Methimazole Is an Inhibitor of Melanin Synthesis in Cultured B16 Melanocytes

To the Editor:

Recently, we have demonstrated in vivo that topical application of the antithyroid agent methimazole (1-methyl-2-mercaptoimidazole: MMI) on dark brown guinea-pig skin produces cutaneous depigmentation (Kasraee, 2002b). The DOPA incubation of the depigmented specimens revealed that MMI induces morphology changes of melanocytes in the treated skin. But unlike most depigmenting chemicals that act as melanocytotoxins, MMI did not cause a significant decrease in the numerical count of melanocytes. This indicated that MMI-induced depigmentation in vivo might result from the inhibition of the melanogenesis pathway in viable melanocytes, rather than a melanocytotoxic effect. To verify this in vitro, we compared the depigmenting and melanocytotoxic effects of MMI with those of kojic acid, hydroquinone, and arbutin, on cultured B16 murine melanocytes.

B16 melanocytes were grown in DMEM/10% fetal bovine serum (FBS) supplemented with 10 nM Cholera toxin, and the murine BDVII keratinocytes were grown in RPMI/10% FBS. MMI was dissolved in vehicle (propylene glycol:ethanol:water (5:3:2)) and was exposed to the cells at final concentrations in medium ranging from 0 to 100 μg per mL (0–880 μM) during four consecutive days according to the Dooley method (Dooley et al., 1994). On the fifth day, the melanocytes were harvested, sonicated, and centrifuged. DMSO was added to the pellet, the suspension was sonicated, and melanin determination was performed spectrophotometrically at 405 nm. The results from triplicate samples were analyzed as percent of control. Cell viability was determined on the fifth day for both keratinocytes and melanocytes, using MTT assay (Sieuwerts et al., 1995). For melanocytes, the specificity of melanogenesis inhibition was assessed by dividing the melanin content by the amount of reduced MTT.

At the highest concentration examined (880 μM), MMI was still well tolerated, as shown by the MTT assay, although it inhibited the melanogenesis activity of B16 melanocytes by more than 50% when compared with control (Fig 1A). The (melanin/MTT) ratio showed a concentration-dependent decrease, indicating that the depigmenting effect of MMI on cultured melanocytes is a result of the inhibition of melanin synthesis and not a melanocytotoxic effect (Fig 1B). Hydroquinone, a well-known depigmenting agent, was shown to be efficient as an inhibitor of melanogenesis in B16 melanocytes (Figs 2A and 2B); however, hydroquinone was highly toxic at 10 μM (Fig 2A), indicating that its observed depigmenting action in vivo may be due, at least in part, to its melanocytotoxicity. Arbutin, a phenolic compound used as a skin-lightening

Abbreviations: FBS, fetal bovine serum; MMI, methimazole

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agent in cosmetics, possesses a hydroquinone moiety bound to glucose. In B16 melanocytes, it began to be cytotoxic between 0.1 and 1 mM, while inducing a significant decrease of melanin already at 10 μM (Fig 3); this indicates that arbutin possesses a specific inhibiting action on melanocytes in vitro at moderate concentrations. Kojic acid, an inhibitor of tyrosinase and other oxidases (Burdock et al, 2001) also used as a lightening agent (Burdock et al, 2001), exhibited a low toxicity but an absence of inhibition of melanogenesis in cultured melanocytes (Fig 4). In order to distinguish between a selective melanocytotoxicity and a general cytotoxicity, an MTT assay was performed with the four compounds in murine BDVII keratinocytes. As shown in Fig 5, hydroquinone was cytotoxic in a concentration-dependent manner, whereas arbutin and kojic acid were non-toxic up to 100 μM, and MMI up to 880 μM.

The depigmenting activity of MMI on guinea-pigs does not go beyond a mild to moderate depigmentation. The moderate depigmenting activity of MMI in vivo is in accordance with its potency to depigment cultured melanocytes in vitro.

Melanocyte peroxidase is presently believed to be involved in several steps of the melanogenesis pathway. These steps include the oxidation of DOPA to dopaquinone (Bertazzo et al, 1999), the oxidative polymerization of indolic melanin monomers to form eumelanin (d’Ischia et al, 1991), and the oxidation of phenothiazine type intermediates in the production of pheomelansins (Kasraee, 2002a). In melanoma cells, pigment deposition begins to appear when peroxidase exhibits its highest levels of activity, i.e., in stages II and III premelanosomes (Shibata et al, 1993). On the other hand, amelanotic melanoma cells are negative for peroxidase activity (Shibata et al, 1993). We have recently reported a high correlation between the melanogenesis-inhibiting activity of chemicals and their ability to inhibit peroxidases (Kasraee, 2002a). The inhibition of peroxidase is a common property among most known melanogenesis inhibitors, whether or not they inhibit tyrosinase. Steroids, ascorbic acid, and indomethacin are examples of melanogenesis inhibitors that do not inhibit tyrosinase but inhibit...
peroxidase activity (Kasraee, 2002a). MMI is a well-known inhibitor of peroxidases from different sources (Balazs et al., 1986) and therefore could interfere with the melanin synthesis pathway at several steps.

Most known skin depigmenting chemicals act by destroying the epidermal melanocytes. Such agents are substrates of melanocytic enzymes such as tyrosinase and/or peroxidase, and their metabolization by these enzymes gives rise to the formation of intracellular cytotoxic species that kill the pigment cells (Kasraee, 2002a). Such a cytotoxic effect, however, is not highly specific for the melanocytes, and other cells such as keratinocytes could also be affected. This non-specific cytotoxicity is, most likely, the result of autooxidation of these compounds (Passi et al., 1987) as well as their peroxidase-mediated metabolization within such cells (Kasraee, 2002a). The non-selective cytotoxicity as well as the mutagenic potential of melanocytotoxins has recently raised serious questions of concern regarding their continual use in the dermatologic products.

MMI has several advantages over known depigmenting agents: (1) unlike hydroquinone that, even at very low concentrations, shows strong cytotoxic effects towards melanocytes and keratinocytes, MMI is non-cytotoxic up to high concentrations; (2) unlike hydroquinone (Joseph et al., 1998) and kojic acid (Wei et al., 1991), which are known to be mutagenic on mammalian cells, MMI has been demonstrated to be devoid of any mutagenic effect (Akatsuka et al., 1979); (3) MMI is relatively stable and does not easily undergo autooxidation; (4) most thiol compounds with depigmenting ability such as mercaptoethylamines have offensive odors, which prohibits their use in topical products (Frenk et al., 1968); MMI, however, is an odorless thiol; (5) Despite the equivocal in vitro results on the melanogenesis-inhibiting effects of kojic acid and arbutin, the skin depigmenting action of these two agents has never been confirmed in any controlled in vivo study, whereas the skin depigmenting effect of MMI is also confirmed in vivo (brown guinea-pig model); and eventually (6) MMI, when used topically, reduces the UV-induced erythema in the human skin (Moseley et al., 1989) and therefore may simultaneously serve as a sun protectant.

Although in our model arbutin was shown to be non-cytotoxic and as efficient as MMI in inhibiting melanogenesis, arbutin is a glycoside containing a hydroquinone moiety, and it is not excluded that epidermal cells, due to the presence of glycosidases (Holleran et al., 1992; Redoules et al., 1999), can hydrolyze it to deliver free hydroquinone. Thus, it is possible that long-term use in humans of topical arbutin could lead to deleterious side effects due to the delivery of hydroquinone.

In conclusion, kojic acid is non-toxic but poorly effective, as confirmed by other studies (Curto et al., 1999; Lei et al., 2002; Springer et al., 2003), hydroquinone is effective but highly cytotoxic and mutagenic (Curto et al., 1999), arbutin is effective in vitro but lacks the data confirming its in vivo efficacy, and is also potentially toxic and mutagenic when used for a long time, whereas MMI is shown to be effective as a depigmenting agent both in vitro and in vivo, and is non-cytotoxic and non-mutagenic. Therefore, MMI could
serve as a lead compound for the discovery of novel safe and efficient skin depigmenting compounds in the future.

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