Inhibitory effect of Hesperidin on tumour initiation and promotion in mouse skin

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Abstract. A flavonoid, Hesperidin was evaluated for its ability to inhibit tumour initiation by a polycyclic aromatic hydrocarbon and tumour promotion by a phorbol ester in the skin of CD-1 mice. Subcutaneous application of Hesperidin did not inhibit 7,12-dimethylbenz(a)anthracene-induced tumour initiation but did inhibit 12-O-tetradecanoyl-13-phorbol acetate-induced tumour promotion. Results provide evidence for a potential chemopreventive activity of Hesperidin.

Key words: Hesperidin – Skin tumorigenesis – Mice

Abbreviations: DMBA 7,12-dimethylbenz[a]anthracene – TPA 12-O-tetradecanoyl-13-phorbol acetate – FOR free oxygen radicals

Introduction

Flavonoids are universally distributed among vascular plants. They are ingested in a normal diet in average quantities of 1 g daily [1]. Accumulating evidence lends support to the theory that certain flavonoids may be inhibitors of carcinogenesis [2–4]. Thus, flavonoids were found to inhibit metabolism of carcinogens in vitro in isolated liver microsomes [5]. Furthermore, a number of hydroxylated flavonoids were found to inhibit the mutagenic activity of bay-region diolepoxides of benzo(a)pyrene [6]. Finally, certain flavonoids inhibited chemically induced tumours in experimental animal models both applied topically or in diet [2–4, 7].

In our previous work, we have shown that the extract of grapefruit, one of the citrus fruits, has some inhibitory effects on cancer cells in vivo [8]. The
flavonoid Hesperidin (7-rhamnoglycosyl mw:610.6 Dalton) (Fig. 1) is found in the edible portion of the majority of citrus fruits, particularly in grapefruit (2–3%) [1].

We then demonstrated one striking feature of Hesperidin; that it plays an important role as a reversible electron transferring substance during ascorbic acid synthesis in nature [9].

It has been also reported that Hesperidin is an effective antioxidative and scavenger of free oxygen radicals (FOR) whose role is well known in carcinogenesis [10–15].

This study aimed to evaluate whether Hesperidin alone has any effect to inhibit experimental skin tumorigenesis. Data indicating that Hesperidin inhibits 7,12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoyl-13-phorbol acetate (TPA) induced tumorigenesis is summarised in this paper.

Materials and methods

Chemicals

DMBA and TPA were obtained from Sigma Chemical (St. Louis, Mo.) and Hesperidin Phosphoric Acid Sodium Salt from Merck.

Animals and treatments

Female CD-1 mice were obtained from Charles Rivers Breeding Laboratories (Germany). Mice weighed between 24 and 26 g at the beginning of the experiment. They were brought to our animal facility at least 1 week before use. Mice were fed a standard chow diet and drinking water ad libitum. All mice were housed in polypropylene cages in a temperature-controlled room. They were kept on a 12-h light, 12-h dark cycle.

The dorsal region of each mouse was shaved with an electric clipper 2 days prior to experimental use and only those mice in the resting phase of the hair growth cycle were used.

DMBA and TPA were applied topically to the shaved area in 0.2 ml of acetone; control mice were treated with equal volumes of the appropriate vehicle alone. Hesperidin was dissolved in distilled water to prepare 1% (v/w) solutions, which was then injected into animals directly. Hesperidin solution was prepared as stocks, protected from the light and kept at 4°C during the experiment.

Mice were injected 125 μl of 1% hesperidin solution everyday subcutaneously at the dorsal skin. The injections either started 1 week prior to DMBA initiation and ended on in-