Reduction of UV-induced Skin Tumours in Hairless Mice by Topical Non-Steroidal Anti-inflammatory Drugs

Suong N. T. Ngo[1,2*]

[1]*School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy, SA 5371, Australia.*
[2]*School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA 5000, Australia.*

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Short Research Article

ABSTRACT

Aims: Inhibition of ultraviolet-A and -B (UVA+B) skin tumour formation by topical treatment with non-steroidal anti-inflammatory drugs (NSAIDs) was investigated in SKH-1 hairless mice.
Methodology: A UV skin tumour study was designed. Group of mice were irradiated with daily doses of UVA+B for approximately 10 min per day, 5 days per week for 10 weeks. After this 10-week, there was no further UV-exposure. The integrated UV-A irradiance (280-320 nm) was 2.4 X 10^{-4} W/cm^2 and the UV-B irradiance (320-400 nm) was 1.8 X 10^{-3} W/cm^2. Mice were divided into 4 groups (n=20 per group). Group 1 was treated with methanol; Group 2 received 2% indomethacin in methanol; Group 3 received 2% paracetamol in methanol; Group 4 received 2% flurbiprofen in methanol. All groups received their treatment once a day, five days per week for 25 weeks. Mice were euthanized after 35 weeks.
Results: The test NSAIDs in methanol were effective in reducing the incidence and size of the skin tumours induced by UVA+B, with a significantly lower average number and/or area of skin tumours observed in the NSAID-treated mice compared to the methanol control animals ($P <$ .05).

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*Corresponding author: Email: suong.ngo@adelaide.edu.au;

Conclusion: The results support the hypothesis that topically applied indomethacin, paracetamol, and flurbiprofen can provide protection against skin cancer, even when applied well after the skin has been exposed to the damaging effects of UV-light.

1. INTRODUCTION

Skin cancer is the most common type of cancer for males and females in the white population [1]. Among the various malignant skin cancer types, non-melanoma skin cancer (NMSC), including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) is most common, and is a source of significant morbidity. Basal cell carcinoma represents about 80% of all reported skin cancers, while squamous cell carcinoma is about 16%, and melanomas represent about 4% [1,2]. Most NMSC occurs in people over 40 years of age, and incidence rates in Australia are believed to be the highest in the world [3]. A 2-fold increase in NMSC incidence has also been seen in the United States from 1994 to 2006 [4]. People with light complexions, fair or red hair and who tend to burn easily on exposure to the sun (Fitzpatrick skin grades 1 and 2) are more prone to develop NMSC than those with dark-skin, and males appear to be at higher risk [3].

Once treated for a NMSC, the risk of developing a new NMSC is highest in the subsequent year [3]. From an early review of 7 studies, it has been established that the 3-year cumulative risk for a subsequent NMSC is on average 44% [5]. There is also a strong association between the risk of developing a subsequent skin cancer and the number of prior skin tumours - that is, the more prior skin cancers, the higher the risk. In one study, the risk was increased from 38% for patients with fewer than 3 previous NMSC to 93% for patients with 3 to 9 previous NMSC [6]. Healthcare costs associated with the treatment of skin cancers are over 500 million annually in the United States alone, and it has been estimated that at current rates, 1 in 5 Americans will develop a skin cancer of some sort during their lifetime [7,8]. Clearly skin cancer is a major worldwide health problem and is a very costly disease to treat and manage.

There is a causal relationship between excessive exposure to solar ultraviolet (UV) radiation and incidence of skin cancers [3,9]. UV light has been reported as being the main aetiology of NMSC due to DNA damages, such as the creation of clyclobutane dimers and nucleotide mutations [9]. Moreover, p53-gene mutations resulted from UV light could inactivate the p53-gene's ability to inhibit tumor promotions in mutated cells [10]. Preventive methods for NMSC include standard sun protection behaviours, such as applying sunscreen creams with high SPF (sun protection factor), avoiding UV peak hours or periods, and minimising exposure to UV light with protective clothing. As total avoidance of sun exposure would seem unrealistic, other interventions to prevent NMSC are needed. This is greatly beneficial for patients with precursor lesions such as Actinic keratosis, as chemoprevention may inhibit the development of the condition into malignancy [11].

Earlier studies have shown that non-steroid anti-inflammatory drugs (NSAIDs) have a potential role in cancer prevention, including NMSC [12-15] by inhibition of cyclooxygenase enzymes COX-1 and COX-2, which are involved in carcinogenesis [16]. Chemically, NSAIDs are divided into a number of subclasses. They include the salicylic acid derivatives - e.g. aspirin, the indole and indene acetic acids - e.g. indomethacin, the heteroaryl acetic acids - e.g. diclofenac, and the arylpropionic acid derivatives - e.g. flurbiprofen [17]. Paracetamol is considered as an atypical NSAID as it is a week inhibitor of COXs. Several studies have reported that COX-2 is up-regulated in many forms of cancer, including human colorectal adenocarcinoma, breast, cervical, prostate, lung and skin tumours [18,19], and have indicated a relationship between the NSAID antiproliferative effect and COX inhibition [20-23]. Several lines of evidence also suggested that antiproliferative effects of NSAIDs are mediated via mechanisms that are at least partly independent of COX inhibition [22,24-27]. The role of COX inhibition in NSAID antiproliferative effect is presently unclear. Recently, a study by Sørensen also reported that the use of NSAID, including non-selective NSAIDs, overall reduced the risk of certain types of skin cancer [28].

To test the relative effectiveness of NSAIDs as anticancer agents, a UV skin tumour study in

hairless mice was designed. It was found that topical NSAIDs from 3 subclasses; indomethacin, flurbiprofen, and paracetamol can provide protection against skin cancer even when applied well after the skin has been exposed to the damaging effects of UV-light. The results of the tumor study and the effects of the three test NSAIDs, including indomethacin, flurbiprofen, and paracetamol that has a weak COX inhibition action are presented in this paper.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Indomethacin, paracetamol, and flurbiprofen were obtained from the Sigma Chemical Company (Castle Hill, NSW, Australia). Methanol and other chemicals were purchased from Biolab (Clayton, VIC, Australia). All other chemicals were purchased commercially and were of analytical grade.

2.2 Mice

All animals received human care according to the "Australian code for the care and use of animals for scientific purposes" (National Health and Medical Research Council, NHMRC, AE-16 7th Edition 2004). The Animal Ethics Committee at The University of South Australia approved the study protocols for all animal experiments.

Female SKH-1 mice were obtained from the Animal Resource Centre (Perth, Western Australia) at four weeks of age. Animals were randomly allocated to one of four treatment groups and allowed free access to a standard diet formulated according to the American Institute of Nutrition (AIN-89 diet, Glen Forrest Feeds, Perth, WA. Australia). The animals were acclimatized and caged together in groups of 10 mice. The mice were maintained under standard conditions of a 12 h dark/12 h light cycle, at the temperature of 24±2ºC, and relative humidity of 50 ± 10%.

2.3 UV Source

A purpose-built apparatus was used for UV irradiation. The UV emission was produced by 6 UV-A lamps (model 40BL, Sylvania) symmetrically housed around a single UV-B lamp (model FL40SE, Philips). The spectrum was measured with a spectroradiometer (model IL 1700, International Light, Newburyport, MA). The

integrated UV-A irradiance (280-320 nm) was 2.4 x 10^{-4} W/cm^2 and the UV-B irradiance (320-400 nm) was 1.8 x 10^{-3} W/cm^2.

2.4 Irradiation of Mice

After one week of acclimatization, animals in all groups were exposed to UVA+B approximating solar UV emission as described above. Irradiation was commenced at 5 min/day and increased to a maximum of 10 min/day. Animals showing undue reddening of the skin (i.e., >2 minimal erythematous dose) were not irradiated further until the erythema had disappeared. Differences between cumulative doses did not exceed 1% of the total UV exposure. No mice exhibited any evidence of blister formation or skin peeling. Mice were irradiated once daily, 5 days per week for 10 weeks. After this 10-week irradiation, there was no further UV exposure.

2.5 Tumor Study

Mice in all groups (n = 20 per group) were irradiated daily for 10 weeks with UVA+B as described above. After exposure to UV for 10 weeks, mice were painted dorsally with (Group 1) 70% methanol, (Group 2) 2% indomethacin in 70% methanol, (Group 3) 2% paracetamol in 70% methanol, (Group 4) 2% flurbiprofen in 70% methanol. The selection of the dose of 2% of topical NSAID was based on preliminary experiments testing a range of concentrations of NSAIDs (from 0.5% up to 3%) on a small number of animals.

All groups received their treatment once a day, five days per week for 25 weeks. Animals were monitored daily for their appearance, and as papillomas or tumours appeared, they were counted and measured regularly. After 35 weeks, the experiment was ended, and all the mice were euthanized with a lethal dose of pentobarbital sodium (Nembutal).

2.6 Statistical Analysis

Statistical analysis was performed using Microsoft Excel software. Tumour multiplicity and total tumor area were compared between the control group and the NSAID treated groups. Tumor multiplicity, expressed as the mean number of tumours, the mean number of tumours plus papillomas per animal, and the total affected area were analysed by Student's *t* test. A *P* value < .05 was considered to be significant. Data concerning the tumor area were

transformed using the square root of the area, prior to performing the Student's *t* test. Data are presented as mean ± SD of total animals/group.

3. RESULTS

3.1 Mice Survival

The majority of the mice survived the full 35-week study (see Table 1). The deaths were higher in two of drug treatment groups than the placebo. A number of mice in the indomethacin group did not survive the entire experimental period and died due to noticeable NSAID's toxicity, such as GI disturbance, bleeding which may be possibly resulted from systemic absorption of indomethacin. Two mice in the flurbiprofen group died in week 30 and 31 of the study. No drug toxicity or other causes of death were observed for other groups.

3.2 Evaluation of Chemopreventive Effects

The incidence of tumours, the mean number of tumours and mean number of tumours plus papillomas, and the total areas found in the various groups at the end of the 35-week study are summarized in Table 2. No tumours or papillomas were present in mice during the 10 weeks of UV exposure. Data are presented as

tumours, and tumours plus papillomas as the natural progression of UVA+B-induced skin cancer is from the initiated cell to a benign papilloma to cancer (defined as an invasive lesion) and in some cases the distinction could not be made unequivocally.

The incidence of UVA+B-induced skin tumours was reduced in the groups treated with paracetamol and indomethacin, to about two-thirds and four-fifths that of the methanol only group, respectively. The mean number of tumours was reduced in the NSAID treated groups and was statistically significant for paracetamol ($P < .05$), but not for indomethacin and flubiprofen possibly due to the large standard errors. The mean number of tumours plus papillomas was also lower and was statistically significant for indomethacin and paracetamol ($P < .05$), not for flurbiprofen.

The mean area of the tumours and the mean area of tumours plus papillomas were also calculated as an index of tumour burden on the mice. Both the mean area of tumours (see Table 2) and the mean area of tumours plus papillomas were significantly reduced in mice treated with indomethacin and flurbiprofen than that in methanol control animals ($P < .05$); but were not statistically significant for paracetamol. The mean area of tumours plus papillomas is presented in Fig. 1.

Table 1. Number of surviving mice in each group during 25 weeks of topical treatment with 2% indomethacin, or 2% paracetamol, or 2% flurbiprofen[a]

| Week number	24	25	26	27	28	29	30	31	32	33	34	35
Placebo	20	20	20	20	20	20	20	20	20	20	19	19
Paracetamol	20	20	20	20	20	20	20	20	20	20	20	20
Flurbiprofen	20	20	20	20	20	20	19	18	18	18	18	18
Indomethacin	20	20	19	19	18	17	15	15	15	15	15	15

[a] *Each group, originally contained 20 mice, which were subjected to 10 week UV-irradiation, then 25 week topical drug treatment*

Table 2. Tumor multiplicity and burden in mice receiving topical treatment with 2% indomethacin, paracetamol, or flurbiprofen after 10-week UV-A + B exposure[a]

	Survival	Percentage of surviving mice with tumours	Tumours[b]		Tumours & Papillomas[b]
			Number	Area (mm^2)	
Methanol	19	68%	1.53 ± 0.96	14.68 ± 11.24	1.76 ± 0.83
Indomethacin	15	53%	0.66 ± 0.51	1.1 ± 0.48[*]	1.16 ± 0.40[*]
Paracetamol	20	45%	1.00 ± 0.57[*]	3.41 ± 4.00	1.28 ± 0.48[*]
Flurbiprofen	18	61%	0.88 ± 0.60	5.27 ± 4.19[*]	1.55 ± 0.78

[a]*Each group originally contained 20 mice: survival values are the number of mice alive after 35 weeks.*
[b] *Mean ± S.D.* [*]*Mean number of tumours, mean area of tumours, and mean number of tumours + papillomas significantly lower than methanol control group at P < .05 by Student's t test.*
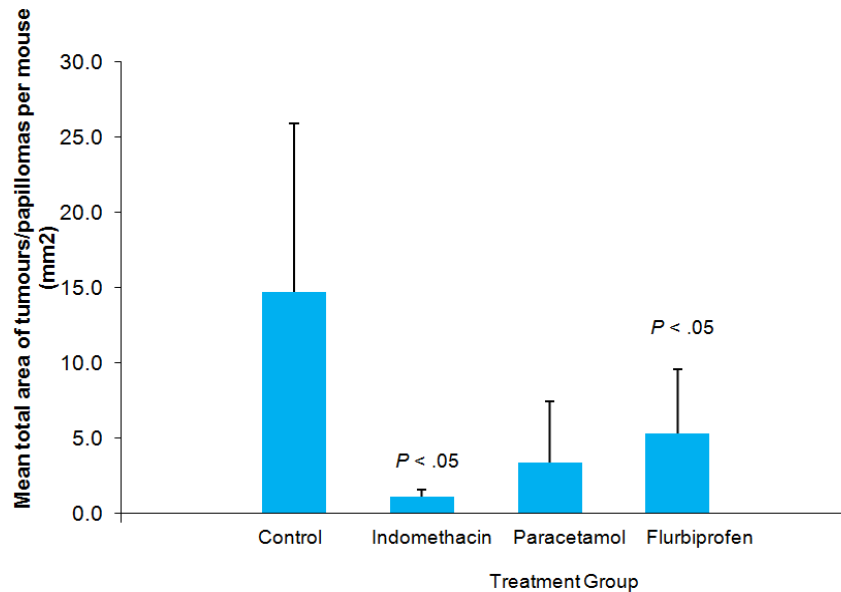
Fig. 1. The mean total area of tumours and papillomas per mouse in the NSAID treated groups compared to the placebo at the end of the study
Data are presented as mean ± SD of total animals/group

4. DISCUSSION

In this study, inhibition of UVA+B-induced skin tumour formation by 3 test NSAIDs from different subclasses; indomethacin, flurbiprofen, and paracetamol adds further support to the hypothesis that NSAIDs can provide protection against skin cancer, even when applied well after the skin has been exposed to the damaging effects of UV-light. This finding is particularly significant given the latency period of skin cancer in humans.

The chemopreventive treatments with systemic NSAIDs, including aspirin, ibuprofen, indomethacin and paracetamol have previously been shown to reduce the risk of developing NMSC [12-14,29]. Previous human population-based case-control studies demonstrated a reduced risk of having NMSC in ibuprofen [12,13], indomethacin [12], paracetamol [13] and aspirin users [13]. Lower risk of NMSC with paracetamol use has also been found in previous human prospective study, with a reduced risk associated with a higher frequency of use (*P = .04*) [29]. An RCT in 1402 AK (actinic keratosis) patients resulted in a reduction in the risk of developing NMSC for aspirin treatment [14]. It has earlier been shown that orally administered NSAID indomethacin [30] and celecoxib could inhibit development of UVB induced skin tumours

in mice [30-33] and in human RCT [34]. Unfortunately, because of the toxicity of indomethacin due to systematic absorption, it was not possible to study its effects at higher concentrations. Regarding the use of topical NSAIDs, only one earlier study has shown a delayed onset and reduced development of skin tumours in mice using celecoxib combined with zileuton, a 5-lipoxygenase inhibitor [35]. In the present study, topical indomethacin was shown to have the greatest protective effects compared to paracetamol and flurbiprofen. However, it was also associated with a lower survival of mice, which was consistent with that reported in the literature. Overall, 3 test compounds, including week COX inhibitor paracetamol, were effective in reducing the mean number and/or area of skin tumours in hairless mice that had been exposed to UVA+B light only during earlier stages of their life (before drug treatment was commenced). The results of this study further support the potential use of NSAIDs as skin cancer chemo-preventive agents.

5. CONCLUSION

The results support the hypothesis that topically applied indomethacin, paracetamol, and flurbiprofen can provide protection against skin cancer, even when applied well after the skin has been exposed to the damaging effects of UV-

light. As topical administration of drugs would lead to less side effects compared with systemic administration, the idea of topical NSAID to prevent UV-induced NMSC with minimal cardiovascular complications and gastrointestinal bleeding is worth being further investigated.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the author(s).

COMPETING INTERESTS

Author has declared that no competing interests exist.

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