Topical 5-Fluorouracil is a Novel Targeted Therapy for the Keratocystic Odontogenic Tumor

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Purpose: The antimetabolite drug, 5-fluorouracil (5-FU), is used in the treatment of various cancers, including basal cell carcinomas (BCCs). The authors hypothesized that keratocystic odontogenic tumors (KOTs) would respond to 5-FU treatment because of their similarities to BCCs in molecular etiopathogenesis.

Materials and Methods: An ambispective cohort study of the treatment efficacy of topical 5-FU on KOTs was conducted. Independent variables included the topical application of 5% 5-FU or modified Carnoy's solution (MC) after enucleation and peripheral ostectomy at the University of Toronto from 2006 through 2014. Outcome variables included time to recurrence and peripheral nerve injury. KOT specimens in these patients were immunostained with p53, Ki-67, thymidylate synthetase (TS), thymidylate phosphorylase (TP), and dihydropyrimidine dehydrogenase (DPD) antibodies. Semiquantitative staining scores were calculated for all immunohistochemistry sections examined. Descriptive statistics were computed using Fisher exact test and Kaplan-Meier analysis as appropriate with the *P* value set at .05.

Results: Thirty-two patients with 32 KOTs were reviewed (41% in women and 59% in men). There were no KOT recurrences in the 5-FU group (n = 11), whereas there were 4 recurrences in the MC group (n = 21; $P = .190$). There was a significantly lower incidence of inferior alveolar nerve paresthesia with 5-FU treatment ($P = .039$). Immunohistochemical staining showed upregulation of TP ($P < .0001$) and DPD ($P < .0001$) and no change in TS ($P > .05$) in inflamed KOTs.

Conclusions: 5-FU effectively treats KOTs with less postoperative morbidity than conventional treatment with MC. Low TS and upregulated TP expressions in inflamed KOTs suggest increased 5-FU efficacy in inflamed KOTs. Topical 5-FU is a novel therapy for KOTs and provides a targeted molecular approach to treatment.

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Keratocystic odontogenic tumors (KOTs) are benign lesions occurring in the maxilla or mandible with potential for serious morbidity. Reports of bone erosion,[1] orbital invasion,[2] skull base extension,[3] and temporal fossa violation[4] show the aggressive nature of this lesion. Previously known as odontogenic keratocysts (OKCs), KOTs were subsequently reclassified as a tumor by the World Health Organization to better represent the neoplastic nature of this lesion.[5]

Historically, treatment of the KOT involved simple enucleation; however, this was suboptimal because of a high recurrence rate. Other treatment options include

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marsupialization, curettage, peripheral ostectomy, adjunctive solution application, removal of overlying mucosa, or resection, alone or in combination.[6-8] Enucleation alone resulted in recurrence rates as high as 56%, whereas resection resulted in recurrence rates closer to 0%.[8,9] Adjunctive application of a chemical fixative called Carnoy's solution (CS; absolute alcohol, glacial acetic acid, chloroform, and ferric chloride) was shown to decrease the rate of recurrence after enucleation. However, chloroform was removed from CS because of its carcinogenicity, giving rise to modified CS (MC).[10,11] A recent study associated the use of MC with markedly higher recurrence rates than with the original CS.[12] Rates of peripheral nerve injury after the application of CS were estimated at 18.2%[13] after direct application of the solution onto the nerve with the 3-minute protocol defined by Frerich et al.[14] Liquid nitrogen after enucleation of the lesion resulted in recurrence rates of 11.5%,[15] which is similar to recurrence rates with CS.[8,13]

A targeted approach to KOT treatment has been proposed based on the current understanding of the molecular genetics of KOTs.[16,17] Molecular studies focusing on the protein patched homolog (PTCH) tumor suppressor gene pathway have yielded a targeted treatment approach for basal cell carcinomas (BCCs). It is known that KOTs develop through PTCH gene mutations similar to BCCs.[18] Mutations in PTCH1 cause smoothened (SMO) activation and sonic hedgehog (SHH) signaling, resulting in neoplastic growth.[19] More recently, Rui et al[20] reported that SMO gene alterations likely play an important role in KOT development. This finding suggests that SHH signaling pathway antagonism might be an efficient way to molecularly target KOTs through SMO inhibition and suppression of SHH transcription factors.[21] A recent study has shown that orally administered vismodegib, an SHH inhibitor, could help decrease the number and morbidity of multiple BCCs and KOTs in patients with nevoid basal cell carcinoma syndrome.[22]

The antimetabolite drug, 5-fluorouracil (5-FU), was shown to induce apoptosis by inhibiting SHH in hepatocellular carcinoma cells.[23] 5-FU has different applications in the treatment of malignant disease, including topical application to treat superficial BCCs.[24] Salonga et al[25] showed that thymidylate synthetase (TS), dihydropyrimidine dehydrogenase (DPD), and thymidine phosphorylase (TP) are independent predictive measurements of tumor responsiveness to 5-FU treatment. Increases in TS mRNA have been used as a marker of resistance to 5-FU.[26-30] Downregulation of TS results in increased efficacy of 5-FU in colorectal cancer cell lines.[31] DPD is an enzyme involved in uracil and thymidine catabolism and is responsible for the breakdown of 5-FU into its excretory metabolites.[32] Low expression of DPD suggests an improved response to 5-FU treatment because DPD is used to break down 5-FU.[25] Conversely, increased expression of TP suggests improved responsiveness to 5-FU because of increased fluorodeoxyuridine monophosphate (FdUMP), an active metabolite of 5-FU.[33]

The purpose of this study was to determine the efficacy of topical 5% 5-FU in the treatment of KOTs. The authors hypothesized that 5-FU would be an effective treatment for KOTs with similar or lower recurrence rates and less morbidity than treatment with conventional application of MC. The specific aims of the study were *1)* to determine the incidence of KOT recurrence and inferior alveolar nerve injury when treated with topical 5% 5-FU compared with MC and *2)* to evaluate the expression of molecular markers (TS, TP, and DPD) that might predict the response to 5-FU.

Materials and Methods

STUDY POPULATION AND DESIGN

To address the research purpose, the authors designed and implemented an ambispective study of patients treated with topical application of 5-FU vs MC after enucleation and peripheral ostectomy of KOTs. The study population was composed of all patients presenting for evaluation and management of KOTs from 2006 through 2014 at the University of Toronto and Mount Sinai Hospital (Toronto, ON, Canada).

To be included in the study sample, patients had *1)* a biopsy-proven KOT (OKC), *2)* a complete history and clinical examination before definitive surgical intervention, and *3)* completed surgical intervention for KOT. Patients were excluded as study subjects if they had *1)* a diagnosed psychiatric condition, *2)* multiple KOTs or diagnosed Gorlin-Goltz syndrome, *3)* a recurrent KOT, *4)* a prior trigeminal nerve injury or existing paresthesia, or *5)* a diagnosis of orthokeratinizing odontogenic cyst or odontogenic keratocyst of the orthokeratinized variant.

STUDY VARIABLES

The independent variables for this study were KOT treatment with topical 5% 5-FU vs MC. Primary outcome variables included *1)* time to KOT recurrence (months) and *2)* incidence of inferior alveolar nerve injury. Independent variables to evaluate the expression of molecular markers with immunohistochemistry included *1)* TS, *2)* TP, and *3)* DPD. The primary outcome variable for immunohistochemistry was the presence of staining. Other study variables included age (years), gender, tumor location (mandible or maxilla), and tumor size (millimeters).

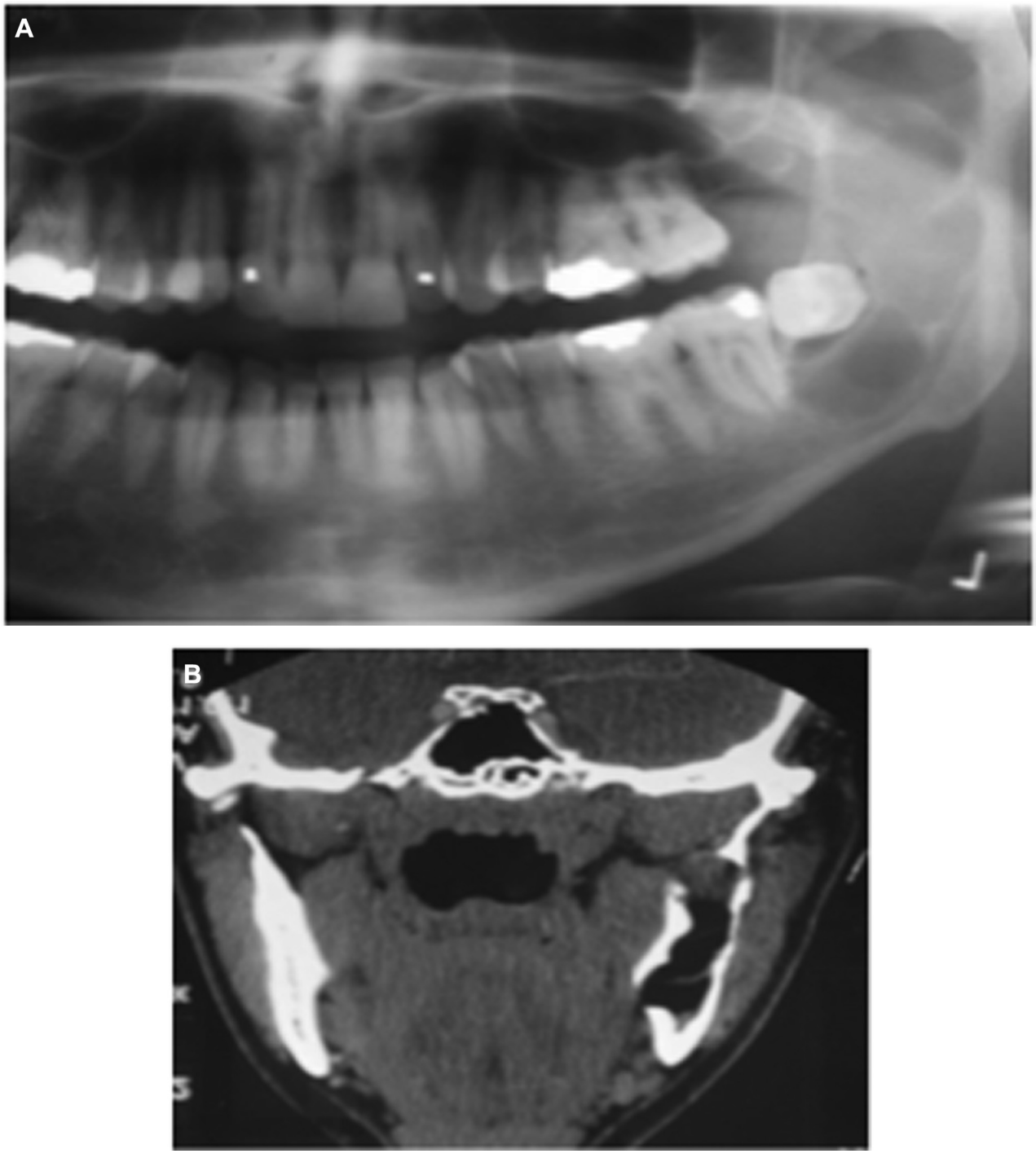

FIGURE 1. 5-Fluorouracil application technique for the keratocystic odontogenic tumor. *A*, Panorex and *B*, computed tomographic images are used as necessary in the preoperative workup and evaluation, which includes incisional biopsy examination. The patient with a biopsy-confirmed keratocystic odontogenic tumor is brought to the operating room and undergoes enucleation and peripheral ostectomy in the standard fashion. **(Fig 1 continued on next page.)**

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DATA COLLECTION METHODS

Ethics approval was obtained from the Mount Sinai Hospital (protocol 15-0011-E) and the University of Toronto (protocol 31638) research ethics boards to perform the ambispective chart review of KOT cases treated with 5-FU or MC. Patient records were located by a retrograde search of operating room case lists and by searching cyst enucleation codes for procedures performed in the clinic as set out by the Ontario Dental Association 2014 fee guide for all attending oral and

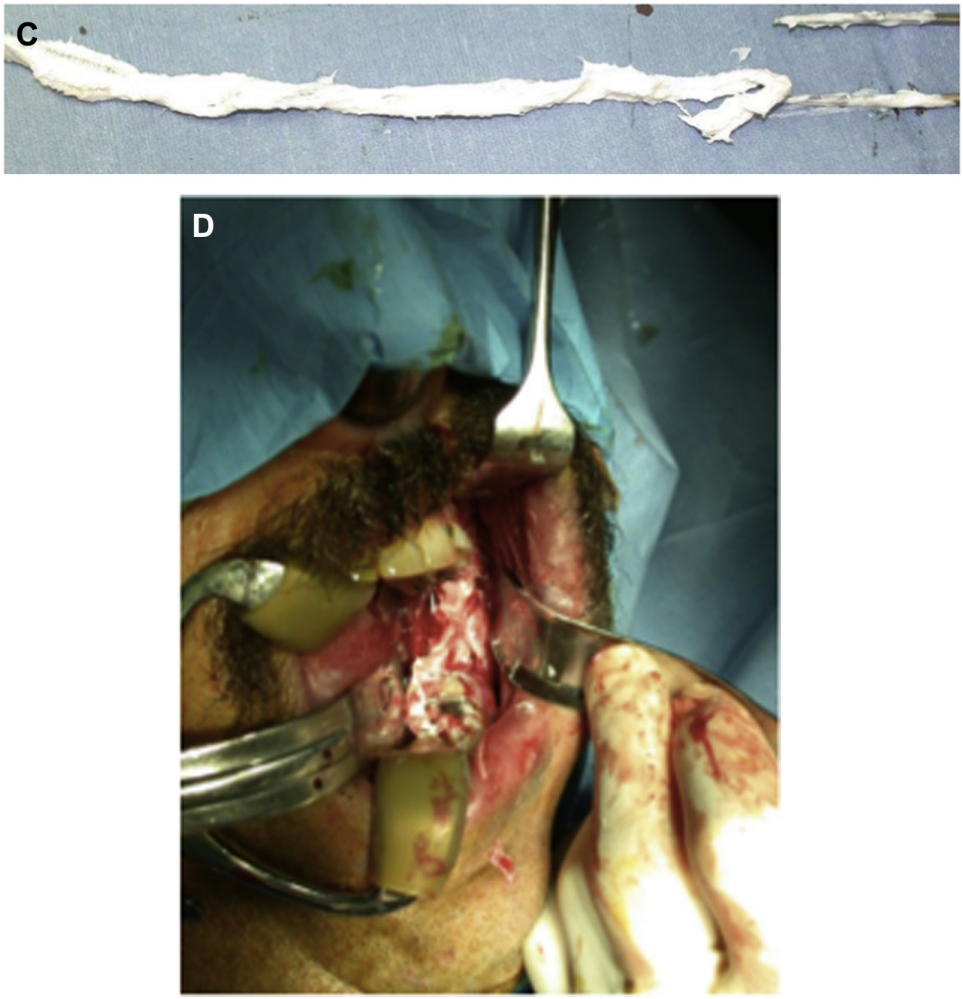

FIGURE 1 (cont'd). Topical 5% 5-fluorouracil cream (Efudex) is *C*, applied generously to a ¼-inch ribbon gauze and *D*, packed into the entire wound covering all surfaces. The wound is closed in a standard fashion leaving approximately 1 cm of ribbon gauze out of the wound. The entire ribbon gauze is removed 24 hours postoperatively. No further lavage or rinsing of the surgical site is performed during the postoperative phase.

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maxillofacial surgeons at Mount Sinai Hospital from 2006 through 2014. Key terms to identify and locate charts included *cyst, enucleation, Carnoy's solution, 5-fluorouracil, KOT, OKC, keratocyst, keratocystic odontogenic tumor,* and *odontogenic keratocyst.* Operative notes, pathology reports, and associated clinical records were reviewed.

CLINICAL AND ORAL BIOPSY EXAMINATIONS

A comprehensive history and examination was performed for all patients to rule out a history of medical conditions or disorders that could alter their trigeminal sensory perception. Oral biopsy specimens of all patients meeting the inclusion criteria were evaluated by the Mount Sinai Hospital or the University of Toronto Oral Pathology Biopsy Service to confirm the diagnosis of KOT. Demographic information was collected for each patient, including age, gender, lesion location, radiographic appearance, and tumor size. The procedure, risks, alternatives, and benefits of treatment with 5-FU or MC were reviewed with the patient and informed consent was obtained.

TOPICAL APPLICATION OF 5-FU

After enucleation and peripheral ostectomy of the KOT lesion, sterile ¼-inch ribbon gauze was coated with 5% 5-FU (Efudex, Valeant Inc, Laval, QC, Canada) and packed into the surgical wound. Then, the wound was closed in the usual manner, leaving a small distal end (approximately 1 cm) of gauze exposed to allow gauze removal at 24 hours postoperatively (Fig 1).

TOPICAL APPLICATION OF MC

After intraoperative enucleation and peripheral ostectomy of the KOT lesion, the surrounding soft tissues were protected with multiple sterile neuro

patties coated with petroleum jelly. Then, MC-saturated neuro patties were carefully placed in the surgical wound so that every discernable surface of the lesional cavity was exposed to MC for 3 minutes[14] followed by thorough normal saline irrigation. Then, all instruments exposed to MC were removed from the operative field, and the surgical team re-gowned and gloved to prevent possible injury to healthy tissues by the caustic MC during wound closure.

IMMUNOHISTOCHEMISTRY

Immunohistochemical staining was performed to evaluate the expression of markers that could predict the response to 5-FU. Proliferative activity and DNA damage response were assessed by staining for Ki-67 (mouse monoclonal antibody against MIB-1; M7240, Dako North America Inc, Carpinteria, CA) and p53 (DO-1 mouse monoclonal antibody; M7001, Dako North American Inc), respectively. Responsiveness to 5-FU was assessed by staining for TS (mouse monoclonal antibody; M3614, Dako North America Inc), TP (mouse monoclonal antibody; ab3151, Abcam, Toronto, ON, Canada), and DPD (rabbit monoclonal antibody; ab134922, Abcam). In 14 of the 32 cases, paraffin blocks with sufficient tissue were available for immunohistochemical analyses using a similar methodology that the authors[34] and others[35] used previously. Four-micrometer-thick sections of formalin-fixed paraffin-embedded tissues were placed on charged slides (VWR Superfrost Plus, catalog number 48311-703; VWR, Radnor, PA), dried at 60°C for 1 hour, deparaffinized, and rehydrated through graded alcohols. Immunohistochemical staining was performed according to the manufacturer's guidelines using the BenchMark XT automated slide stainer (Ventana Medical System, Tucson, AZ) with standard antigen retrieval (CC1, Tris, borate, ethylenediaminetetraacetic acid; pH 8.0; catalog number 950-124). The dilution and incubation time for each primary antibody are presented in Table 1. Positive controls were selected according to information published in the Human Protein Atlas (http://www.proteinatlas.org) and included tonsil (TS, TP, and p53), spleen (DPD), and colon (Ki-67/MIB-1). A Ventana Ultraview Universal DAB Detection Kit (catalog number 760-500), containing a cocktail of enzyme-labeled secondary antibodies that locate the bound primary antibody, was used. The complex was visualized with hydrogen peroxide substrate and 3,3′-diaminobenzidine tetrahydrochloride chromogen, which produced a dark brown reaction product. The slides were counterstained with Gill modified hematoxylin, dehydrated in graded alcohol, cleared in xylene, and placed in coverslips coated with Permount.

Ki-67, p53, TS, TP, and DPD labeling indices (LIs) were calculated as percentages of positive cells among at least 500 epithelial cells in 5 randomly selected fields, which were compared with their respective positive controls.[35] A Leica DM2500 microscope equipped with a DFC320 camera and application suite 4.4.0 (build:454) software was used to obtain photomicrographs at ×200. A semi-quantitative scoring system was used initially: 0 to 10% staining was considered negative (−), 11 to 50% was considered positive (+), and 51 to 100% was considered strongly positive (++).[36] For comparing non-inflamed and inflamed fields of KOTs, a simplified scoring system was used in which positive and strongly positive staining were scored as 1 and negative staining was scored as 0. All LIs were analyzed independently by 2 blinded reviewers (N.L. and D.L.) and the results were compared. Any differences were resolved by direct comparison together at the microscope. Percentages of immunoreactive positive cells from LIs were summarized as mean percentage.

DATA ANALYSIS

Data are reported as mean ± standard error; Fishers exact tests and Kaplan-Meier analysis were used as appropriate ($P < .05$ considered to reflect statistical significance) using SPSS 22.0 (SPSS, Inc, Chicago, IL) for analysis.

Table 1. DILUTION AND INCUBATION TIMES FOR PRIMARY ANTIBODIES

Antibody	Source	Host	Product Number	Dilution	Incubation Time (minutes)
Ki-67	Dako	mouse	M7240	1:100	60
p53	Dako	mouse	M7001	1:250	32
TS	Dako	mouse	M3614	1:50	60
TP	Abcam	mouse	ab3151	1:1,000	60
DPD	Abcam	rabbit	ab134922	1:2,000	60

Abbreviations: DPD, dihydropyrimidine dehydrogenase; TP, thymidine phosphorylase; TS, thymidylate synthetase.

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Results

PATIENT DEMOGRAPHICS

Thirty-two patients with 32 KOTs were reviewed (41% in women and 59% in men; $P > .05$). The mean age at diagnosis was 42 years 2 months ± 2.9 years ($P > .05$). Mandibular lesions accounted for 27 of 32 KOTs, with the remaining 5 found in the maxilla ($P > .05$). Twenty-one KOTs were treated with enucleation, peripheral ostectomy, and topical application of MC, and 11 KOTs were treated by enucleation, peripheral ostectomy, and topical application of 5% 5-FU cream (Table 2). There were no significant differences in patient demographics between the 2 treatment groups ($P > .05$).

KOT RECURRENCES

Mean KOT measurements were of 34.8 × 44.5 mm (3.9 × 4.8 mm) in the MC group and 28.4 × 30.1 mm (4.1 × 6.1 mm) in the 5-FU group ($P > .05$). In the MC group (n = 21), there were 4 recurrences (19.0%) with a mean recurrence time of 26.3 ± 1.8 months and a mean follow-up time of 41.3 ± 3.8 months. In contrast, there were no recurrences in the 5-FU group (n = 11) with a mean follow-up time of 35.0 ± 8.5 months ($P = .19$; Table 3). All 5-FU–treated cases exhibited normal bony healing (Fig 2). A Kaplan-Meier analysis (Fig 3) was performed to illustrate differences in the time to recurrence between the 2 treatment groups ($P > .05$).

PATIENT MORBIDITY

There were no adverse local or systemic events in response to 5-FU or MC application. In 14 of 18 mandibular cases (77.8%) treated with MC, postoperative inferior alveolar nerve paresthesia was noted with a mean recovery time of 29.0 ± 10.6 weeks. Four of these cases (22.2%) resulted in permanent paresthesia. In contrast, only 3 cases (33.3%) of 5-FU–treated patients had transient paresthesia that resolved in a mean time of 42.0 ± 10.0 weeks ($P = .039$; Table 4).

IMMUNOHISTOCHEMISTRY

Ki-67 and p53 immunopositivity was observed in the basal and suprabasal nuclei of KOT epithelium (LI, 18.64 and 16.56%, respectively; Fig 4).

TS and DPD staining was observed in the cytoplasm, whereas TP yielded nuclear and cytoplasmic staining (Fig 4). Sum scores calculated for TS, TP, and DPD showed mainly negative TS immunostaining, whereas positive TP and DPD staining was seen in 8 of 14 cases (Table 5).

Of the 14 cases evaluated, 10 (71.4%) showed inflammation in the cyst lining in at least 1 area of

Table 2. DEMOGRAPHICS OF KERATOCYSTIC ODONTOGENIC TUMOR CASES TREATED USING MC OR 5-FU

	MC	5-FU	Total
Cases, n	21	11	32
Age, mean (SE)	42 yr 3 mo (3.7 yr)	42 yr 1 mo (4.8 yr)	42 yr 2 mo (2.9 yr)
Gender			
Women	9	4	13
Men	12	7	19
Location			
Mandibular body	3	6	9
Mandibular ramus	1	1	2
Mandibular condyle	0	0	0
Anterior mandible + body	1	0	1
Mandibular body + ramus	12	2	14
Mandibular body + ramus + coronoid process	1	0	1
Anterior maxilla	1	1	2
Maxillary premolar + molar	2	0	2
Anterior maxilla + maxillary premolar + molar	0	1	1
Radiographic appearance			
Unilocular	12	9	21
Multilocular	7	4	11
Lesion size (mm, width × height), mean (SE)	34.8 × 44.5 (3.9 × 4.8)	28.4 × 30.1 (4.1 × 6.1)	31.6 × 37.3 (2.9 × 3.9)

Abbreviations: 5-FU, 5-fluorouracil; MC, modified Carnoy's solution; SE, standard error.

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Table 3. RECURRENCES IN KERATOCYSTIC ODONTOGENIC TUMOR TREATED WITH MC OR 5-FU

	MC (n = 21)	5-FU (n = 11)
Recurrences	4	0
Time to recurrence (mo), mean (SE)	26.25 (1.8)	N/A
Follow-up time (mo), mean (SE)	41.3 (3.8)	35.0 (8.5)

Abbreviations: 5-FU, 5-fluorouracil; MC, modified Carnoy's solution; N/A, not applicable; SE, standard error.

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all sections examined. Inflamed areas of KOT showed significantly higher expression of TP ($P < .0001$) and DPD ($P < .0001$) in the epithelial lining compared with non-inflamed areas (Fig 4). TS expression was low in non-inflamed and inflamed areas (Fig 4). There were no differences in the LI for Ki-67 or p53 between non-inflamed and inflamed fields ($P > .05$).

All inflamed fields stained with TP (27 of 27) and DPD (24 of 24) were positive, whereas TS (16 of 26) stained minimally in the inflamed fields. This is in contrast to the non-inflamed fields where minimal positive staining was observed for all 3 markers (TS, 18 of 44; TP, 7 of 43; DPD, 6 of 41; Table 6, Fig 4).

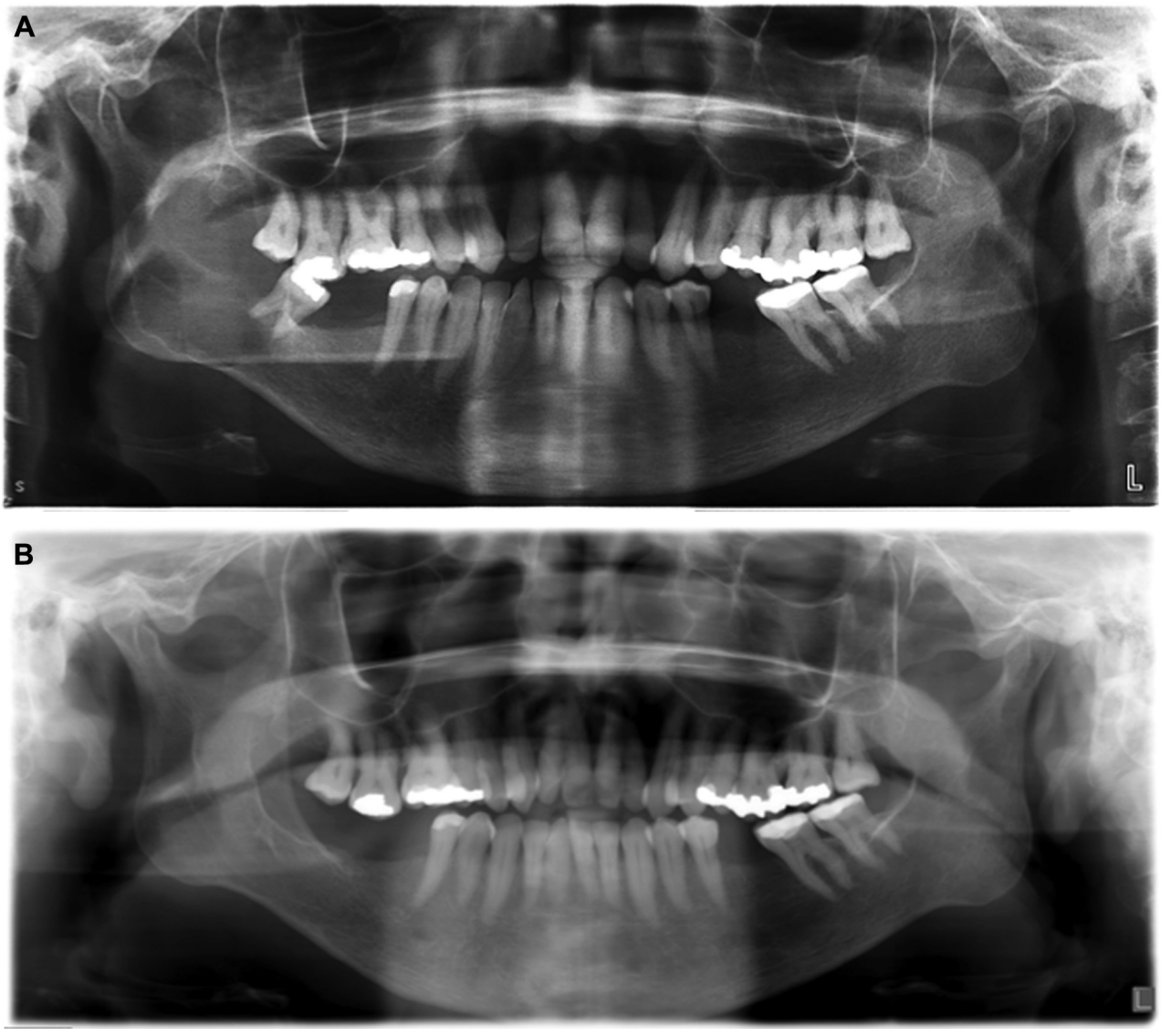

FIGURE 2. Representative example of keratocystic odontogenic tumor treated with 5-fluorouracil. *A*, Preoperative Panorex radiograph showing a biopsy-confirmed keratocystic odontogenic tumor involving the right mandibular body, ramus, and coronoid process. *B*, Two-year postoperative Panorex radiograph showing a well-healed, tumor-free right mandible treated with enucleation, peripheral ostectomy, and topical application of 5% 5-fluorouracil cream (Efudex).

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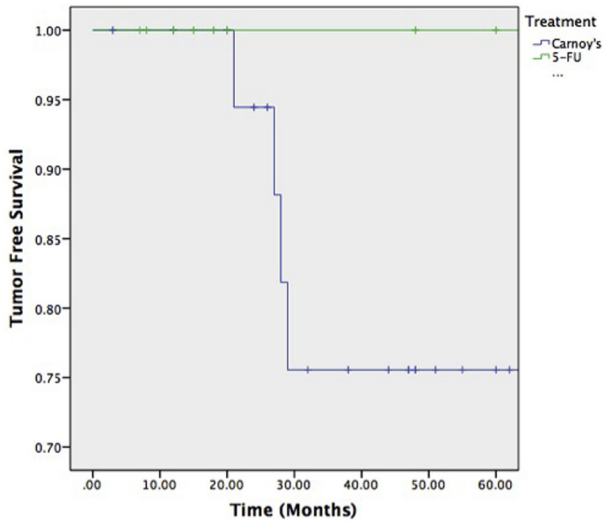

FIGURE 3. Kaplan-Meier survival analysis comparing 5-year data for patients treated with 5-FU or modified Carnoy's solution. The *y*-axis represents the percentage of patients free of tumor and the *x*-axis denotes time in months ($P > .05$). 5-FU, 5-fluorouracil.

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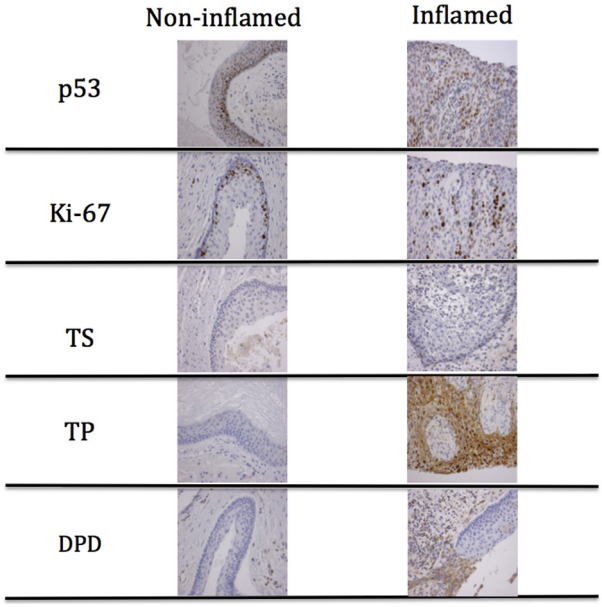

FIGURE 4. Representative immunohistochemical staining of non-inflamed and inflamed areas of keratocystic odontogenic tumors for p53, Ki-67, TS, TP, and DPD (magnification, ×200). P53 and Ki-67 staining was observed in the nuclei of basal and suprabasal epithelial cells. Minimal to no staining for TS was seen in non-inflamed or inflamed areas, TP showed positive nuclear and cytoplasmic staining in the epithelial lining of inflamed areas, and DPD showed positive cytoplasmic staining in the epithelial lining of inflamed areas. DPD, dihydropyrimidine dehydrogenase; TP, thymidine phosphorylase; TS, thymidylate synthetase.

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Discussion

The purpose of this study was to determine the efficacy of using topical 5% 5-FU for the treatment of KOTs. The authors hypothesized that 5-FU would be an effective treatment for KOTs with similar or lower recurrence rates and lower incidence of nerve injury than treatment with conventional application of MC. The authors aimed *1)* to determine the incidence of KOT recurrence and inferior alveolar nerve injury when treated with topical 5% 5-FU compared with MC and *2)* to evaluate the expression of molecular markers TS, TP, and DPD that could predict a response to 5-FU. The authors found for the first time that 5-FU is an effective and novel targeted treatment for KOTs. Topical application of 5-FU, after enucleation and peripheral ostectomy, effectively treats KOTs, resulting in normal bony healing with no adverse local or systemic effects.

5-FU could be more ideal than MC because of its ready availability, technical ease, shorter operating time, similar efficacy, and decreased morbidity compared with MC. 5-FU is simply coated onto ¼-inch ribbon gauze and packed into the residual bony cavity in a manner that allows for easy retrieval at 24 hours postoperatively. In contrast, there is substantially increased operating time when MC is used, because of the need for multiple precautions as described earlier. There were no KOT recurrences in patients treated with 5-FU. Conversely, the 19.0% recurrence rate observed with MC in this study is slightly lower compared with a recent report,[12] which might be explained by the addition of a peripheral ostectomy as a procedural adjunct in the present cohort. The mean recurrence time of 26.3 months also is in line with prior studies.[37] MC can result in major local tissue destruction if not carefully handled. When MC is used to cauterize and fix the perilesional cavity, the blood components and bone turn black, which is likely due to protein precipitation and reaction with ferric chloride.[38] Contact of MC with peripheral

Table 4. INFERIOR ALVEOLAR NERVE INJURY DATA FOR MANDIBULAR CASES TREATED WITH MC OR 5-FU

	MC	5-FU
Total cases	18	9
Postoperative nerve injury cases	14	3*
Neurosensory recovery (wk), average (SE)	29.0 (10.6)	42.0 (10.0)
Permanent nerve injury cases	4	0

Abbreviations: 5-FU, 5-fluorouracil; MC, modified Carnoy's solution; SE, standard error.
* $P < .05$.

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Table 5. IMMUNOHISTOCHEMICAL STAINING SCORES FOR PREDICTORS OF 5-FLUOROURACIL RESPONSE IN KERATOCYSTIC ODONTOGENIC TUMORS

Case	TS	TP	DPD
1	−	+	+
2	−	+	+
3	−	−	−
4	+	+	+
5	−	−	−
6	−	−	N/A
7	−	−	−
8	−	+	+
9	−	++	++
10	−	−	−
11	−	++	+
12	−	+	+
13	−	+	+
14	−	−	−

Note: Sum scores were calculated by counting the total positive cells among 500 cells in 5 randomly selected fields (magnification, ×200) for TS, TP, and DPD.

Abbreviations: −, negative (0 to 10%); +, positive (11 to 50%); ++, highly positive (51 to 100%); DPD, dihydropyrimidine dehydrogenase; N/A, not available; TP, thymidine phosphorylase; TS, thymidylate synthetase.

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nerves causes damage to the perineural tissues when following the 3-minute application protocol defined by Frerich et al.[14] In agreement with prior studies,[13,39] a large majority of the patients with mandibular KOTs treated with MC in the present study developed postoperative paresthesia and a substantial number had permanent neurosensory deficits.

The authors examined 14 representative cases by immunohistochemical staining for markers that could predict responsiveness to 5-FU treatment. All KOTs in this study exhibited a moderate proliferation index, which is in agreement with previous studies[40] and suggests that KOTs are amenable to treatment by inhibition of DNA synthesis with an antimetabolite agent such as 5-FU. The overall low expression of TS in KOTs is suggestive of susceptibility to 5-FU treatment. Studies in cancer cell lines have shown an inverse relation between TS expression and efficacy of 5-FU treatment.[30,31]

The expression of TP and DPD was markedly altered by the presence of inflammation. Areas of inflammation in the cyst wall were seen in most cases (10 of 14) in this study and were likely induced by prior incisional biopsy examination. There was no change in the low expression of TS in inflamed KOT linings, but there was increased expression of TP and DPD. TP can be upregulated by multiple proinflammatory cytokines, including tumor necrosis factor-α, interferon-γ, and interleukin-1α.[41] Greater TP expression in the lining of inflamed KOTs could promote conversion of 5-FU to active metabolites, including FdUMP, and thereby enhance destruction of any residual KOT inadvertently left behind after enucleation. The low TS and high TP expressions in inflamed KOTs suggest that procedures such as prior incisional biopsy examination, marsupialization, and intraoperative enucleation and curettage, which induce inflammation, could increase the efficacy of 5-FU treatment of KOTs. 5-FU has been suggested to induce an intense inflammatory reaction when applied topically to skin within the first 24 hours.[42] Previous studies have suggested increased DPD expression can result in decreased efficacy of systemic 5-FU treatment.[43] This is likely related to metabolic inactivation of systemic 5-FU by hepatic DPD or tumor DPD. The topical application of 5-FU in the present study avoids the problem of inactivation by hepatic DPD metabolism. The effect of increased DPD expression in inflamed KOTs on the efficacy of 5-FU treatment is unclear.

This is the first study that shows the efficacy and versatility of topical 5-FU application by packing the surgical site with 5-FU–impregnated ribbon gauze. This technique can be used for hard-to-treat areas of cortical perforation, in contrast to the relative contraindications for MC use in areas of cortical perforation. Similarly, 5-FU could be more amenable than MC for lesions in the posterior maxilla in close proximity to major vessels of the head and neck, orbital contents, and the maxillary sinus, where there are concerns of vascular injury, neurovascular injury, and sinus necrosis. Periorbital connective tissues also seem to be unaffected by twice-daily application of topical 5-FU when used to treat ocular surface squamous neoplasia.[44] No studies to date have shown the effect of direct application of topical 5-FU to major blood vessels; however, twice-weekly application of topical 5% 5-FU for 4 weeks after medial maxillectomy and sphenoethmoidectomy for ethmoidal adenocarcinoma showed no adverse effects on the infraorbital nerve or the remaining sinus mucosa.[45,46]

There were no adverse effects from topical application of 5-FU in the present study. However, systemic administration of 5-FU can result in adverse responses, including mucositis, granulocytopenia, neuropathy, cardiac toxicities, nausea, vomiting, pallor, hypotension, general malaise, and death.[47,48] Approximately 3 to 5% of the population is partially deficient in DPD, which can cause an intense systemic toxicity when 5-FU is used in any treatment. This is most prevalent in African-American women, with up to 12% of this particular demographic reported to be deficient in DPD; therefore, caution should be exercised when treating with 5-FU. The benefit of topical application

Table 6. IMMUNOHISTOCHEMICAL STAINING SCORES FOR PREDICTORS OF 5-FLUOROURACIL RESPONSE ACCORDING TO THE PRESENCE OF INFLAMMATION IN KERATOCYSTIC ODONTOGENIC TUMORS

	TS		TP		DPD	
Case	Not Inflamed	Inflamed	Not Inflamed	Inflamed	Not Inflamed	Inflamed
1	0/2	3/3	0/2	3/3	0/2	3/3
2	0/2	3/3	0/2	3/3	0/2	3/3
3	1/5	—	1/5	—	0/5	—
4	2/3	2/2	0/2	3/3	1/2	3/3
5	4/5	—	2/5	—	0/5	—
6	0/3	0/2	0/3	2/2	N/A	N/A
7	0/5	—	1/5	—	0/5	—
8	2/2	1/3	0/2	3/3	0/2	3/3
9	1/2	2/3	1/2	3/3	3/3	2/2
10	3/4	1/1	1/4	1/1	0/4	1/1
11	1/2	2/3	1/2	3/3	1/2	3/3
12	0/2	2/3	0/2	3/3	0/2	3/3
13	1/2	0/3	0/2	3/3	1/2	3/3
14	3/5	—	0/5	—	0/5	—
Total	18/44	16/26	7/43	27/27*	6/41	24/24*

Note: TS, TP, and DPD staining values were assessed in 5 fields (magnification, ×200) for each case (100 cells per field). Non-inflamed and inflamed fields were separated for each case. Positive and highly positive staining values were scored as 1 and negative staining was scored as 0. The proportion of positively stained fields was indicated for non-inflamed and inflamed fields.

Abbreviations: DPD, dihydropyrimidine dehydrogenase; N/A, not available; TP, thymidine phosphorylase; TS, thymidylate synthetase.

* $P < .001$.

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of 5-FU in a controlled fashion as shown in this study is the avoidance of untoward side effects.

KOTs are reasonably rare, which makes having large-scale prospective studies difficult to achieve. As such, this study was limited by the number of cases and follow-up times in the MC and 5-FU groups. A simple power calculation suggests that 80 cases should be included to better extrapolate the findings to the general population. This ambispective study with a heavy retrospective component had disadvantages, such as possible selection bias and information bias. It is often difficult to assess temporal relations among study variables.

Reports of KOT recurrences have been noted up to 25 years after the initial treatment.[49] Therefore, it is prudent that well-designed, long-term, prospective, randomized, double-blinded, controlled (multicenter) clinical trials are developed to truly analyze these factors and determine optimal sequences for treatment and follow-up based on outcomes and recurrence patterns. Future studies comparing the efficacy of 5-FU with new medication classes such as SHH inhibitors should be considered.

5-FU is a novel, effective, targeted treatment for KOTs with lower recurrence rates and less morbidity compared with MC. Inflamed KOTs might be more likely to respond to 5-FU treatment based on the present immunohistochemical findings. The advantages of topical 5-FU include decreased postoperative morbidity, lower risk of reoperation, lower cost, and straightforward technique. It also is a known, accessible, and well-studied drug. Further molecular characterization, prospective clinical trials, and consideration of additional targeted medications such as SMO or SHH inhibitors are suggested for the treatment of KOTs.

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