In Vivo Antineoplastic Effects of the NSAID Sulindac in an Oral Carcinogenesis Model

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Abstract

The antineoplastic properties of the NSAID sulindac have long been studied. The purpose of this study was to explore sulindac’s in vivo effects on oral squamous cell carcinoma (SCC) oncogenesis using the hamster cheek pouch oral carcinogenesis model (HOCM). Thirty Syrian golden hamsters were divided into three experimental and two control groups (n = 6 each). The animals’ right buccal pouches were treated with carcinogen for 9 weeks in one experimental and one control group and for 14 weeks in all other three groups. The animals of two experimental groups received sulindac from the 1st week until the end of the third experimental group from the 10th week. After the end of carcinogenesis, treated buccal pouches were removed and examined. In animals treated with carcinogen for 14 weeks, development of oral SCC and tumor volume were significantly lower in animals that received sulindac from the first week of the experiment. Oral SCC developing in animals that received sulindac were more frequently well differentiated compared with the control group. In animals treated with carcinogen for 9 weeks, the animals that received sulindac developed lower grade of epithelial dysplasia. Proliferation index Ki-67 and positivity for the antiapoptotic molecule survivin were lower in the animals that received sulindac. Treatment with sulindac appears to delays the progression of oral premalignant lesions to oral SCC in the HOCM, also resulting in smaller and better differentiated tumors. These in vivo antineoplastic effects may be related to sulindac’s ability to decrease cell proliferation and to prevent survivin expression. Cancer Prev Res; 8(7) July 2015.

Introduction

Each year oral cancer accounts for 300,373 new cases worldwide (1), with 90% of them being oral squamous cell carcinoma (SCC; ref. 2). Despite the progress achieved in cancer therapy in the last decades, the prognosis of oral SCC has not been drastically improved and the 5 years survival rate does not exceed 50% (3, 4). Epidemiological data, in vitro studies in cell cultures, as well as in vivo animal studies and controlled clinical trials have demonstrated that nonsteroidal anti-inflammatory drugs (NSAIDs) exert antineoplastic effects in various types of cancer, such as colon, lung, breast, prostate, and head and neck cancer (5–10). NSAID sulindac has shown growth inhibitory and proapoptotic effects on various cancer cell lines in vitro, including colon and breast cancer cell lines (11–13). Furthermore, the use of sulindac has shown an inhibitory effect on tumor growth in vivo in gastric, lung, urinary bladder, colorectal, and head and neck cancers in animal models (14–17). Studies using sulindac in combination with other anti-cancer drugs, such as cisplatin, paclitaxel, and docetaxel, have shown a synergistic antineoplastic effect (18, 19).

Sulindac and other cyclooxygenase (COX) inhibitors exert their anti-inflammatory, analgesic, and antiinflammatory effects through the inhibition of prostaglandins, but the exact mechanism of their antineoplastic effects remains largely unknown (20). The antiproliferative and proapoptotic effects of sulindac on cancer cells have been attributed to both COX-dependent and COX-independent mechanisms, including alterations of important cell signaling and apoptotic molecules, such as survivin (21).

Survivin belongs to the inhibitor of apoptosis family of proteins (IAP; ref. 22). In contrast to most IAP members, survivin is normally expressed in developing tissues, the thymus, basal colonic tissues, endothelial tissues, and neural stem cells, but not in normally differentiated tissues (23, 24). Survivin expression is detected in a number of cancers and is implicated in cell-cycle regulation and inhibition of apoptosis (22). Furthermore, its expression has been detected in preneoplastic lesions, suggesting a possible participation in the induction of malignant transformation (23). Survivin expression is also detected in oral SCC but not in normal oral mucosa (24, 25).

It has been shown that sulindac treatment of oral SCC cell lines causes cell growth inhibition and promotes apoptosis (21). Furthermore, it has been shown that cell growth inhibition of apoptosis and survivin downmodulation is a specific effect of sulindac that is not shared by other COX inhibitors and that survivin is a downstream target and effector of oncogenic Stat3 signaling in oral SCC, which is targeted by sulindac in a COX-2–independent manner (25). An in vivo study in athymic nude mice that developed tumors after subcutaneous injection of HEP-2 SCC cells showed significant regression of HEP-2 xenograft growth and downregulation of survivin protein levels in sulindac-treated mice versus untreated control mice (26).
The hamster cheek pouch oral carcinogenesis model (HOCM) is the best known animal system that closely correlates with sequential common events involved in the development of human oral premalignant and malignant lesions (27, 28). In the HOCM, development of epithelial dysplasia occurs at weeks 6 to 9 and development of invasive SCC at weeks 10 to 14 of the carcinogenesis process (27, 29).

The antineoplastic effects of sulindac have not been extensively explored in a primary carcinogenesis model such as the HOCM. The significance of sulindac-induced survivin down-regulation in oral SCC has not been elucidated in the HOCM. Currently, there are no studies to our knowledge that have explored the therapeutic effects of sulindac in the management of oral epithelial dysplasia. Therefore, the present investigation was undertaken to assess the effects of sulindac in the chemoprevention of oral epithelial dysplasia and SCC using the HOCM.

Materials and Methods

Animals

The experiments were carried out using 30 healthy male Syrian golden hamsters (Mesocricetus auratus) 5 weeks old purchased from Harlan Laboratories S.r.l. The animals were housed three per cage and were maintained in a controlled environment at a room with controlled temperature (22°C ± 1°C) and relative humidity (60 ± 5) with 12-hour light–dark cycles at the facilities of the Laboratory of Experimental Surgery and Surgical Research in School of Medicine, University of Athens, Greece. All animals were given lab chow ad libitum and tap water. Each cage was labeled with the group, number, and laboratory number of each animal and type and time of diet modification. The protocol was approved by the prefecture of Athens (k/363/21/01/2009) according to the European legislation (160/91).

Experimental and control groups

After 13 days of acclimatization, the animals were randomly divided in three experimental groups (A1, A2, and B) and two control groups (C1 and C2) according to diet modification with or without addition of sulindac and sacrifice day (Fig. 1). Each group included six animals (n = 6) each. The animals were briefly anesthetized with ether and individually numbered by ear-punching.

The groups were as follows:

- **Group A1** (n = 6): the animals were treated with carcinogen and received sulindac in their diet from the beginning of the experiment until the end of the 9th week when they were sacrificed.
- **Group A2** (n = 6): the animals were treated with carcinogen and received sulindac in their diet from the beginning of the experiment until the end of the 14th week when they were sacrificed.
- **Group B** (n = 6): the animals were treated with carcinogen from the beginning of the experiment until the end of the 14th week. They did not receive sulindac during the first 9 weeks of the experiment, but they started to receive sulindac in their diet from the beginning of the 10th week of the experiment until the end of the 14th week when they were sacrificed.
- **Group C1** (n = 6): the animals were treated with carcinogen from the beginning of the experiment until the end of the 9th week when they were sacrificed. They did not receive sulindac.
- **Group C2** (n = 6): the animals were treated with carcinogen from the beginning of the experiment until the end of the 14th week when they were sacrificed. They did not receive sulindac.

Carcinogenesis

All animals’ right buccal pouches were treated three times per week with 0.5% 9,10-dimethyl-1,2-benzanthracene (DMBA; D3254, Sigma Chemical Co.) diluted in paraffin oil using a #4 camel’s hair brush. The amount of carcinogen delivered to each animal was quite uniform using the “wiped brush” method (28). The right buccal pouches of the animals in groups A1 and A2 were treated with DMBA for 9 weeks, whereas the right buccal pouches of the animals in groups A2, B, and C2 were treated with DMBA for 14 weeks (Fig. 1). The pouches of all animals were examined weekly in order to observe the development and growth of lesions/tumors on the mucosa. The animals’ weight was monitored and their general health was grossly evaluated weekly. Photographs of the animals’ right buccal pouches were taken after the end of the 6th, 9th, and 14th week of the experiment and after the animals’ sacrifice.

Diet and sulindac administration

During acclimatization, all animals of all groups received regular lab chow (Teklab Global Diet 2018; Harlan Laboratories S.r.l.). The animals of group A1 received 500 ppm sulindac (S8139; Sigma Chemical Co.) in their diet (Teklab 2018 added with 500 ppm Sulindac; Harlan Laboratories S.r.l.) for 9 weeks, the animals of group A2 received 500 ppm sulindac in their diet for 14 weeks, whereas the animals of group B received sulindac from the beginning of the 10th week until the end of the 14th week. The animals of groups C1 and C2 did not receive sulindac but regular lab chow (Teklab Global Diet 2018; Harlan Laboratories S.r.l.; Fig. 1). The consumption of sulindac enriched or regular lab chow, according to the animals group and the time of the experiment, was monitored.
Animals’ sacrifice and buccal pouch removal
Before sacrifice each animal was weighed and its general health was grossly examined. The animals’ buccal pouches were examined and photographs of each animal and its right buccal pouch were taken.

The animals were sacrificed under isoflurane anesthesia. The animals of groups A1 and C1 were sacrificed after the end of the 9th week of the experiment, whereas the animals of groups A2, B, and C2 were sacrificed after the end of the 14th week of the experiment (Fig. 1).

The treated buccal pouches were removed after the sacrifice of the animals, photographed and examined macroscopically, histopathologically and immunohistochemically.

Macroscopical evaluation
The whole cheek pouch was excised and flattened on a transparency. Visible tumors were counted and the length, width, and height of each tumor in millimeters were measured with the help of a magnifying lens. The tumor volume in \( \text{mm}^3 \) of each tumor was calculated with the mathematical type: \( 4/3 \pi r_1 r_2 r_3 \), with \( r_1, r_2, \) and \( r_3 \) representing the radius of each one of the three dimensions of each tumor (30). Finally, the total tumor volume in \( \text{mm}^3 \) for each animal was calculated adding the tumor volumes of its tumors (30).

Histopathologic study
The animals’ right buccal pouches were fixed in 10% formalin, embedded in paraffin, cut into 5-µm-thick sections and mounted on polylysine-coated glass slides. One section from each specimen was stained with hematoxylin and eosin for histopathologic examination.

The specimens were examined under light microscopy by two independent evaluators (K.K. and N.N.) and classified according to WHO criteria (31) in normal oral mucosa, epithelial hyperplasia, mild, moderate and severe epithelial dysplasia, carcinoma in situ, microinvasive SCC and well, moderately and poorly differentiated SCC (31). Mild epithelial dysplasia was considered as architectural disturbance limited to the lower third of the epithelium accompanied by cytologic atypia, moderate as architectural disturbance extending into the middle third of the epithelium (consideration of the degree of cytologic atypia may require upgrading) and severe when greater than two thirds of the epithelium shows architectural disturbance with associated cytologic atypia (31).

Immunohistochemistry
Paraffin-embedded tissue sections of the animals’ right buccal pouches were deparaffinized, immersed in ethanol 100% and 95%, and heated for antigen retrieval in 0.01 M citrate buffer for 25 minutes in a pressure cooker inside a microwave oven. After dehydration in hydrogen peroxide, the sections were incubated with primary antibodies at room temperature for 1 hour. The applied antibody for Ki-67 was a monoclonal Ki-67 antibody (Dako) diluted at 1:150. The applied antibody for survivin was Survivin Rabbit Polyclonal Antibody (Thermo Fisher Scientific) diluted at 1:50.

Immunostaining was assessed by two independent evaluators (K.K. and N.N.). Immunohistochemical reactivity for Ki-67 and survivin was graded according to the percentage of positive cells and intensity of staining in comparison to negative control tissues.

Statistical analysis
Data are presented as mean ± SE. Tumor volume, development of epithelial dysplasia or oral SCC, degree of epithelial dysplasia, oral SCC differentiation, and depth of tumor invasion were statistically evaluated and the results for groups A1 and B were compared with group C2, respectively. Results for groups A1 and C1 were compared with each other. Furthermore, the proportion of Ki-67 and surviving staining in tissue specimens was compared between groups A1 and C1, and between groups A2 and C2 and B and C2, respectively. Statistical evaluation was performed with the program IBM Statistics (former SPSS) with analysis of variance (ANOVA) between the experimental groups and with Dunnett’s post hoc control comparing group A1 with the control group C1 and group A2 and B with the control group C2.

Results
Results of macroscopical evaluation
There were no significant differences regarding the body weight and intake of food between the animals of all groups. Sulindac administration did not affect the body weight and no general toxicity was shown.

Animals in groups C1 and A1 did not develop tumors but only erythema in their carcinogen-treated right buccal pouches. In contrast, animals in groups C2, B, and A2 developed tumors of varying number and size, with the exception of one animal in group A2 that did not develop tumor but only erythema. The total tumor volume for group C2 was 532.80 \( \text{mm}^3 \), for group B 340.21 \( \text{mm}^3 \), and for group A2 54.72 \( \text{mm}^3 \) (Fig. 2). The mean tumor volume for the animals of group C2 was 88.80(±46.81) \( \text{mm}^3 \), for the animals of group B was 56.70(±13.70) \( \text{mm}^3 \) and 9.12(±6.86) \( \text{mm}^3 \) for the animals of group A2.

Tumor volume was lower in group B compared with group C2, but there was no statistically significant difference in tumor volume between the two groups (\( P = 0.22 \)). Tumor volume in group A2 was significantly lower compared with group C2 (\( P = 0.047 \) and 95% confidence interval (CI), −0.764 to −0.0424).

Histopathologic results
All six animals of group C1 developed severe epithelial dysplasia. In group A1, five animals developed moderate and one animal severe epithelial dysplasia (Fig. 3 and 4A).
Animals in group A1 developed less severe epithelial dysplasia compared with animals in group C1 \( (P = 0.008) \).

All six animals of group C2 developed invasive SCC, superficial in four cases and deeply invasive in two cases.

One of the animals with superficial invasive SCC developed a well-differentiated SCC, whereas the remaining five animals developed moderately differentiated SCC (Fig. 3 and 4B).

Figure 3.
Composite of photomicrographs of representative cases. A–E, hematoxylin and eosin staining. A, severe epithelial dysplasia (group C1). B, moderate epithelial dysplasia (group A1). C, moderately differentiated SCC (group C2). D, Mild epithelial dysplasia (group B). E, severe epithelial dysplasia (group A2). F–J, immunohistochemical expression of Ki-67. Representative examples showing higher positivity in group C1 (F) than in A1 (G) and in group C2 (H) compared with B (I) and A2 (J). K–O, Immunohistochemical expression of survivin. Representative examples showing higher positivity in group C1 (K) than in A1 (L) and in group C2 (M) compared with B (N) and A2 (O). All pictures except (C) are in 400 × magnification; C, 100 × magnification.
In group B, only five animals developed invasive SCC, of which three superficial and two deeply invasive SCC; whereas the last animal showed only mild dysplastic changes. One of the animals with deeply invasive SCC developed moderately differentiated SCC, whereas the remaining four animals had well-differentiated SCC (Fig. 3 and 4B).

In group A2, three animals developed SCC, all of them superficial and well differentiated, with two of the remaining animals showing carcinoma in situ and one severe dysplasia (Figs. 3 and 4B).

The proportion of animals developing SCC in group A2 was significantly lower compared with group C (P = 0.079) with 90% CI (−0.9705)−(−0.0295). SCC developing in groups A2 and B were more frequently well differentiated compared with group C2 (P = 0.020 and 0.037, respectively) with 95% CI (−1.4175)−(−0.2492) and (−1.1336)−(−0.1331), respectively.

**Immunohistochemical results for Ki-67**

Regarding the immunohistochemical results for Ki-67 positivity, animals in group C1 exhibited epithelial cell positivity in a proportion ranging from 10% to 30% with a mean of 19.17 (±4.54)% and animals in group A1 expressed epithelial cell positivity ranging from 3% to 20% with a mean of 11.30 (±2.92)% (Fig. 5). Animals in group C2 showed epithelial cell positivity in a proportion ranging from 25% to 55% with a mean of 37.50 (±5.78)%; animals in group B in a proportion ranging from 2% to 25% with a mean of 14.50 (±4.54)% and animals in group A2 in a proportion ranging from 20% to 40% with a mean of 28.30 (±4.16)% (Fig. 5). The proportion of Ki-67 positivity was significantly higher in group C1 compared with group A1 (P = 0.066).

**Immunohistochemical results for survivin**

Regarding the immunohistochemical expression of survivin, lesions developing in animals of group C1 showed positivity for survivin in a proportion ranging from 33% to 57% with a mean of 45.17 (±4.33)%; whereas lesions in animals of group A1 exhibited positivity ranging from 22% to 30% with a mean of 24.67 (±1.32)% (Fig. 5). Animals in group C2 expressed epithelial cell positivity for survivin in a proportion ranging from 40% to 75% with a mean of 52.50 (±5.87)%; animals in group B showed positivity in a proportion ranging from 10% to 44% with a mean of 23.67 (±5.47)% and animals in group A2 expressed positivity ranging from 16% to 45% with a mean of 29.83 (±4.67)% (Fig. 5). The proportion of survivin positivity was significantly higher in group C2 than in group A1 (P = 0.00057). The proportion of survivin positivity was also significantly higher in group C2 than in group A2 [P = 0.024, 95% CI (−0.1396)−(−0.0179)] and in group C2 compared with group B [P = 0.007, 95% CI (−0.167)−(−0.0088)].

**Discussion**

The HOCM is the best known in vivo carcinogenesis model for the investigation of oral cancer development and evaluation of chemopreventive agents (30, 32). The similarities of SCC developing in humans and in the HOCM provide a rationale for analyzing the effects of putative chemopreventive agents in the HOCM (30, 32). In this study, the effects of the NSAID sulindac on the chemoprevention and management of oral epithelial dysplasia and SCC were explored using the HOCM.

The duration of DMBA application and time of sacrifice were carefully planned according to the literature (27, 29) at 9 weeks for the animals of the control group C1 in order to achieve development of epithelial dysplasia, but not invasive SCC, at a rate of 100%; and at 14 weeks for the animals of the control group C2 in order to achieve development of invasive SCC at a rate of 100% (27, 29). Furthermore, the time of sulindac treatment in group B was carefully planned in order to initiate sulindac treatment at the beginning of the 10th week of the carcinogenesis at a point when all animals of group B develop epithelial dysplasia but not
invasive SCC (27, 29). As a matter of fact, groups A1 and C1 had the same possibility for epithelial dysplasia development and groups A2, B, and C2 had the same possibility for SCC development, so that any differences between these groups could be attributed only to sulindac administration.

In our study, the development of less severe epithelial dysplasia in animals of group A1 compared with those of group C1 suggests that sulindac administration reduces the degree of epithelial dysplasia in the HOCM. Therefore, it can be assumed that sulindac reduces the rate of epithelial dysplasia generation in the face of constant exposure to carcinogens.

When examining the potential chemopreventive effects of sulindac, in other words its ability to prevent or retard the malignant transformation of epithelial dysplasia, animals of group A2 developed invasive cancer in only 50% of cases; further, SCC developing in these animals had a significantly lower average tumor volume compared with group C2, suggesting that sulindac administration from the beginning of carcinogenesis inhibits tumor development and growth in the HOCM. Even when sulindac administration was started after the development of epithelial dysplasia, as in animals of group B, a delay in malignant transformation was also seen, manifested as reduction in tumor volume, better differentiation and, in one case, complete abolition of invasion. Overall, it appears that sulindac administration partially prevents oral SCC formation and transformation of already established oral epithelial dysplasia into oral SCC in the HOCM after 14 weeks of carcinogenesis.

Ki-67 is a large nonhistone protein that has been used as a marker of proliferative activity during the G1, S, G2, and M-phases of the cell cycle (33). Although the exact role of the Ki-67 protein during the cell cycle has not been completely elucidated, it is widely accepted that it represents a reliable marker for cell proliferation and could therefore be used to identify the proliferative activity of tumor cell population (34). The significantly higher proportion of Ki-67 positivity in group C1 compared with group A1 suggests that sulindac administration leads to reduction of cell proliferation in the early stages of carcinogenesis in the HOCM. The proportion of Ki-67 positivity was also significantly higher in group C2 than in group B. The proportion of Ki-67 was higher in group C2 than in group A2, but there was no statistically significant difference in Ki-67 positivity between the two groups. This finding may be interpreted by the fact that cell proliferation and thus Ki-67 positivity may be higher in the early stages of oncogenesis and decreases in well and moderately differentiated SCC (35). Because the animals in group A2 had lesions that were at earlier stages of oncogenesis than those in group C2, it is possible that the antiproliferative effect of sulindac is counterbalanced by the increased cell proliferation in earlier stages of oncogenesis. On the contrary, groups B and C2 did not exhibit statistically significant difference in the development of invasive SCC and thus the difference in Ki-67 positivity between those two groups can more directly reflect the antiproliferative effects of sulindac.

Besides its antiproliferative activity, sulindac has been known to induce apoptosis in oral SCC cells (21). These proapoptotic effects have been shown to be mediated through downregulation of survivin activity (25, 26). In accordance with these findings, our current results also indicate that sulindac decreases survivin expression in vivo, in all stages of carcinogenesis in the HOCM.

Taken together, the results of this study in the face of the available literature (25, 26) may support the ability of sulindac to downregulate cell proliferation and survivin expression in the HOCM, which may provide a molecular explanation for its potent antineoplastic properties in the HOCM.

In vitro studies showed that sulindac treatment of oral SCC cell lines caused cell growth inhibition and correlated with increased levels of apoptosis (21). Both sulindac sulfide, the active metabolite of sulindac that inhibits COX enzymatic activity and the sulfone derivative of sulindac that lacks COX inhibitory effect, induced a significant dose- and time-dependent cell growth reduction accompanied by increase in apoptosis without concomitant cell-cycle arrest, suggesting that the anticancer effect of sulindac is due not only to COX-dependent mechanisms but also to COX-independent mechanisms (21). Furthermore, it has been shown that cell growth inhibition, induction of apoptosis, and survivin downmodulation is a specific effect of sulindac that is not shared by other COX inhibitors (25). Survivin has proven to be a downstream target and effector of oncogenic Stat3 signaling in oral SCC, which is targeted by sulindac in a COX-2–independent manner (25).

An in vivo study in athymic nude mice that developed tumors after subcutaneous injection of HEP-2 cells showed significant regression of HEP-2 xenograft growth in sulindac-treated mice and downregulation of survivin protein levels in sulindac-treated tumors versus untreated control mice (26).

Apart from our study, only one other study explored the inhibitory effect of sulindac in the HOCM (36). In that study, 18 male Syrian golden hamsters 5 weeks old were treated three times a week with a 0.5% solution of DMBA dissolved in acetone for a period of 13 weeks. Six animals did not receive sulindac and served as the control group, whereas six animals received 200 and six 400 ppm sulindac in their diet, respectively. The onset of carcinoma formation was detected in 8.7 weeks for the animals who did not receive sulindac and 14.8 and 11.8 weeks for the animals that received 200 and 400 ppm, respectively (36). The mean survival time also extended for the animals that received sulindac. In that study, the group of animals that received 200 ppm sulindac had better results than those receiving 400 ppm sulindac. The authors suggested that the aforementioned results could have been attributed to an ideal dose of sulindac for inhibition of carcinogenesis (36).

In our study, the animals were treated with 500 ppm sulindac in their diet with better results than the 400 ppm group and similar results with the 200 ppm group of the aforementioned study, since after 14 weeks of treatment with 0.5% DMBA dissolved in paraffin oil, only three out of six animals developed SCC. It is worth mentioning that in our study we adopted a more aggressive carcinogenesis method as the animals’ buccal pouches were treated for 14 and not for 13 weeks with DMBA.

Celecoxib, a selective COX-2 inhibitor, has also been shown to exert chemopreventive effects in the HOCM (29). The administration of celecoxib in the animals’ diet delayed the onset of carcinoma formation that started at 10.0 and 11.2 weeks in the 500 and 1,500 ppm celecoxib group, instead of 6.7 weeks in the control group. In addition, tumor growth was inhibited and survival was increased in the group of celecoxib treatment, with increase of the apoptotic cells in the tumor parenchyma and significant inhibition of angiogenesis in the stroma (29).
In another study (37), hamsters were treated with 0.5% DMBA thrice every week for 6 weeks. Topical treatment thrice every week for 18 weeks with 3% and 6% zileuton (a specific 5-lipoxygenase inhibitor) reduced the incidence of SCC from 76.9% to 45.8% (P < 0.05) and 32.1% (P < 0.0001) respectively, and treatment with celecoxib 6% to 50% (P < 0.05). The combination of celecoxib 3% with zileuton 3% reduced the SCC incidence to 36% (P < 0.05), suggesting an additive effect of celecoxib and zileuton in the HOCM.

Regarding clinical trials, chemoprevention with NSAIDs have shown both a reduction in the number and size of colorectal adenomas in patients with familial adenomatous polyposis (38). Most importantly, sulindac has been approved by the Food and Drug Administration as an adjunct in the treatment of familial adenomatous polyposis (39, 40).

In conclusion, our results indicate that sulindac appears to exert antineoplastic effects in vivo in the HOCM. Sulindac seems to prevent the development of epithelial dysplasia and oral SCC in the HOCM. Furthermore, our observations support that sulindac delays the transformation of epithelial dysplasia to oral SCC, and reduces tumor growth in oral SCC in the HOCM. Sulindac appears to exert antiproliferative and apoptotic effects in carcinogenesis and to downregulate survivin expression in the HOCM.

The results of this study suggest that sulindac may play a potential role in the management of oral epithelial dysplasia and in the chemoprevention of malignant transformation into oral SCC in humans and may exert beneficial effects in patients with prominent risk factors for the development or relapse of epithelial dysplasia or SCC. Furthermore, it may be proved helpful as an adjuvant chemotherapeutic factor in patients with oral SCC. We consider that the results of this study taken together with the results of the aforementioned in vitro (21, 25, 26) and in vivo (26, 36) studies pave the way for clinical trials.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: K. Katoumas, N. Nikitakis
Development of methodology: K. Katoumas, N. Nikitakis, D. Perrea, I. Dontas
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Katoumas, D. Perrea, I. Dontas
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K. Katoumas, D. Perrea
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