for chemoprevention is the decreased level of the proteins of tumor suppressor genes that regulate cell proliferation and the cell cycle. The tumor suppressor genes, p21 and p27 form complexes with CDK-cyclins to inhibit their kinase activity. The p21 protein also binds PCNA to inhibit DNA replication directly and induces G1 and G2 arrest (15). The p27 protein inhibits transition from S to G2 phase by apparently interacting with CDK2 (13, 20). The protein levels of p21 and p27 were decreased by ~80% in mouse lung tumors relative to normal lung tissue. Short-term treatment with budesonide restored the protein level of both genes back to the level found in normal lung tissue. The protein level of p21 has been reported to be low in human A549 lung adenocarcinoma cells and to be increased by flavone, another potential chemopreventive agent (21). The protein level of p27 has been reported to be decreased in human non-small-cell lung carcinomas and to be a possible prognostic factor predicting a poor outcome (13, 20, 22, 23). The decreased p21 and p27 protein levels in both human and mouse lung tumors suggest that the ability of budesonide to restore the protein level of the two genes back to the level found in normal lung tissue could be developed into a biomarker for the efficacy of the drug.

Budesonide increased the protein level of p21 and p27 in lung tumors after only a few days of treatment, indicating that protein levels of the genes were controlled by reversible mechanisms. Reversible mechanisms that can decrease the protein level of these tumor suppressor genes in tumors include decreased transcription of the gene and increased turnover of the protein (14, 24–26). As budesonide also increased the mRNA level of p21, it would indicate that increased transcription was involved in this reversal. Another glucocorticoid, dexamethasone, has been shown to prevent lung tumors in strain A mice (19, 27–30).
In rat hepatoma cells, dexamethasone-induced G1 cell cycle arrest has been associated with increased expression of the p21 protein (31). Dexamethasone appeared to activate the p21 gene promoter by two pathways; one involving the glucocorticoid receptor-transcription factor and the other involving the CCAAT/enhancer binding protein-α (CEBPα) transcription factor (31–34). Thus, it is possible that budesonide up-regulation of the mRNA and protein levels of the p21 gene involved these two transcription factors.

The protein level of p27 has been reported to be mainly controlled by a post-translational mechanism such as phosphorylation (13,20,35,36). The ability of budesonide to increase the protein level of p27 in the absence of increased mRNA levels of the gene supports a post-translational mechanism. Degradation of the p27 protein appears to involve phosphorylation and ubiquitination of the protein that leads to proteosome-mediated degradation (13,20,35,36). Thus, it is proposed that chemopreventive agents increase the protein level of p27 by preventing its phosphorylation or ubiquitination so that it is not degraded.

The changes in cell proliferation and molecular markers observed after only 7 days of budesonide treatment would appear to reflect altered gene expression in the tumor cells themselves. An alternative interpretation might be that the lesions after 7 days of treatment had a higher percentage of ‘normal’ infiltrating cells than the control tumors. This interpretation is not probable because histopathological examination of the tumors from budesonide treated mice did not reveal evidence of infiltrating normal cells. In summary, 7 days of treatment with budesonide increased the protein level of p21 and p27 and the mRNA level of p21. This would suggest that the ability to increase the protein level and in some cases, the mRNA level of these and other tumor suppressor genes might prove suitable surrogate end-point biomarkers for chemoprevention of lung tumors. The involvement of the two genes in human lung cancer further suggests that the ability to increase their expression is also likely to be a useful biomarker in humans.

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References


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