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REVIEW ARTICLE

An Experimental Approach to the Chemotherapy of Melanoma

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Malignant melanoma represents an important challenge to the clinician and scientist. Although substantial progress has been made in the diagnosis of early lesions, the systemic therapy of advanced disease urgently requires new agents. This situation exists despite the fact that malignant melanoma possesses features that should render it particularly susceptible to selective chemotherapy. First, melanocytes serve a nonessential role in the host, and therefore, potential agents need not discriminate between normal and malignant cells, but rather between melanocytic and nonmelanocytic cells. This feature obviates the dilemma that traditionally faces the cancer chemotherapist; specifically, the incorporation of tumor selectivity into the design of the drug. Second, melanocytes possess a unique phenotype (Figure), containing the enzyme tyrosinase, a polyphenol oxidase, which catalyzes the oxidation of tyrosine to levodopa to dihydroxyindole and ultimately to the biopigment melanin. Although there is controversy as to whether tyrosinase alone or peroxidase are the enzymes involved, it is clear that these conversions are carried on to a greater degree within melanocytes. Since this enzymatic activity is restricted to melanocytes and is generally present in larger amounts in melanoma cells, a defined biochemical target is present. Third, melanoma cells are responsive to a specific polypeptide hormone, melanocyte stimulating hormone (MSH) which causes an increase in pigmentation and a decrease in growth, thereby further enhancing the difference between melanocyte and nonmelanocyte. Fourth, conventional agents are most effective against rapidly dividing cells with tumor cells generally demonstrating an inverse relationship between rate of growth and phenotypic maturation. As a correlary of these features, melanoma specific agents should be complementary to conventional agents and most effective against the slowly growing, deeply pigmented cells which are least susceptible to conventional agents therefore suggesting the possibility of combination chemotherapy. During the past several years, advances have been made toward specific approaches to the therapy of melanoma and we shall review some recent developments.

BACKGROUND

Human depigmentation signifying the destruction of melanocytic cells occurs in a variety of spontaneous forms including vitiligo, halo nevi, and halo metastasis of melanoma. Depigmentation has also been reported to occur as a result of exposure of human skin to a variety of compounds in common use and 2 of these compounds, hydroquinone and the monobenzylether of hydroquinone, have been employed extensively for the treatment of pigmentary disturbances characterized by increased pigmentation [1]. Several studies have confirmed the inherent cytotoxicity of mono- and dihydroxylated benzene derivatives for the pigment cell *in vivo* and *in vitro* and it has been suggested that melanin producing cells contain and produce compounds that might be autotoxic [2-4]. It is apparent from the Figure that the major metabolites in the pigment pathway may be grouped into 1 of 3 categories and considered to be related to either tyrosine (monohydroxy), levodopa (dihydroxy), or 5,6-dihydroxyindole. Derivatives representing each of these categories have been examined as potential models for the design of compounds that will exhibit useful clinical cytotoxicity for melanoma cells. It should be stressed that the rationale for these approaches is not to interfere with melanin biosynthesis because melanin production is not essential to the general metabolic economy of the melanoma cell, but rather to design compounds which are converted to cytotoxic derivatives by the action of a unique enzymatic apparatus within the melanoma cell.

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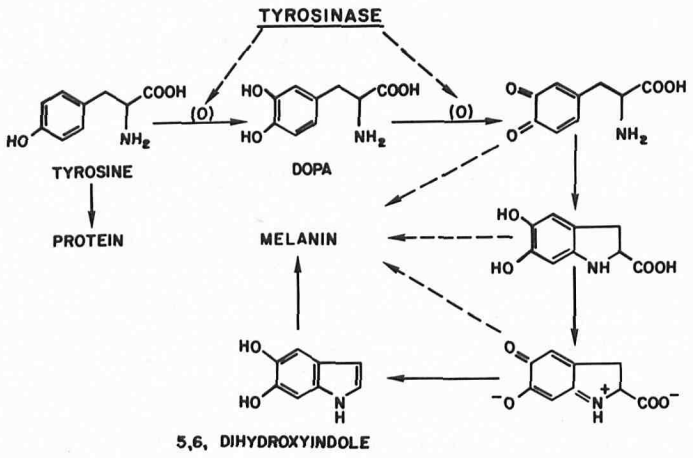
TYROSINE

The initial intermediate in the biosynthetic pathway for the formation of melanin is tyrosine. Tyrosine may be considered to be a momohydroxy derivative of benzene and electronically corresponds to a phenol. Tyrosine serves as the starting material for the biosynthesis of melanin and is also an important substrate for melanoma cells *in vitro*. The degree of pigmentation of melanoma cells can be influenced by changing the concentration of tyrosine in the growth medium [5]. Raising the concentration of tyrosine causes an increase in cellular pigmentation. Furthermore, tyrosine levels may be elevated sufficiently to cause toxicity to pigment producing cells as compared to their nonpigmented counterparts [6]. Further discussion of the mechanism of cytotoxicity will be discussed under Levodopa Analogs. This technique has proved to be a biologically useful method of generating amelanotic clones of melanoma cells starting from mixed populations. A major obstacle to applying this approach for the chemotherapy of melanoma has been that tyrosine is one of the naturally occurring amino acids and is therefore used extensively in general cellular protein synthesis. Rapidly growing pigmented cells incorporate only about 5% of exogenous tyrosine into pigment with the remainder entering protein synthesis [7]. Since tyrosine levels present in mammals like those of other amino acids are under rigid homeostatic control, attempts to raise or lower levels of tyrosine *in vivo* have met with limited success.

A major factor influencing the cytotoxicity of tyrosine to melanoma cells is the ratio of tyrosine level to specific activity of tyrosinase within the cell. A potential solution to the problem of elevation of tyrosine levels may be to elevate the level of tyrosinase present in the melanoma cells themselves. The availability of a hormone for enhancing pigmentation, α-MSH, suggests a possible method for implementing this approach. α-MSH has been shown to cause a marked enhancement of pigmentation of the B-16 melanoma *in vivo* and there was a suggestion that the tumor volume itself was somewhat inhibited [8]. *In vitro* experiments suggest that α-MSH is inhibitory to the growth of pigment-producing cells, an effect that may be mediated either through the elevation of intracellular cyclic



The Raper-Mason scheme for melanin biosynthesis. The major classes of intermediates are represented by tyrosine, levodopa, and 5,6-Dihydroxyindole.

AMP levels or alternatively through the effects of tyrosine and enhanced pigmentation directly [9]. Since the phenotypic differentiation could be further induced by the addition of phosphodiesterase inhibitors such as theophylline which result in a synergistic increase in pigmentation, it is possible that clinically useful cytotoxicity could be obtained. Sufficient cytotoxicity can be generated using this approach to reduce significantly both the clonogenic and tumorigenic potential of murine melanoma cells reinjected into animals. Evaluation of this approach in humans needs detailed clinical pharmocologic control of α-MSH administration [10].

Several other approaches using phenol derivatives have been explored. An interesting approach has recently been described using the synthesis and production of naturally occurring phenolic precursors isolated from the common mushroom, *Agaricus bisporus*. These compounds are derivatives of L-glutamic acid (γ-4-hydroxyanilides) and appear to function as natural growth regulators in the mushroom. Under the influence of tyrosinase present in the mushroom, these compounds are readily converted to quinone-like intermediates which are capable of inducing a cryptobiotic state in the mushroom characterized by the inhibition of macromolecule biosynthesis [11]. Recently, it has been demonstrated that the parent phenol is inhibitory to the growth of murine B-16 melanoma *in vivo* and *in vitro*, presumably because mammalian melanoma cells also possess tyrosinase, and are able to convert the phenol to the inhibitory quinone [12]. We have prepared and examined the effects of a variety of monohydroxy and dihydroxy analogs of this quinol in an attempt to enhance the potency of these compounds [13].

LEVODOPA-DOPAMINE

The second point of entry into the biochemical apparatus of the melanoma cell occurs at the level of levodopa conversion. It has been shown that levodopa, unlike tyrosine, is selectively incorporated into melanoma cells. Although levodopa is an amino acid, it is not normally found in cellular proteins. Through the use of levodopa decarboxylase inhibitors, which limit the catabolism of levodopa, enhanced incorporation into melanoma tissues could be achieved *in vivo* [14]. This approach appeared attractive, since levodopa is intrinsically more selective than tyrosine and we proceeded to examine the effect of levodopa and dopamine upon the growth of melanoma cells *in vitro*. We were gratified to observe that levodopa was selectively toxic to pigmented cells *in vitro* and that through the use of its more water soluble analog, levodopa methyl ester, *in vivo* antitumor activity in the experimental B-16 melanoma was observed for the first time using a melanin precursor [15,16]. Interestingly, it was noted that levodopa was also capable of inhibiting the growth of other tumor cells including the C1300 neuroblastoma and murine L1210 lymphocytic leukemia cells, although quantitatively, the degree of inhibition observed in these nonmelanocytic cells was always less than that observed for tyrosinase positive cells [17,18]. The major metabolite of levodopa, dopamine, which is also effectively incorporated into melanocytic cells, was observed to be a highly potent inhibitor against the B-16 melanoma *in vivo* and *in vitro* [19].

Recent studies have revealed several interesting features concerning the mechanism of action of these derivatives [18]. First, these compounds appear to exert a selective inhibitory effect on thymidine incorporation with relatively lesser effects upon uridine or leucine incorporation. Second, these drugs exhibited a specific effect upon the cell cycle traverse, namely, the production of a block at the G_1S interface. Third, the inhibition of thymidine incorporation was noted to occur very early, being essentially complete as soon as 5 min after exposure to drug. Fourth, similar kinetics of inhibition were observed for other nucleotides suggesting that the inhibition occurred at a point where each of these precursors of DNA synthesis were equally utilized. DNA polymerase itself was suggested to be the site of action. This hypothesis was tested by examining the effect of these analogs upon isolated mammalian DNA polymerase [20]. A highly effective inhibition of this enzyme was noted when the analog and tyrosinase enzyme were present in the reaction mixture together. The parent quinols themselves, in the absence of tyrosinase, were not inhibitory to DNA polymerase. Addition of either preformed quinone or tyrosinase which permits the generation of quinone *in situ*, restored inhibitory properties of these drugs. A second consequence of these experiments indicated that the quinones rather than the quinols were the inhibitory species. Furthermore, one of the analogs, 3,4-dihydroxybenzylamine, which was unable to cyclize to a dihydroxy indole derivative, was the most potent derivative. This observation suggests that catechols and quinones themselves were capable of possessing cytotoxicity and that subsequent conversion to an indole derivative was not necessary for activity.

These experiments suggested a model of the melanoma cells *in vitro*, with tyrosinase representing the unique phenotype of the melanoma cell and DNA polymerase representing its proliferative thrust. Following entry of quinol into the cell, it is converted to quinone by tyrosinase present. The quinone in turn interacts with DNA polymerase which is known to be a sulfhydryl dependent enzyme and sensitive to oxidizing conditions. The result of these interactions is inactivation of DNA polymerase with a consequent inhibition of cellular growth and ultimately cell death. The experimental model also provides a convenient approximation of conditions present within the melanoma cell with a close juxtaposition of tyrosinase and DNA polymerase thereby permitting the evaluation of the effectiveness of new derivatives. The model permits the drugs to be judged for their ability to be activated by tyrosinase as well as to subsequently inhibit DNA polymerase. Although the mechanisms of activation of these drugs within other tumor cells such as leukemia cells and neuroblastoma cells is not clear, it is possible that other enzymes are found in these cells such as myeloperoxidases. Alternatively, selectivity may be related to differing levels of superoxide dismutase which has recently been shown to be deficient in tumor cells [21].

Several analogs of levodopa have now been examined which provide insight into the relationship of structure and activity. Levodopa is somewhat unique among antitumor agents in that the dose limiting toxicity which appears to be neurotoxicity operates by a mechanism other than its antitumor effect. For example, one of the analogs, 6-hydroxydopa, has been shown to be highly potent in terms of selective toxicity for melanoma cells *in vitro* [22]. The IC_{50} of 6-hydroxydopa was approximately 90 times less than that observed for levodopa itself. After extensive *in vivo* evaluation of this derivative, it appears to be too toxic for the demonstration of *in vivo* antitumor activity

since 6-hydroxydopa has unique neurotoxic properties that result in the selective degeneration of adrenergic nerves.

In an attempt to retain *in vitro* antitumor activity while limiting neurotoxicity several other analogs of levodopa have been prepared [18]. Recent results have suggested that one of these derivatives, 3,4-dihydroxybenzylamine, may represent compounds at the opposite extreme. With this derivative, we are able to completely limit the neuroagonist effect intrinsic in these drugs and separate them from their antitumor effects. For example, 3,4-dihydroxybenzylamine has shown equivalent antitumor activity *in vitro* yet possesses a 2-fold greater *in vivo* tolerated dose and therefore, an enhanced therapeutic response was observed by increased delivery of drug.

DIHYDROXYINDOLE

The third intermediate in the melanin pathway is 5,6-dihydroxyindole. It has been reported that dihydroxyindole is cytotoxic to melanoma cells *in vitro* [22]. This compound is an extremely reactive nucleophile and electrophile, and this reactivity might limit the role of indole as antitumor agents. Furthermore, when dopachrome derivatives, which are dihydroxy derivatives related to dihydroxyindoles are examined for DNA polymerase inhibitory activity, they appear to be less effective than orthoquinones as inhibitors of this enzyme thereby suggesting that their cytotoxicity is mediated by a different mechanism. As mentioned previously, these mechanisms may be related to the generation of superoxide radical.

SUMMARY

Significant progress has been made toward a rational approach to the chemotherapy of melanoma. Because of the limitations of this review, we have restricted our comments to those approaches that utilize the melanin synthetic apparatus. Although the exact details of the mechanism of action of these agents remain to be elucidated, the general phenomena outlined here might serve as a model for chemotherapy of cancer. The unique features are yet to be identified at the molecular level in most tumors, but this situation may well change as detailed biochemical differences of the different tumor cell types are uncovered and will ultimately permit the design of drugs that are selective against other tumors.

In addition, important biochemical properties of catecholamines have been identified where intracellular effects are involved. These findings may have implications for other biologic effects of these drugs and it is possible that the action of levodopa now observed in the treatment of neurological disorders may be mediated to some extent by intracellular mechanisms identified in the antitumor studies. Similarly, if DNA polymerase is the target of these drugs, additional insights into the mechanism of action of this important enzyme may be obtained, especially since the quinol-quinone interaction in the dopa series probe into the fundamental redox properties of a tumor cell. Finally, since levodopa and dopamine have had extensive use in humans, an opportunity exists for the rapid clinical evaluation of the potential role of these compounds. With the availability of analogs of improved activity, the rapid application of basic scientific advances to clinical medicine may be anticipated.

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