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Chemopreventive activities of etodolac and oxyphenbutazone against mouse skin carcinogenesis

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ABSTRACT

Previous cancer chemoprevention studies have demonstrated that the non-steroidal anti-inflammatory drugs (NSAIDs) can be effective in suppressing the development of various human malignancies. Recently we identified the possible anti-tumor promoting potentials of 14 new NSAIDs in the Epstein–Barr virus early antigen activation assay induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). In this study we report the inhibition of 7,12-dimethylbenz (a) anthracene (DMBA) induced two-stage mouse skin carcinogenesis by etodolac (ETD), one of the most potent NSAIDs identified in our in vitro cancer chemopreventive screening of this group of drugs. Topical administration of ETD at a very low dose of 85 nmol showed a significant decrease in both tumor incidence and burden. This effect is also accompanied by a delay in the tumor latency period. Since ETD showed potent chemopreventive activity in both in vitro and in vivo studies, it warrants prompt consideration for trial in humans as a potential cancer chemopreventive agent. We also investigated oxyphenbutazone (OPB) another commonly used NSAID for its cancer chemopreventive effect on peroxynitrite (PN) induced-TPA promoted skin tumors in the mouse. Following tumor initiation with 390 nmol of PN, the skin tumor promotion with 1.7 nmol of TPA was significantly inhibited by oral administration of 0.0025% OPB. The results demonstrate that OPB is a potent cancer chemopreventive agent in the highly sensitive in vivo mouse test model we used.
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A wide variety of chemical agents have been shown to possess chemopreventive properties against a broad spectrum of tumor types.[1] These agents are thought to function through mechanisms which include carcinogen detoxification, suppression of genetic mutation, inhibition of signal transduction, and proliferation and induction of apoptosis.[2] Some of the best characterized candidate chemopreventive agents to-date are the non-steroidal anti-inflammatory drugs (NSAIDs), which have been shown to be effective against the development of several types of tumors.[3] NSAIDs include the arylalkanoic acid derivatives (etodolac, fenoprofen calcium, ibuprofen, and naproxan), pyrazolone derivatives (oxyphenbutazone and phenylbutazone), the indoles (indomethacin and tomentin sodium), indene (sulindac) and anthranilic acid derivatives (meclofenamic sodium) and a number of salicylic acid derivatives (aspirin, *p*-aminosalicylic acid, diflunisal, etc.). We have earlier identified etodolac (ETD, **1**) as the most potent NSAID chemopreventive agent in the in vitro Epstein–Barr virus early antigen activation assay.[4]

In this Letter, we demonstrate that ETD (Fig. 1) is also effective in the in vivo 7,12-dimethylbenz(a)anthracene (DMBA)-induced, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) promoted two-stage skin cancer chemopreventive model in the mouse. The pyrazolone derivative oxyphenbutazone (OPB, **2**) also showed high activity in the in vitro Epstein–Barr virus early antigen activation assay. This Letter also includes the study of the effect of OPB in the peroxynitrite (PN)-induced, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) promoted tumors in the mouse skin.[5,6]

In the ICR mouse, topical application of 0.0025% ETD resulted in a significant inhibition of the tumor burden by approximately 40%, in the DMBA-TPA induced model (Fig. 2).[7] Control animals that received DMBA and promoted with TPA developed skin tumors as

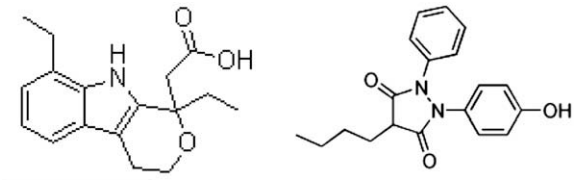

Figure 1. Structures of the NSAIDs evaluated in this study.

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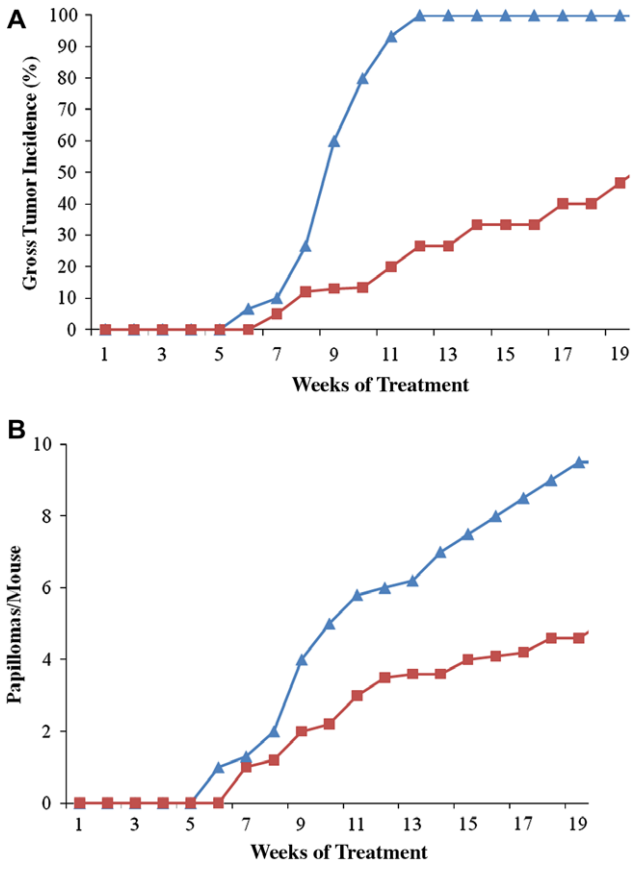

Figure 2. Effects of Etodoloac (ETD) on mouse skin carcinogenesis induced by DMBA and TPA. Six weeks old female ICR mice were divided into groups of 15 animals per group. All mice were initiated with DMBA (390 nmol) and promoted with TPA (1.7 nmol) twice weekly starting one week after initiation. The back of each mouse was topically treated once daily with 0.0025% ETD in 100 μl of acetone in their groups. Control animals were included in the study. (A) Percentage of mice bearing papillomas; (B) average number of papillomas per mouse. Group 1 (■) positive control versus Group 2 (▲) comprising of DMBA + 0.0025% ETD at 20 weeks of tumor promotion.

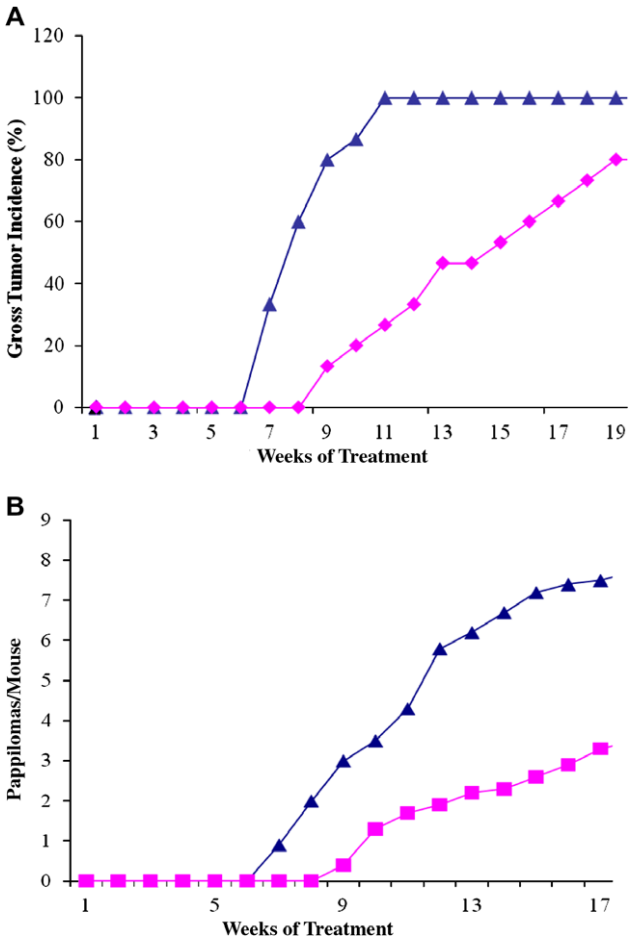

Figure 3. Effects of oxyphenbutazone (OPB) on mouse skin carcinogenesis induced by PN and promoted by TPA. Studies were performed in HOS-HR-1 specific pathogen free mice (5 animals/cage and 15 animals/group). Following tumor initiation with a single dose of 390 nmol of PN, the skin tumor promotion was done with TPA (1.7 nmol//100 μl of acetone) twice weekly for 20 weeks. Oral administration of OPB (0.0025%) in drinking water was started one week prior to tumor initiation and stopped one week after that. Control animals were included in the study. (A) Percentage of mice bearing papillomas; (B) average number of papillomas per mouse. Group 1 (▲) positive control versus Group 2 (■) comprising of PN initiated and TPA induced + 0.0025% OPB at 20 weeks of tumor promotion.

early as in six weeks. Treatment with ETD one week prior to and after the tumor initiation resulted in the delay of the tumor induction and significantly reduced the average number of tumors per mouse at 20 weeks of treatment. Figure 3 shows the inhibitory effect of OPB on the PN-induced TPA-promoted skin tumors in the HOS-HR-1 mouse.[8] The cancer chemopreventive effects were evident as a significant reduction in both tumor incidence and tumor multiplicity was observed at the end of the assay. At a low dose of 0.0025% OPB treatment for two weeks increased the tumor latency period by 29%, decreased the tumor incidence by >20% and the burden by >50%, which are all characteristics of a potent cancer chemopreventive agent. In this study we did not observe any toxicity associated with OPB treatment. Between the two NSAIDs studied here, ETD exhibited higher cancer chemopreventive potential and no side effects as compared to OPB which is known to have toxic side effects.

Recently, genotoxicity and carcinogenicity studies have been reviewed for 120 anti-inflammatory, analgesic and antipyretic drugs including the two NSAIDs studied by us in this Letter.[9] ETD and OPB were listed among the drugs that gave a positive genotoxic test result. Carcinogenicity assays performed in both mice and rats at various doses lower than those recommended by current guidelines for carcinogenicity testing for pharmaceuticals, gave negative results for ETD. However, according to the International Agency for Research on Cancer guidelines, OPB is considered non-classifiable as to its carcinogenicity to humans.[9]

Many retrospective epidemiological studies have established an inverse correlation between long-term NSAID use and the risk of developing cancer.[3] In addition, many prospective chemoprevention studies using animal tumor models have demonstrated the efficacy of several classical NSAIDs such as indomethacin, sulindac and piroxicam.[10] These analyses are in agreement with our present studies. Human clinical trials using sulindac in the treatment of benign polyps have also demonstrated a chemopreventive benefit.[11] ETD is a novel selective COX-2 inhibitor and prostaglandin E2 inhibitor,[12] and is reputed as one of the most well-tolerated NSAIDs with a few or no gastric and renal side effects.[13] In this study on OPB we have shown that it is a potent cancer chemopreventive agent in the highly sensitive in vivo mouse model employed.

Since ETD and OPB are prescribed for long-term use, data on their chemopreventive potential, such as those presented in this study, should be of value in the evaluation of the suitability and safety of this drug for extended use among the general patient population.

The potent chemopreventive activity exhibited by ETD in both in vivo and in vitro studies warrants its prompt consideration for trial in humans as a potential cancer chemopreventive agent.

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References and notes

1. Lippman, S. M.; Benner, S. E.; Hong, W. E. *Clin. Oncol.* **1994**, *12*, 851.
2. Kellof, G. J.; Boone, C. W.; Crowell, J. A.; Steele, V. E.; Lubert, E.; Sigman, C. C. *Cancer Epidemiol. Markers Prev.* **1994**, *3*, 85.
3. Singh, D.; Lippman, S. *Oncology* **1998**, *12*, 1787.
4. Kapadia, G. J.; Azuine, M. A.; Takayasu, J.; Konoshima, T.; Takasaki, M.; Nishino, H.; Tokuda, H. *Cancer Lett.* **2000**, *161*, 221.
5. Kapadia, G. J.; Azuine, M. A.; Nishino, H.; Konoshima, T.; Kuchide, M.; Mukainka, T.; Tokuda, H. *Abstracts of Papers*, 43rd Annual Meeting of the American Society of Pharmacognosy, New Brunswick, NJ, 2002; Abstract P:30.
6. Kapadia, G. J.; Azuine, M. A.; Tokuda, H.; Konoshima, T.; Kumugai, A.; Nishino, H. *Abstract of Papers*, International Congress on Natural Products, Phoenix, AZ, 2004; Abstract P:147.
7. The procedure followed for the DMBA-induced and TPA-promoted mouse skin carcinogenesis was essentially the same as the one adapted by us in an earlier study.[14] Briefly, the protocol involved treatment of female ICR mice, which were housed five animals per cage (15 mice per group). Prior to the study, the dorsal side of the skin was shaved using surgical clippers. On the day of tumor initiation, the back of each mouse was treated topically with a single dose of DMBA (390 nmol per mouse in 100 μl of acetone). After one week, each mouse was treated topically with TPA (1.7 nmol in 100 μl of acetone) twice a week for 20 weeks. One week before tumor initiation, the back of each mouse was topically treated once daily with 0.0025% ETD in 100 μl of acetone or acetone alone. Treatment of the animals with ETD was continued for an additional one week, and thus the total treatment period being two weeks. Five experimental groups were studied as follows. Group 1: DMBA + TPA alone, Group 2: DMBA + TPA + 0.0025% ETD, Group 3: DMBA only, Group 4: ETD only, and Group 5: TPA only. Animals in all groups were observed for 20 weeks. Animal survival, mean latency period of tumor formation and gross incidence of papillomas were observed and recorded weekly.
8. Tumors were induced with PN and promoted with TPA as described earlier.[14] Studies were performed in HOS-HR-1 specific pathogen free mice (5 animals / cage and 15 animals/group). Following tumor initiation with a single dose of 390 nmol of PN, the skin tumor promotion was done with TPA (1.7 nmol// 100 μl of acetone) twice weekly for 20 weeks. Oral administration of OPB (0.0025%) in drinking water was started one week prior to tumor initiation and stopped one week after that. Similar groups (1–5) were set up as described in the previous experiment described above[7] with the exception that PN replaced DMBA in this study. Like in the previous experimental model, animal survival, mean latency period of tumor formation and gross incidence of papillomas were observed and recorded weekly.
9. Brambilla, G.; Martelli, A. *Pharmacol. Res.* **2009**, *60*, 1.
10. Dubois, N.; Giardiello, F. M.; Smalley, W. E. *Gasteroenterol. Clin. N. Am.* **1996**, *25*, 773.
11. Eberhart, C. E.; Dubois, R. N. *Gasteroenterology* **1995**, *109*, 285.
12. Takahashi, M.; Katayama, Y.; Takuda, H.; Kuwayama, H.; Terano, A. *Ailment. Pharmacol. Therap.* **2000**, *1*(Suppl.), 44.
13. Hatori, M.; Kokuban, S. *Curr. Med. Res. Opin.* **1999**, *15*, 193.
14. Azuine, M. A.; Tokuda, H.; Takayasu, J.; Enjo, F.; Mukainka, T.; Konoshima, T.; Nishino, H.; Kapadia, G. J. *Pharmacol. Res.* **2004**, *49*, 161.