A Reduction in Inflammatory Macrophages May Contribute to Skin Cancer Chemoprevention by Nicotinamide

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TO THE EDITOR

Nicotinamide (NAM), an amide form of vitamin B3, has been recognized as an effective chemopreventive agent for keratinocyte cancers (KCs) (basal cell carcinoma and squamous cell carcinoma [SCC]) and actinic keratoses. The ONTRAC study (Oral Nicotinamide To Reduce Actinic Cancer, Australian New Zealand Clinical Trials Registry number ACTRN12612000625875) was a multicenter, phase 3, double-blinded, controlled trial in which NAM (500 mg) or placebo was given twice daily for 12 months to immunocompetent patients who had at least two KCs in the previous 5 years. In this high-risk population, NAM reduced the incidence of new KCs by 23%, with similar efficacy in preventing both SCC and basal cell carcinoma (Chen et al., 2015). Chemopreventive efficacy has also been reported in immunocompromised transplant recipients (Bostom et al., 2016; Drago et al., 2016).

UVR diminishes antigen-presenting capability and induces immunosuppressive cytokines (Halliday, 2005). The photoprotective effects of NAM on skin immunity (Damian et al., 2008) may be due to its ability to enhance DNA repair, thereby reducing DNA photolesions (Surjana et al., 2013), which are a trigger for UV-induced immunosuppression (Kripke et al., 1992). Understanding the immune mechanisms in skin cancer, including the roles of T and B lymphocytes, macrophages, and dendritic cells, can provide a basis for chemopreventive and therapeutic opportunities (Rangwala and Tsai, 2011).

We analyzed KCs collected at the Royal Prince Alfred Hospital ONTRAC study site (Sydney, Australia) (n = 130), using a range of immunological markers to better understand the mechanisms by which NAM reduced skin carcinogenesis. We also assessed DNA damage in tumors arising at sun-exposed sites on NAM and on placebo by staining for cyclobutane pyrimidine dimers (CPDs) and 7,8-dihydro-8-oxoguanine (8oxoG).

A total of 130 tumors from 78 patients were included in the study (70 and 60 arose in patients receiving placebo and NAM, respectively). The study was approved by the Ethics Committees of the University of Sydney and the Sydney Local Health District, and all patients provided written informed consent. Selection of tumors was made blinded to treatment allocation (see Supplementary Figure S1 online).

To minimie the risk of false-p ositive results arising from multiple hypothesis testing, we a priori grouped the immunological markers, on the basis of their intercorrelation, to form four indices (see Supplementary Figure S2 online for statistical methods). Thus, the primary analyses of immunological markers comprised a family of four hypothesis tests. Index 1 reflected the abundance of lymphocyte markers and was derived from CD3 (Novocastra, Leica Biosystems, Newcastle, UK), CD4 (Cell Marque, Rocklin, CA), CD8 (Dako, Carpenteria, CA), and FoxP3 (Abcam, Melbourne, Australia). Index 2 reflected the abundance of macrophage markers and was derived from CD68 (Dako) and CD163 (Novocastra). Index 3 was the count of the dendritic cell marker CD11c (Novocastra). Index 4 was the count of the proliferative cell marker, Ki-67 (Ventana, Tuscan, AZ) (see Supplementary Materials and Methods). Tumors arising on sun-exposed sites (head, neck, elbows, forearms, and hands) were stained for DNA damage markers (CPDs and 8oxoG, n = 49 tumors). Comparisons between randomized groups were performed using a mixed linear modeling approach. The detailed methods are described in the Supplementary Materials and Methods and elsewhere (Thompson et al., 2014).

Analysis of the immunological markers showed no convincing statistical evidence of a treatment effect on Index 1 (lymphocyte markers CD3, CD4, CD8, and FoxP3; $P = 0.06$), Index 3 (dendritic cell marker CD11c; $P = 0.06$), or Index 4 (proliferative cell marker Ki-67; $P = 0.94$ peritumoral and $P = 0.77$ intratumoral) (Figure 1). The effect of NAM on Index 2 (macrophage markers CD68 and CD163 counts) was significant ($P = 0.0029$). Secondary analyses showed this to be driven by a prominent reduction in CD68 in patients receiving NAM ($P = 0.0018$), although no clear treatment effect on CD163 was noted ($P = 0.15$). A post hoc analysis of the ratio of CD163 to CD68 provided some evidence of a NAM effect ($P = 0.0482$). This reflects the disproportionately larger decrease in CD68 cells compared with CD163 cells in the NAM group of tumors (Figure 1).

Analysis of the DNA damage markers found nonsignificant reductions with NAM in (i) CPD lesions of 49% in the epidermis ($P = 0.21$) and 21% intratumorally ($P = 0.73$) (Figure 2a) and (ii) 8oxoG lesions in the epidermis of 10.5% ($P = 0.76$) and in the tumoral region of 28.5% ($P = 0.37$) (Figure 2b).

CPDs and 8oxoG have previously been observed in human skin SCCs and actinic keratoses (Agar et al., 2004). DNA repair, essential for the prevention of UV-induced carcinogenesis, is a

Abbreviations: 8oxoG, 7,8-dihydro-8-oxoguanine; CPD, cyclobutane pyrimidine dimer; KC, keratinocyte cancer; NAM, nicotinamide; ONTRAC, Oral Nicotinamide to Reduce Actinic Cancer; SCC, squamous cell carcinoma

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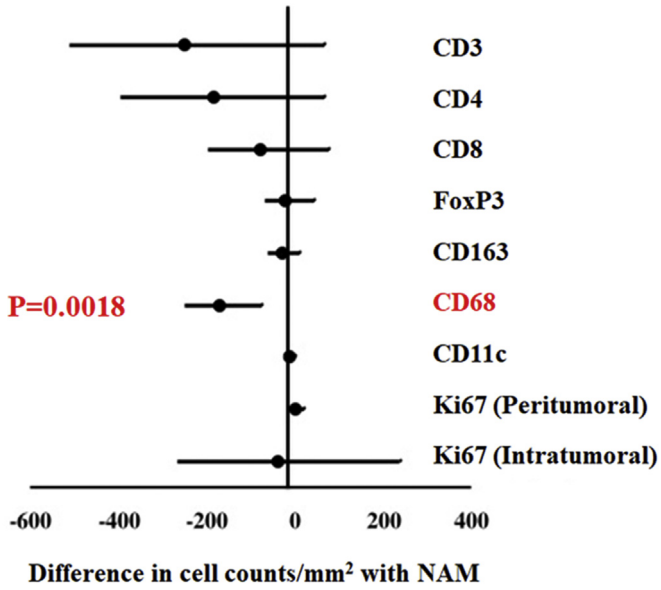

Figure 1. Forest plot to illustrate mean difference in cell counts between the tumors arising in patients receiving NAM compared with placebo and associated standard deviations. 130 tumors were stained with seven different immune markers and one proliferative marker. In the plot, the vertical axis represents the placebo group set to 0. The circles indicate the back-transformed adjusted mean differences in cell counts/mm^2, and the horizontal lines represent the standard deviation. NAM caused a significant decrease in the tumor infiltration of macrophages (CD68) ($P = .0018$). NAM did not have a significant effect on the other cell markers. NAM, nicotinamide.

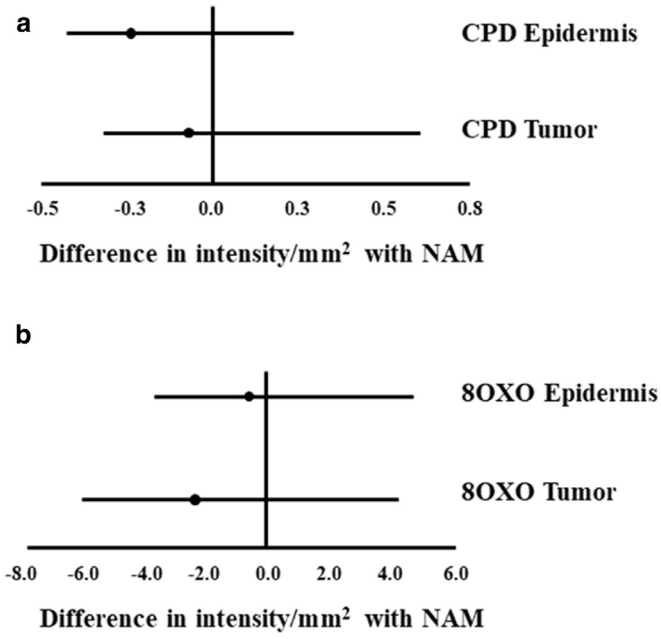

Figure 2. Forest plot to illustrate mean difference in DNA photolesions between tumors arising in patients receiving NAM compared with placebo and associated standard devations. (**a**) 49 keratinocyte cancers from sites that are normally sun exposed (tumors on the head, neck, elbow, forearm, or hand) were stained for CPDs. In the plot, the vertical axis represents the placebo group set to 0. The circles indicate the back-transformed adjusted mean differences in cell counts/mm^{2}with NAM compared with placebo, and the horizontal lines represent the standard deviation. NAM reduced the CPD lesions in the epidermis of the tumors by 49% compared with placebo and by 21% intratumorally. (**b**) The results of 48 keratinocyte cancers that were stained for 8oxoG. NAM reduced the 8oxoG lesions in the epidermis by 10.5% and the tumoral region by 28.5%. For both **a** and **b,** results did not reach significance. 8OXO, 7,8-dihydro-8-oxoguanine; CPD, cyclobutene pyrimidine dimer; NAM, nicontinamide.

highly energy dependent process. NAM is a precursor of nicotinamide adenine dinucleotide and an essential coenzyme in adenosine triphosphate production (Park et al., 2010). Consistent with previously observed effects of NAM on DNA repair in ex vivo UV-irradiated human skin (Surjana et al., 2013), tumors arising in NAM-supplemented participants tended to have lower levels of CPD and 8oxoG photolesions, although this failed to reach significance. It is likely that there was high variability in intensity and time since sun exposure, as well as variability in DNA repair efficiency, in our study participants. This would have resulted in a large spread in the data.

We found a significant decrease in the number of macrophages in KCs that arose in patients receiving NAM compared with placebo. This was restricted to a significant reduction in tumor-associated cells expressing CD68$^+$, which is a marker identifying both M1 and M2 macrophages. This is consistent with the observation of higher numbers of CD68$^+$ macrophages in SCC compared with normal skin (Pettersen et al., 2011) and the association of CD68$^+$ cells with inflammation and higher 10-year uveal melanoma mortality (Mäkitie et al., 2001). CD163, which is associated with an M2 macrophage functional program, was not significantly reduced. This suggests that M1 macrophages are specifically depleted by NAM. NAM's effects on macrophages have been sparsely documented, although it has been reported that in diabetic patients NAM suppresses inflammatory cytokine production by monocytes and macrophages (Krętowski et al., 2000). Inflammation and reactive oxygen/nitrogen species, which are produced by M1 macrophages (Tan et al., 2016), are recognized promoters of skin carcinogenesis (Halliday, 2005), and NAM inhibition of inflammatory cytokine production by macrophages may therefore contribute to its chemopreventive effects on skin cancer. The tumors studied, however, are the subset that resisted chemoprevention by NAM, and the effects of NAM on cancers that failed to grow could not determined.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.08.018.

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Incidence and Mortality of Pemphigus in France

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TO THE EDITOR

The incidence of pemphigus varies from 0.5 to 34 cases/million inhabitants/year, with the highest incidence rates in Brazil (Hans-Filho et al., 1996; Ishii et al., 2008; Langan et al., 2008; Meyer and Misery, 2010). Additionally, although the prognosis of pemphigus patients is considered good in the literature, recent findings reported unusually high mortality rates (Almugairen et al., 2013; Langan et al., 2008).

We estimated the incidence and mortality of pemphigus among 13 regions in France (Figure 1a) over a 10-year period. Inclusion criteria were: (i) patient living in 1 of the 13 regions and (ii) newly-diagnosed pemphigus. Cases were identified using the computerized databases of the pathology laboratories of the university and general hospitals and private-practice laboratories that perform direct immunofluorescence. Statistical analyses are described in Supplementary Material online.

From January 2004 to December 2013, 629 patients were identified in included regions, which corresponded to a population size of 13.75 million inhabitants (Figure 1a). Among them, 380 were excluded: (i) diagnosis of pemphigus not confirmed (n = 74); (ii) patient not domiciled in the selected regions (n = 194), and (iii) diagnosis of pemphigus made before or after the study period (n = 112). A total of 249 incident cases (125 women, 124 men) were included. Mean age at diagnosis was 59.4 ± 18.7 years and was similar between male and female patients ($P = 0.93$). The age distribution of the population is shown in Figure 1b. Pemphigus types were pemphigus vulgaris (PV) (n = 155 [62%], pemphigus

Abbreviations: CI, confidence interval; IQR, interquartile range; PF, pemphigus foliaceus; PNP, paraneoplastic pemphigus; PV, pemphigus vulgaris

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