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Analgesic and Non Steroidal Anti-Inflammatory Use in Relation to Non Melanoma Skin Cancer: A Population-Based Case-Control Study

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Abstract

Background—Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) are potentially chemopreventive.

Objective—We examined the relation between NSAID use and non-melanoma skin cancer in a population-based case-control study.

Methods—NSAID and analgesic use was analyzed in 1484 subjects: 535 squamous cell carcinoma (SCC), 487 basal cell carcinoma (BCC), and 462 controls.

Results—Use of NSAIDs, particularly aspirin, related to reduced odds ratios (OR) of SCC especially tumors positive for p53 (OR = 0.29; 95% CI= 0.11-0.79) or with *PTCH* loss of heterozygosity (OR = 0.35; 95% CI = 0.13 – 0.96). Although not considered an NSAID, decreased odds ratios of both BCC and SCC were observed in relation to use of paracetamol. Risk of BCC was unrelated to NSAID use.

Limitations—Self reported drug use.

Conclusions—This study supports the hypothesis that NSAIDs, aspirin in particular, may reduce risk of SCC and additionally may affect specific molecular subtypes of SCC.

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Keywords

NSAIDS; non-melanoma skin cancer; basal cell carcinoma; squamous cell carcinoma; p53; PTCH; case-control study

Introduction

Nonmelanoma skin cancer (NMSC) is the most common form of cancer in the United States with more than one million skin cancers diagnosed annually. Between 40 and 50 percent of Americans who live to age 65 will have NMSC at least once [1]. The major environmental risk factor for the development of NMSC is ultraviolet (UV) light exposure [2]. In addition to UV, risk factors for NMSC include exposure to ionizing radiation, arsenic or organic chemicals, human papillomavirus infection, and immuosuppression [2].

Two important tumor suppressor genes in the pathogenesis of NMSC are *TP53* and *PTCH*. *PTCH* is the human homolog of the drosophila tumor suppressor gene, Patched, and encodes a receptor that mediates Hedgehog signaling [3], a key pathway in the regulation of cell growth and differentiation and tumorigenesis. PTCH represses hedgehog target gene expression through its interaction with Smoothened (*SMO*), and this repression is relieved when Sonic Hedgehog (*SHH)* binds *PTCH*, or after *PTCH* has been inactivated through mutation. Inactivating mutations in *PTCH* result in constitutive activation of hedgehog signaling and are a common event in sporadic BCC [4]. Mutations in *PTCH* are associated with Gorlin syndrome, a rare autosomal dominant hereditary nevoid BCC syndrome [5]. Although less commonly recognized, *PTCH* is also frequently mutated in SCC. In a previous report, we demonstrated a high prevalence of any *PTCH* LOH in both BCC (75.5%) and SCC (60.8%) tumors although BCCs were more likely to contain LOH than SCCs ($P<0.009$) [6].

TP53 is the most frequently mutated gene in human cancer [7], and is mutated in both SCC and BCC [8]. The p53 protein regulates signaling pathways involved in cell division and apoptosis, and serves as a sensor of cytotoxic stress. Mutations in *TP53* occur early in the progression of SCC, and may facilitate genomic instability and subsequent acquisition of additional genetic mutations [9].

In addition to mutations in these tumor suppressor genes, another genetic event associated with NMSC is overexpression of cyclooxygenase 2 (*COX2*) [now designated prostaglandin-endoperoxide synthase 2 *(PTGS2)*]. *COX2* is involved in the synthetic pathway of prostaglandins. The *COX2* isoenzyme induces antiapoptotic, proangiogenic and other tumorigenic pathways [10]. Although *COX2* is not overexpressed in BCC, it is in 40% of SCC tumors [11]. This has led to the suggestion that non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, that inhibit *COX2*, may be useful in the treatment or prevention of SCC. Consistent with this hypothesis, in mouse carcinogenesis models, both systemic and topical application of NSAIDs inhibited the formation of SCC, and specifically UVR-induced lesions [12, 13]. A number of clinical trials have demonstrated regression of actinic keratoses in response to the topical NSAID, diclofenac (a potent *COX2* inhibitor) [14-16].

Although both animal models and small clinical trials suggest a protective effect of NSAIDs on NMSC, there are limited epidemiologic data. A nested case-control study of SCC from Australia (n=86 cases and 187 controls) found a dramatically reduced risk among long-term NSAIDs users (i.e., OR = 0.37 for use ≥2 times per week and OR = 0.07 for use ≥8 times per week for 5 years or longer) compared to non-users [17]. An analysis of NSAID use in 1402 participants in the SKICAP-AK trial in Arizona indicated reduced hazard ratios for

both BCC (HR=0.43, 95% CI = 0.25-0.83) and SCC (HR = 0.49, 95% CI = 0.28-0.87) among subjects who were new users of NSAIDs, but not among continuous users [18]. Similarly, data from a prevention trial found evidence of a decreased risk of subsequent SCC occurrences in NMSC patients who used NSAIDs in the year prior to diagnosis (OR = 0.71; 95% CI = 0.48 – 1.04) [19].

To test whether NSAIDs might exert a chemopreventive effect on NMSC development, we assessed NSAID and analgesic use in a population-based case-control study of 1484 subjects. We explored potential associations between BCC and SCC tumors separately, and among SCC tumors, with specific histologic findings (adjacent actinic keratoses) and anatomic location. We also examined whether any relation between NSAIDs and SCC tumors was associated with molecular alterations in *TP53* or *PTCH*.

Material and Method

Study group

To identify cases for our study, we enlisted the collaboration of dermatologists and pathology laboratories throughout New Hampshire and bordering regions. We selected a random sample of incident BCC cases (for efficiency) and all cases of incident invasive SCC diagnosed from 1 July 1997, through 31 March 2000. The sample of BCC cases was drawn concomitantly with the SCC cases (at a ratio of about 1 to 1). These BCC cases were selected to represent the entire diagnosis group for anatomic site, age, and sex. Eligible subjects included New Hampshire residents who were age 25 to 74 years at the time of diagnosis, spoke English, and had a listed telephone number. A small percentage (<1%) were excluded due to physician refusal to contact. We identified 1403 potentially eligible participants. Of these, we contacted and confirmed the eligibility on 1373 (98%), of whom 1118 (81%) were interviewed and enrolled (540 BCC and 578 SCC cases).

We chose controls from New Hampshire residents age 25–74 years who were frequency-matched on age (25–34, 35–44, 45–54, 55–64, 65–69, and 70–74 years) and sex to the combined distribution of the SCC and BCC cases. We selected controls (roughly equal to the number of BCC cases) from lists of New Hampshire residents provided by the New Hampshire Department of Transportation (for those less than 65 years old) and Health Care Financing Administration's Medicare Program (for those 65 years and older). As with the eligibility criteria for cases, controls were required to speak English and to have a listed telephone number. For interviewing purposes, controls were randomly assigned reference dates corresponding to the cases' diagnosis dates. A total of 526 controls (76%) were interviewed and enrolled from a potential 694 confirmed eligible participants.

Personal Interview

All participants provided informed consent in accordance with the Committee for the Protection of Human Subjects at Dartmouth College. Study participants completed an interviewer-administered structured personal interview, usually at their homes. To minimize potential reporting bias, we did not reveal the specific hypotheses of interest to either the interviewer or participant, and we did not inform the interviewers of the case-control status of participants. The interview included sociodemographic information (level of education), use of tobacco, assessment of skin pigment and nevi, and skin sensitivity to the sun after first exposure in the summer (i. e., tendency to sunburn). To estimate sun exposure, we used a standardized questionnaire developed for a case-control study conducted in Australia [20, 21] to inquire about the amount of time spent outdoors on work days, nonwork days, and vacations (both in the summer and other times of the year); history of sunbathing; number of

painful and blistering sunburns; lifetime residential history; and frequency of specific outdoor activities (i.e., swimming, sailing).

Drug use assessment

Subjects were asked if they used a pain medication at least four times a week for at least one month any time prior to the reference date (diagnosis date of the cases and a comparable date randomly assigned to the controls). Those who responded positively were asked the brand names of each medication they took, and for each medication, the age began and stopped, total duration of use and the condition for which the drug was used. Those who responded that they did not use pain medications at the specified frequency were considered non-users.

Brand names were re-coded into active ingredients based on a drug matrix that took into account variations in the composition of the drugs over time, and specifically addressed the exact years of withdrawal/substitution of phenacetin from the formulation. Past editions of the Physician Desk Reference ® were used as the main source of information on drug composition to generate the matrix. Whenever necessary, a variety of other sources were used, such as direct contact with pharmaceutical companies, documents from regulatory agencies and others. Drug categories considered for the analysis were: phenacetin, paracetamol, any NSAID (including aspirin, ibuprofen, diclofenac and any other drug generally included in this category), aspirin, and ibuprofen.

Tumor P53 and *PTCH* LOH

We requested the original paraffin-embedded tumor specimens for histopathology re-review by the study pathologist. A comprehensive pathology review was conducted by the study dermatopathologist, including classification of tumor morphology, grade, associated actinic keratoses, degree of histologic solar elastosis, and percent of tumor present in the tissue specimen. In addition, immunohistochemical analysis of a subset of tumors was performed for p53 and scored for intensity and percent of tumor cells staining positively. We considered a positive cut point a score of 3+ intensity in >10% of the tumorous cells. We further tested for the presence of *TP53* mutations and *PTCH* LOH. For these analyses, tumor DNA was extracted using a standard protocol as previously described [6]. Single strand conformation polymorphism (SSCP) analysis of exons 5-9 of *TP53* was performed with fluorescently labeled primers described by Toguchida, et al. [22]. PCR products were run on an Applied Biosystems 310 capillary based DNA autosequencer. Samples with band shifts were identified for sequencing using Gene Scan 3.1 software (Applied Biosystems), amplified by PCR with unlabeled primers, resolved on agarose gels, and bands were gel extracted with QIAquick Gel Extraction Kit (Qiagen). Extracted products were purified using Centrisep purification columns (Princeton Separations) and cycle sequencing was performed using forward primers and Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing analysis was performed with DNA Sequencing Analysis Software v3.4.5 (Applied Biosystems) and aligned with Sequencher v4.1.2 software (Gene Codes Corporation, Ann Arbor, MI). For LOH analysis, corresponding peripheral blood DNA was isolated for each case (Qiagen). Five microsatellite loci, D9S15, D9S53, D9S196, D9S176, including an intragenic microsatellite in *PTCH* (exon 1a, 1AJL; [23]) were examined, as described [6]. For each informative locus, the allelic ratio was calculated using the peak heights from the blood and tumor-derived DNA samples. LOH was determined by dividing the allelic ratio in the blood-derived DNA by the allelic ratio in the tumor-derived DNA. Ratios of <0.5 or >1.5 were scored as positive for LOH. Two hundred and eighty-two tumors from 247 cases were included in the analysis of LOH. Six cases were not informative at any of the tested loci, leaving a total of 276 tumors from 241 cases. Slides from tumors in 356 cases were immunohistochemically stained and scored for p53. Three hundred and

seventy tissue samples from the diagnostic tumor for each case were included in the analysis of *TP53* mutations. We obtained informed consent from each participant and all procedures used study materials approved by the Committee for the Protection of Human Subjects at Dartmouth College.

Statistical analysis

We computed odds ratios (ORs) and their 95% confidence intervals (CIs) for BCC and SCC for the studied drugs using logistic regression with those who responded that they did not use the pain medications at all or with the specified frequency as the reference category. Risk estimates were adjusted by: age in quintiles according to the age distribution among the controls (<52, 52 to 61, 62 to 67, 68 to 71, and >71 years), sex, the number of cigarettes smoked per day (none, less than 1 pack per day, more than one pack per day), skin response to first sun exposure in the summer (tan or mild burn followed by a tan, painful burn or burn with blistering), the lifelong number of painful sunburns (none, 1-2, >2), and lifelong cumulative number of hours of sun exposure (≤ median hours exposure, > median hours exposure). When assessing the effects of specific types of NSAIDs, we assessed the possibility that other types of pain medications could act as a potential confounder (e.g. effects of aspirin, with ibuprophen, paracetamol, and other NSAIDs included in the model) but the inclusion of these variables did not appreciably influence our results and therefore were not included in our final models. Likelihood ratio tests of interaction were used to assess whether specific NSAID effects were modified by the number of painful sunburns, skin response to sun exposure, or by number of cigarettes smoked.

Duration was classified according to median of control distribution among users for each drug. Tests for trend were performed by including a single term for categorical exposure variables in logistic regression models using non-users as the reference category. Further, we examined a model in which the drug use variables excluded the year prior to the reference date in order to assess the possibility of drug use consequent to undiagnosed tumor symptoms. In addition to evaluating histologic types of tumors separately (BCC and SCC), we analyzed subgroups of SCC tumors according to presence or absence of actinic keratoses in the adjacent skin, anatomic site, p53 immunohistochemistry (3+ intensity in >10% of the tumorous cells present or absent), *TP53* mutation (present or absent), and *PTCH* LOH (present or absent). The statistical package SAS v9.1 was used for all the analyses.

Results

Of 1644 subjects who were eligible and interviewed, pain medication data were available on 1484 (90%). Of these, 487 were BCC patients, 535 were SCC patients, and 462 were controls. Demographic characteristics, sun sensitivity and sunburn history of these subjects are shown in Table 1. Approximately 80% of subjects were over age 52, and more than half were men. As expected, BCC and SCC patients were more likely than controls to report an increased propensity to burn rather than to tan in response to the sun, a higher number of painful sunburns, and greater lifetime cumulative sun exposure (SCC only). Smoking status of patients was not appreciably different from that of controls.

The proportion of NSAID and analgesic use was 58.1% among subjects with BCC, 53.3% among subjects with SCC and 63.3% among controls. Overall there was little to no association between use of NSAIDs and BCC overall (OR = 0.91; 95% CI 0.69-1.21), nor with use of specific types of NSAIDs, although the odds ratio for aspirin was slightly reduced (Table 2). A reduced odds ratio for BCC was found with use of paracetamol, especially among current users (OR = 0.56; 95% CI 0.33-0.97) and those who reported a longer duration of use (OR = 0.54; 95% CI 0.29-1.03) (Table2).

Use of NSAIDs (OR = 0.78 95% CI = 0.59-1.03), and particularly use of aspirin (OR = 0.75; 95% CI = 0.55-1.02), was associated with a modestly reduced risk of SCC (Table 2). While there was no duration effect for NSAIDs overall, the association with aspirin use was strongest for ≤6 years of use (OR = 0.66, 95% CI 0.43 – 1.00) (Table 2). Associations were not observed for propionic acid NSAIDs or ibuprofen. As with BCC, use of paracetamol was associated with reduced risk of SCC (OR = 0.62, 95% CI = 0.40-0.97), especially among current users (OR = 0.56, 95% CI = 0.33-0.97) and users of ≤7 years (OR 0.51 95% = CI 0.27-0.97) (Table 2). Odds ratios for BCC and SCC and NSAID use did not appear to vary by number of cigarettes smoked (data not shown).

We further explored whether effects of NSAID or analgesic use on SCC might be modified by sunlight-related factors. Odds ratios for SCC did not appreciably differ by tumor site (head and neck versus other sites) nor by the presence or absence of associated actinic keratosis (Table 3). The association with paracetamol was evident for the subset of SCC tumors without associated actinic keratosis (OR 0.43, 95% CI 0.20-0.91) but not in those with associated actinic keratosis (OR 1.03, 95% CI 0.57-1.87) (Table 3). No significant interactions were detected between any specific NSAID or analgesic drug type and the number of painful sunburns or to skin response to initial solar exposure (data not shown).

We next analyzed use of NSAIDs and analgesics in relation to subgroups of SCC tumors, defined by presence of molecular alterations in *TP53* or *PTCH* (Table 4). Stratifying cases by presence of molecular alteration, ever user of NSAIDs was associated with a lower risk of developing tumors with altered p53 or *PTCH* (Table 4). Similarly, ever use of aspirin or ibuprofen was related to a lower risk of SCC tumors positive for p53 (by IHC or *TP53* mutation analysis) or with *PTCH* loss.

Discussion

Overall, our data provide some support for the hypothesis that NSAID use may reduce risk of SCC, but not BCC. Decreased ORs associated with NSAID use, and aspirin specifically, were seen primarily in the subset of SCC tumors positive for p53 (by IHC or *TP53* mutation analysis), and with *PTCH* LOH. We did not find evidence of effect modification by sun exposure. Additionally, although not considered an NSAID, we found decreased odds ratios for both BCC and SCC with current use of paracetamol.

Previous studies have suggested a potential protective effect of NSAIDs on SCC risk; however, the magnitude of association, as well as effects by duration of use, has varied among studies. A nested case control study of 86 SCC patients and 187 controls from Queensland Australia reported a substantially lower risk (OR = 0.07; 95% CI = 0.01-0.07) associated with >5 years of NSAID use [17]. However, no association was found among current users. An inverse relation with NSAIDs was observed in a subsequent cohort analysis of a retinol prevention trial from Arizona [18]; but with the opposite trend for timing of use: the HR was 0.49 (95% CI = 0.28-0.87) for new users, and was 1.11 for continuous users. A third cohort study, based on a prevention trial [19], observed an OR=0.71 (95% CI = 0.48-1.04) for SCC for users in the year prior to diagnosis. Approximately half of the patients in this study were drawn from clinical centers in northern latitudes (New Hampshire, Minnesota), similar to our study population in New Hampshire. It is possible that geographical factors, including extent and duration of UV exposure in southern versus northern latitudes, impacts the magnitude of the protective effect of NSAIDs, with stronger effects among individuals receiving more intense UV exposure in lower latitudes. But, there was no difference in the magnitude of our risk estimates for NSAIDs according to subgroups indicative of higher UV exposure (i.e. tumors associated with actinic keratoses or on the head and neck) or effect modification by sunlight-related factors, i.e., sunburns. Perhaps

potential preventive effects of NSAIDs cannot overcome those induced by high UV exposure, or they operate via other pathways that contribute to the development of SCC.

Our findings suggest that a possible protective effect of selected NSAIDs and analgesics may be greater in a molecular subset of tumors, in particular those with *PTCH* LOH or mutant p53 (by IHC or mutational analysis). It is conceivable that NSAIDs selectively suppress the growth or progression of SCC in which these mutations are present. An alternative or additional possibility is that NSAIDs exert their protective effect by mimicking some activities of wild type p53. P53 is a multifunctional tumor suppressor that inhibits tumor development by multiple mechanisms. For example, in response to DNA damage, p53 can induce either cell cycle arrest and repair of the damage, or trigger an apoptotic pathway to eliminate the damaged cell[7]. In addition, p53 suppresses COX2 and the synthesis of its product, prostaglandin E2 [24]; tumors with wild type p53 show reduced COX2 protein levels relative to tumors with mutated p53 [25]. By blocking COX2, NSAIDs may functionally replace the normal activity of wild type p53, and thus inhibit the progression of cells with mutant p53 to the fully malignant state. Further work will be required to confirm our results and to elucidate the mechanism underlying the possible preventive effects of NSAIDs on the development of molecular subtypes of SCC.

In addition to possible differences in the subgroups of tumors across studies, methodological differences among these studies may explain some of the inconsistencies. One is the formulation of the NSAIDs consumed. In our study, results varied by type of NSAID, in that a reduced risk was found largely for aspirin, but not propionic acid-based NSAIDs. Use of different formulations of NSAIDs in the Australian population may account for the strong inverse association found in that study [17] or their ability to evaluate high frequent use (≥ 8 times per week). Comparable assessments of frequency were not made in other studies. Thus, while long duration and high frequency of use in theory could each affect risk, larger and more detailed studies will be required to distinguish among these possibilities.

Our findings agree with our *a priori* hypothesis that NSAIDs would not likely affect risk of BCC because COX1 and COX2, the primary targets of NSAIDs, are only weakly expressed in BCC relative to SCC tumors [26]. Our results are similar to those reported in one study [19], but differ from those described in another study [18] that reported a HR=0.43 (95% CI = 0.25-0.73) associated with NSAID use and BCC. The reason for discrepancies among studies is unclear, and warrants further investigation.

An unexpected result was a reduced odds ratio for SCC in relation to paracetamol use. Paracetamol only weakly inhibits COX1 and COX2; however, recent studies have uncovered a new COX isoform, COX3, produced by alternative splicing of the COX1 gene [27]. Interestingly, COX3 is effectively inhibited by analgesics including paracetamol, and it has been suggested that inhibition of COX3 may represent an important mechanism by which paracetamol and other analgesics decrease pain and fever [27]. Although expression of COX3 in the skin has not yet been studied, it is possible that the protective effect of paracetamol may be attributable to COX3 inhibition. This possibility will require further exploration.

Strengths of this study include that it is a population based study of histologically confirmed cases, in which cases were re-reviewed by a single study pathologist and subgrouped by molecular phenotype of the tumor. The study population is from New Hampshire, a lower sun exposure area than previous studies (i. e., Australia and Arizona). Detailed interviews along with histopathological assessment allowed us to evaluate the impact of potential modifying or confounding factors (such as UV exposure and smoking).

Limitations include the observational nature of the study, with self reported drug use. There is potential for confounding by medical indication of use. With limited statistical power, we did not find substantial differences in associations by reason for use (i.e., condition). For example, we found that the inverse associations with SCC were present for aspirin use for pain relief, cardiovascular disease prevention, and autoimmune diseases. Additionally, while the data were adjusted for sunburn history and sun sensitivity, there is potential for confounding by sunscreen use, as it is virtually impossible to separate out the effects of sunscreen use from those of skin sensitivity to the sun in observational studies, as those with sun-sensitive skin are more likely to use sunscreen. Recall bias is a general limitation of case-control studies such as ours and may affect our conclusions as well. Selection bias in our study population must also be considered (i.e., non participants or those for whom drug information was not available may have differed from participants), although overall participants and nonparticipants were similar according to factors that we were able to evaluate such as age and sex. Additionally, non differential misclassification likely exists in our data. We attempted to minimize this by defining drug use as frequent use (e.g., four times a week during a month period or more). By doing this, the “non-users” category included subjects who could have used the drugs but did not fulfill our user definition. Such misclassification likely would lead to an underestimate of effects.

In conclusion, our results suggest that use of NSAIDs, particularly aspirin, may reduce risk of SCCs. Additionally, NSAIDs may target specific molecular subtypes of SCC, including those exhibiting p53 alterations and *PTCH* LOH.

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Appendix

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Table I

Demographic characteristics, sun sensitivity and sun burn history by basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) status.

Characteristic	Control (n=462) N(%)	BCC (n=487) N(%)	SCC (n=535) N(%)
Age			
< 52	97 (21.0)	131 (26.9)	65 (12.2)
52-61	102 (22.1)	136 (27.9)	117 (21.9)
62-67	101 (21.9)	87 (17.9)	124 (23.2)
68-71	91 (19.7)	84 (17.3)	134 (25.1)
72 +	71 (15.4)	49 (10.1)	95 (17.8)
Gender			
male	271 (58.7)	261 (53.6)	328 (61.3)
female	191 (41.3)	226 (46.4)	207 (38.7)
Skin reaction to acute sun			
tan	313 (67.9)	260 (53.6)	307 (57.7)
burn	148 (32.1)	225 (46.4)	225 (42.3)
Lifetime # painful sunburns			
0	138 (31.2)	117 (24.7)	134 (26.0)
1	79 (17.8)	61 (12.9)	77 (15.0)
2	43 (9.7)	38 (8.0)	39 (7.6)
3+	183 (41.3)	257 (54.3)	265 (51.5)
Lifetime cumulative sun exposure in warm months			
< 15328 hours	216 (50.0)	252 (54.2)	190 (37.6)
15328+ hours	216 (50.0)	213 (45.8)	315 (62.4)
Number of cigarettes smoked per day			
0	167 (36.4)	196 (40.3)	172 (32.3)
>0-20	208 (45.3)	209 (42.9)	246 (46.3)
>20	84 (18.3)	82 (16.8)	114 (21.3)

Missing data: skin reaction to acute sun (1 control, 2 BCC, 3 SCC); lifetime sunburns (19 controls, 14 BCC, 20 SCC); lifetime sun exposure (30 controls, 22 BCC, 30 SCC); number of cigarettes (3 controls, 3 SCC)

Table II

Risk of basal cell carcinoma (BCC) of the skin and squamous cell carcinoma (SCC) of the skin and use of NSAIDs and other analgesics

	Control	BCC		SCC	
	N	N	OR (95%CI)*	N	OR (95%CI)*
NSAIDs					
No	283	308	1.00 (ref)	349	1.00 (ref)
Yes	179	179	0.91 (0.69-1.21)	186	0.78 (0.59-1.03)
Yes, current use	137	133	0.89 (0.65-1.22)	144	0.77 (0.57-1.05)
Yes, past use	42	46	0.99 (0.62-1.58)	42	0.80 (0.50-1.29)
Duration ≤ 6yrs	83	92	0.99 (0.69-1.41)	89	0.78 (0.54-1.11)
Duration > 6yrs	96	87	0.83 (0.58-1.19)	96	0.77 (0.55-1.10)
Aspirin					
No	338	377	1.00 (ref)	411	1.00 (ref)
Yes	124	110	0.81 (0.59-1.12)	124	0.75 (0.55-1.02)
Yes, current use	100	85	0.79 (0.55-1.13)	100	0.71 (0.51-1.01)
Yes, past use	24	25	0.90 (0.49-1.66)	24	0.87 (0.47-1.62)
Duration ≤ 6yrs	60	51	0.77 (0.50-1.18)	55	0.66 (0.43-1.00)
Duration > 6yrs	64	58	0.88 (0.58-1.35)	68	0.82 (0.55-1.23)
Propionic acid NSAIDs					
No	450	469	1.00 (ref)	520	1.00 (ref)
Yes	12	18	1.51 (0.66-3.44)	15	1.19 (0.51-2.79)
Yes, current use	8	13	1.43 (0.54-3.78)	7	0.88 (0.29-2.61)
Yes, past use	4	5	1.71 (0.37-7.85)	8	1.91 (0.47-7.76)
Duration ≤ 3yrs	8	6	0.81 (0.25-2.63)	6	0.78 (0.22-2.70)
Duration > 3yrs	4	12	2.68 (0.81-8.88)	9	1.75 (0.52-5.90)
Ibuprofen					
No	405	419	1.00 (ref)	472	1.00 (ref)
Yes	57	68	1.05 (0.70-1.56)	63	0.91 (0.61-1.35)
Yes, current use	29	39	1.13 (0.66-1.94)	41	1.13 (0.67-1.91)
Yes, past use	28	29	0.97 (0.56-1.69)	22	0.68 (0.38-1.24)
Duration ≤ 4yrs	25	33	1.18 (0.68-2.05)	33	1.01 (0.58-1.76)

	Control	BCC		SCC	
	N	N	OR (95%CI)*	N	OR (95%CI)*
Duration > 4yrs	32	35	0.94 (0.55-1.61)	30	0.83 (0.48-1.42)
Paracetamol					
No	403	438	1.00 (ref)	490	1.00 (ref)
Yes	59	49	0.65 (0.42-1.01)	45	0.62 (0.40-0.97)
Yes, current use	40	30	0.56 (0.33-0.97)	31	0.56 (0.33-0.97)
Yes, past use	19	19	0.82 (0.41-1.63)	14	0.72 (0.34-1.50)
Duration ≤ 7yrs	30	26	0.74 (0.42-1.32)	19	0.51 (0.27-0.97)
Duration > 7yrs	29	23	0.54 (0.29-1.03)	25	0.68 (0.37-1.23)

*OR adjusted for age, gender, number of cigarettes smoked per day, skin type, lifelong number of painful sunburns and lifelong cumulative number of hours of sun exposure

*P-values are <.05 if the 95% CI does not include the value of 1

Table III

Odds ratios (95% confidence intervals) for SCC among regular users of anti-inflammatory and analgesics* – stratified by presence of actinic keratosis and anatomic site

Drug use	Presence of actinic keratoses		Anatomic Site	
	No (n=222)	Yes (n=176)	Head and neck (n=305)	Other sites (n=208)
	OR (95%CI) *	OR (95%CI) *	OR (95%CI) *	OR (95%CI) *
NSAIDs	0.84 (0.56-1.26)	0.77 (0.52-1.15)	0.85 (0.62-1.16)	0.69 (0.48-1.00)
Aspirin	0.75 (0.47-1.19)	0.73 (0.46-1.15)	0.78 (0.55-1.12)	0.71 (0.47-1.08)
Proprionic Acid	0.68 (0.14-3.25)	0.72 (0.15-3.44)	1.21 (0.45-3.23)	1.58 (0.56-4.47)
Ibuprofen	1.08 (0.62-1.90)	0.95 (0.53-1.71)	0.98 (0.62-1.54)	0.77 (0.45-1.32)
Paracetamol	0.43 (0.20-0.91)	1.03 (0.57-1.87)	0.66 (0.40-1.11)	0.58 (0.32-1.06)

* Referent category is never use of NSAID medications.

ORs adjusted for age, gender, number of cigarettes smoked per day, skin type, lifelong number of painful sunburns and lifelong cumulative number of hours of sun exposure

* P-values are <.05 if the 95% CI does not include the value of 1

Table IV

Odds ratios (95% confidence intervals) for SCC among regular users of anti-inflammatory and analegics[*]– stratified by p53 IHC, *TP53* Mutation and *PTCH* LOH

Drug use	p53 IHC		TP53 Mutation		PTCH	
	Negative (n=136)	Positive (n=51)	Negative (n=132)	Positive (n=63)	Retained (n=77)	Lost (n=39)
	OR (95%CI) [*]	OR (95%CI) [*]	OR (95%CI) [*]	OR (95%CI) [*]	OR (95%CI) [*]	OR (95%CI) [*]
NSAIDs	0.91 (0.58-1.44)	0.32 (0.14-0.73)	0.81 (0.50-1.32)	0.56 (0.30-1.07)	1.21 (0.72-2.01)	0.61 (0.28-1.34)
Aspirin	0.68 (0.40-1.14)	0.29 (0.11-0.79)	0.68 (0.40-1.14)	0.42 (0.19-0.91)	0.93 (0.53-1.64)	0.35 (0.13-0.96)
Ibuprofen	1.40 (0.77-2.56)	0.19 (0.03-1.45)	1.25 (0.65-2.42)	0.72 (0.27-1.94)	1.65 (0.86-3.18)	0.91 (0.29-2.84)
Paracetamol	0.70 (0.33-1.52)	1.50 (0.56-4.00)	0.91 (0.42-1.99)	0.82 (0.32-2.10)	0.66 (0.40-1.11)	0.58 (0.32-1.06)

[*] Referent category is never use of NSAID medications.

ORs adjusted for age, gender, number of cigarettes smoked per day, skin type, lifelong number of painful sunburns and lifelong cumulative number of hours of sun exposure

[*] P-values are <.05 if the 95% CI does not include the value of 1