ANTI PROLIFERATIVE ACTIVITY OF NEXRUTINE, A HERBAL EXTRACT OF PHELLODENDRON AMURENSE CAUSES CHEMOPREVENTION OF CHEMICALLY INDUCED SKIN TUMORIGENESIS

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INTRODUCTION

Skin cancer is the most common form of cancer and the incidence is increasing steadily. The average increase in new skin cancer cases has been around 3% to 8% per year since the 1960s, with approximately 1 million new cases diagnosed each year in United States alone. This is particularly important because the available options have proven to be inadequate for the management of cancer. Thus, novel mechanism based approaches for management of skin cancer should be pursued. Chemoprevention, recognized as an important approach to control malignancy, have focussed on the search for desirable chemopreventive agents. Phytochemicals from plants, consumed by ethnic population are a promising group of potential cancer chemopreventive agents because of their low or no toxicity.

BACKGROUND AND PURPOSE

Nexrutine, a commercial herbal extract from a plant, Phellodendron amurense, is widely used for the treatment of inflammation, gastroenteritis and other chronic diseases. Recent studies have shown that Nexrutine may possess preventive effects against prostate cancer. In the present study, the antitumor-promoting effects of Nexrutine were explored in 7,12-dimethyl benz[a]anthracene-(DMBA)/ 12-O-tetradecanoylphorbol-13-acetate (TPA) induced mouse skin tumorigenesis model and to understand the mechanism involved therein.

METHODS

Animals and treatment for biomarker studies

Swiss female mice (6 to 7 weeks old) obtained from Animal Breeding Facility of IITR were used in the study. For short-term biomarker studies, mice were divided into four groups, shaved on the dorsal side for topical application. The mice in the first group received a topical application of 0.1 ml acetone, while those in second and third group received 1.0 mg Nexrutine per mouse. Thirty minutes after Nexrutine treatment, the mice in group 3 and 4 were treated with a single topical application of TPA (10 nmol per mouse) and sacrificed at different time intervals (6, 24 and 48 hrs).

Edema, hyperplasia and immunostaining for inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX2)

To assess the inhibitory effect of Nexrutine on TPA-induced edema, 1 cm diameter punches of skin from all the above groups were removed and weighed. After drying for 24 h at 500C, the skin punches were reweighed and loss of water content was determined. For the epidermal hyperplasia and Immunostaining studies, skin was fixed in 10%

formalin and embedded in paraffin. Vertical sections (5 μm) were cut and one set was stained with hematoxylin and eosin, while another set of sections was used for immunostaining for iNOS and COX2.

Thymidine Incorporation, ornithine decarboxylase (ODC) activity and Western Blot analysis

For assaying cell proliferation, all the above mentioned groups were given i.p. injection of 3H-Thymidine, 2 hrs prior to sacrifice, for thymidine uptake in DNA. From the same treatment groups cytosolic lysate was prepared for ODC activity. Western blot analysis of ODC, COX-2, iNOS protein expressions and phosphorylation of extracellular signal-regulated kinase1/2 (ERK1/2) c-jun N-terminal kinases (JNK), p38 and activation of NF-κB/p65 in whole cell lysate and nuclear extracts, was carried out using specific antibodies available commercially.

Skin tumourigenesis

Two-stage DMBA/TPA mouse skin tumorigenesis protocol was used (Int. J. Cancer: 117, 709, 717: 2005). Tumor induction was initiated by a single topical application of DMBA (120 nmol) followed by twice-weekly applications of TPA (4 nmol) alone or with Nexrutine (1.0 mg/0/1ml) with a time gap of 30 min., for 24 weeks. Skin tumor formation was recorded weekly.

RESULTS

In the short-term study, single topical application of Nexrutine (1.0 mg per mouse), 30 min prior to TPA (10 nmol per mouse) application to mice afforded significant inhibition against TPA-mediated increase in skin hyperplasia and edema (25, 27 & 32 % at 6, 24 and 48 hrs respectively) and hyperplasia. Topical application of Nexrutine resulted in substantial inhibition against TPA-induced epidermal (i) Thymidine incorporation (42%) and ODC activity (69%) (ii) COX-2 and iNOS protein expressions; (iii) phosphorylation of ERK1/2, JNK and p38; (iv) activation of NF-κB/p65. Subsequently, the effect of topically applied Nexrutine DMBA/TPA induced mice was assessed. Pre-treatment with Nexrutine showed significantly reduced tumor incidence (69%), lower tumor body burden (60%) and a significant delay inthe latency period for tumor appearance from 5 to 9 weeks in DMBA/TPA induced animals after 24 week interval.

CONCLUSION

These results provide evidence for anti-skin tumor-promoting effects of Nexrutine in mice and the chemoprevention is due to antiproliferative activity. Thus, it is conceivable to design Nexrutine containing emollient or patch, as well as sunscreen and skin-care products for prevention of skin cancer and other skin diseases.