DERIVATIVES OF p-METHOXYPHENOL [4-HYDROXYANISOLE] AS MELANOMA SPECIFIC DRUGS: BIOCHEMICAL TARGETING TO TYROSINASE AND β-GLUCURONIDASE

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Biochemically targeted drugs increase the specificity of chemotherapeutic drugs. The prodrugs are not toxic in normal tissues but are metabolized to cytotoxic compounds by enzymes that exist at high levels in tumor cells as compared to those in normal ones. Tyrosinase is the only enzyme that is required for the synthesis of melanin. It is expressed exclusively in melanocytes. Tyrosinase is an oxidase that converts, among many other phenols, tyrosine into DOPA which is further oxidized to *o*-dopaquinone. Most quinones generate reactive oxygen and are toxic due to oxidative damage. Tyrosine analogs have thus been considered as drugs that specifically kill melanocytes. Indeed, the tyrosinase substrate *p*-methoxyphenol (PMP) is used as a skin depigmentation agent.

Most melanomas are rich in tyrosinase and in β-glucuronidase. Tyrosinase- and β-glucuronidase-targeted drugs are therefore expected to specifically kill melanoma cells. *p*-Methoxyphenol (PMP) is a prodrug targeted to tyrosinase.

PMP readily penetrates cells, most probably by virtue of its non-polar methoxy moiety. Within cells, the weakly toxic PMP is oxidized by tyrosinase to a highly toxic *o*-methoxy benzoquinone. PMP is converted to a toxic quinone upon metabolism by tyrosinase.

Tyrosinase-rich tumor cells in culture were readily killed by treatment with PMP, whereas tyrosinase-deficient cells were much more resistant to PMP.

We have established that the cytotoxicity of PMP to murine and human melanoma cell lines and to other tumor cell lines was largely correlated with the level of tyrosinase, and that inhibition of tyrosinase by kojic acid decreased the toxicity of PMP. In addition, PMP exerted tyrosinase-independence toxicity, in that PMP was toxic to tyrosinase-deficient cells and to kojic acid-treated tyrosinase-rich cells. Cytochromes P450 did not seem to metabolize PMP in that melanoma and other cell lines contained very low level of CYP450, and inhibition of CYP450 by α-naphthoflavone or by octylamine did not rescue PMP-treated cells. Likewise, PMP appeared not to be metabolized by cyclooxygenase in that its inhibition by indomethacin did not affect the toxicity of PMP. The biochemical reason for the tyrosinase-independent portion of PMP toxicity remains, as of yet, unknown.

Patients with tyrosinase-rich melanomas have relatively high concentrations of the enzyme in their blood, and treatment with PMP led to systemic toxicity.

Melanoma cells are rich also in lysosomal β-glucuronidase as compared to normal tissues.

In order to minimize the systemic toxic effect of PMP, we have synthesized *p*-methoxyphenyl glucuronide (PMPG) which is resistant to tyrosinase. The activation of PMPG depends on both β-glucuronidase and tyrosinase. PMPG was toxic to melanoma cells in the presence, but not in the absence of exogenous β-glucuronidase, indicating that the polar glucuronide did not penetrate cells.

In order to render the drug permeable, we synthesized a non-polar PMP derivative (2'-3'-4'-hydroxy-6'-carboxymethyl)-*p*-methoxyphenyl-O-glucuronide named protected PMPG (P-PMPG).

P-PMPG is insoluble in water and did not kill cells in culture. Cells were thus exposed to multilamellar phosphatidyl choline liposomes containing P-PMPG.

Liposomal P-PMPG was toxic to tyrosinase-and β-glucuronidase- rich melanoma cells, and less toxic to cells containing lower levels of these enzymes, and inhibition of tyrosinase by kojic acid decreased the toxicity of liposomal P-PMPG.

The effect of P-PMPG on the development of tumors in vivo was determined in C57Bl mice injected s.c. with B16F10 melanoma cells and fed with a diet containing P-PMPG. In treated mice, death was postponed by 3-6 days and the rate of tumor growth was lower as compared to untreated mice. P-PMPG also decreased the incidence of metastases into the lungs.