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Enhancement of L-Dopa Incorporation into Melanoma by Dopa Decarboxylase Inhibition

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Melanoma cells possess a special biochemical pathway for the conversion of L-dopa to melanin. Selective incorporation of exogenous L-dopa into melanoma cells *in vivo* has been limited by extensive decarboxylation to dopamine. Pretreatment of animals bearing the S-91 Cloudman or ACI melanomas with Ro4-4602, a potent dopa decarboxylase inhibitor limited incorporation of label into adrenal tissue and enhanced entry of label into tumor. Six hours following pretreatment, the ratio of tumor to adrenal specific activities was altered from 0.25 to 1.5 for the S-91 melanoma and 0.68 to 1.99 for the ACI melanoma indicating diversion of metabolism away from catecholamine formation. The possibility of a selective diagnostic and/or therapeutic approach is proposed.

Malignant melanoma is a therapeutically resistant tumor that possesses a special biochemical pathway for the conversion of L-3,4-dihydroxyphenylalanine (L-dopa) to the biopigment melanin [1]. The biosynthesis of melanin is catalyzed by the enzyme tyrosinase, which is restricted to normal and malignant melanocytes. Attempts to utilize this pathway for the design of specific chemotherapeutic or diagnostic agents have focused on the selective incorporation of melanin precursors. Recently, we have demonstrated the highly selective, temperature-dependent incorporation of L-dopa by pigmented melanoma cells *in vitro* and have restudied incorporation *in vivo* [2].

Three previous reports have examined the partition of L-dopa in animals bearing melanoma with conflicting results. Blois and Kallman showed a highly selective incorporation of radiolabeled L-dopa into a spontaneously arising murine melanoma [3]. Hempel and Deimel, utilizing the Harding-Passey model, demonstrated extensive incorporation into tumor but there was even greater incorporation into the adrenal medulla [4]. Finally, Meier, Beierwaltes and Counsell were able to show selective incorportation into the Fortner hamster melanoma but not into B-16 tumor [5]. In each study, it was apparent that decarboxylation of precursor L-dopa to dopamine and diversion to catecholamine biosynthesis was a major limitation since incorporation of label into adrenal medulla was always extensive.

Recent understanding of the human clinical pharmacology of L-dopa confirms that of an administered dose 69% is diverted to catecholamine biosynthesis, 1% excreted unchanged, 10% excreted as metabolites of L-dopa and 20% of the label not recovered [6]. Potent inhibitors of the initial decarboxylation by the enzyme aromatic L-amino acid decarboxylase (dopa decarboxylase) have been developed for use in conjunction with L-dopa in the therapy of Parkinson's disease, where their use permits a large reduction in required therapeutic dose of L-dopa [7,8].

We report our results of the effect of a recently available inhibitor of dopa decarboxylase on the tissue distribution of radiolabeled L-dopa in experimental melanoma. One of these inhibitors, Ro4-4602, causes a marked inhibition of incorporation of label into the adrenal medulla with a concomitant enhancement of tumor incorporation in the ACI and S-91 Cloudman transplantable melanomas.

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MATERIALS AND METHODS

Chemical Agents

L-3,4-dihydroxyphenylalanine (L-dopa) was obtained from Sigma Chemical Co., St. Louis, Mo. Ro4-4602 (N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl) hydrazine was a gift from Hoffman LaRoche Co., Nutley, New Jersey. Uniformly radiolabeled ^{3}H-L-dopa (specific activity 11 Ci/mmole) was obtained from New England Nuclear Co., Boston, Mass. All other chemicals were reagent grade and used without further purification.

Tumors

The origin and maintenance of the cell lines has been described [2]. S-91A refers to a pigmented clone of Cloudman melanoma while S-91B refers to a hypomelanotic clone. The ACI rat melanoma, a heavily pigmented tumor, was from the Mason Research Laboratory, Worcester, Mass. Tumors were maintained by serial subcutaneous implantation of tumor cells following standard National Cancer Institute protocols [9]. Experiments were conducted on tumors when they were approximately 1 cm in diameter.

Tissue Radioactivity

All injections were given intraperitoneally in normal saline. L-dopa was administered at a dose of 2.5 mg/kg with 20 μCi for rats and 25 mg/kg with 10 μCi for mice. Ro4-4602 was administered at a dose of 250 mg/kg in rat experiments and 500 mg/kg in mouse experiments, 1 hr prior to L-dopa.

Animals were sacrified by cervical dislocation. Serum samples were obtained by retro-orbital bleeding. 20–30 mg samples of tissue were obtained 6 hr following injections and transferred directly to preweighed scintillation vials. Three samples of each tissue were removed from each animal. The use of the rat melanoma permitted the dissection of the adrenal medulla away from the remainder of the gland. 0.5 ml of Protosol (New England Nuclear, Boston, Mass.) was added and samples were heated at 50°C for 12 hr. 10 ml of scintillation fluid (Aquasol, New England Nuclear Co.) were added and samples were counted on a Beckman LS-335 scintillation counter. Quenching was corrected by addition of an internal toluene standard.

RESULTS

Figure 1 shows the tissue distribution of L-dopa and dopamine in normal mice (BDF_1, 6–8 weeks of age, Jackson Laboratory, Bar Harbor, Maine) at 24 hr. Dopamine is specifically incorporated into the adrenal gland; L-dopa without decarboxylase inhibition, displays a pattern similar to dopamine reflecting its extensive conversion to the latter compound. Pretreatment with Ro4-4602 effects a profound alteration in distribution. Adrenal incorporation is minimal, while the predominantly renal excretion of metabolites is reflected in high kidney specific activity. This activity was equal to that observed in a sample of urine taken at the time of sacrifice, thus suggesting its nonparenchymal location. The adrenal radioactivity, although repre-

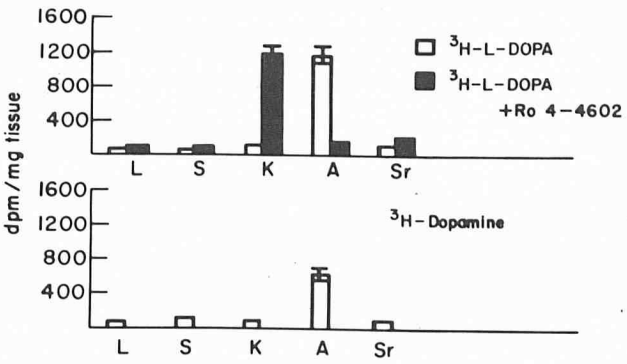

FIG 1. Tissue distribution of radioactivity 24 hr following administration of labeled precursor. *L* (liver), *S* (spleen), *K* (kidney), *A* (adrenal), *Sr* (serum). Values represent mean ± S.E.M. for 3 animals/group.

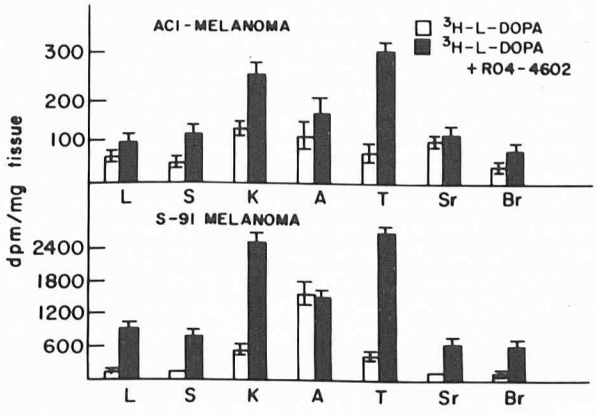

FIG 2. Effect of decarboxylase inhibition on tissue distribution of radioactivity. Values represent mean ± S.E.M. for 3 animals/group. ACI melanotic melanoma, S-91A melanotic melanoma. *L* (liver), *S* (spleen), *A* (adrenal), *Sr* (serum), *Br* (brain), *T* (tumor).

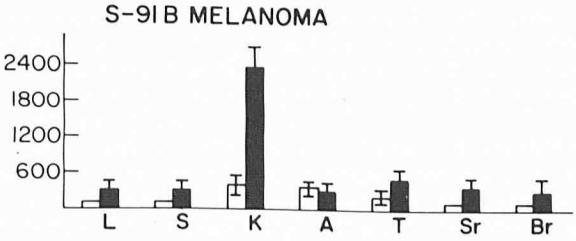

FIG 3. Effect of decarboxylase inhibition on tissue distribution of radioactivity in S-91B hypomelanotic melanoma. Values represent mean ± S.E.M. for 3 animals/group. *L* (liver), *S* (spleen), *A* (adrenal), *Sr* (serum), *Br* (brain), *T* (tumor).

senting only a small fraction of the overall administered dose is therefore a convenient biologic index of the degree to which L-dopa has been converted to dopamine.

Figure 2 depicts the effect of decarboxylase inhibition in both the ACI and S-91 melanotic tumors, as measured 6 hr after administration. Since adrenal radioactivity is stable over several days, 24 hr is the optimum time at which to demonstrate the effects of decarboxylase inhibition on adrenal incorporation. We have found however, that 6 hr is the time at which tumor radioactivity is maximal. This result is to be expected since melanin is a continuously growing polymer that must lose label as it grows. The difference in time at which the tissues were sampled accounts for the elevated serum activity in Fig 2 as compared to Fig 1 and also for the elevated adrenal activity, since whole tissue was sampled including extracellular fluid.

In each case, the effect of decarboxylase inhibition is most apparent when one examines the ratio of tumor/adrenal activity. For the ACI melanoma, the ratio was altered from 0.68 to 1.99 with pretreatment. In the S-91 melanotic melanoma, the tumor/adrenal ratio for controls was 0.25 while animals treated with the decarboxylase inhibitor showed a ratio of 1.5.

The hypomelanotic melanoma S-91B, shows much less incorporation of label (Fig 3). Furthermore, although serum levels are enhanced by decarboxylase inhibition there is no specific increase found in the tumor. In vitro, S-91B incorporates about one-tenth of the label that S-91A incorporates [2].

DISCUSSION

The role of L-dopa as a precursor of catecholamine biosynthesis has severely limited potential selective chemotherapeutic or diagnostic intervention. It is apparent from our experiments that administering L-dopa in the presence of a potent decarboxylase inhibitor, like Ro4-4602, results in higher serum levels and enhancement of incorporation into melanoma. The major diversion of label into adrenal tissue that was apparent in all previous *in vivo* studies of L-dopa incorporation by melanoma could be largely obviated. No attempt was made in these studies to maximize L-dopa incorporation into tumor but rather we attempted to use conditions similar to those of previous studies to afford a basis for meaningful comparison.

The possibility of selective localization of radiolabeled compounds for tissue identification has been a useful one with the principle example being that of adrenal gland scanning with ^{131}I-iodocholesterol [10]. This technique is completely dependent on biologic selectivity in concentration of precursor. Decarboxylase inhibition may afford the possibility of a similar opportunity with L-dopa and melanoma. The proposal of Hempel and Deimel, therefore, may now be reexplored [4]. They initially suggested the use of radiolabeled L-dopa for the radiotherapy of melanoma. They concluded from the results of their studies, however, that the concentration of activity in the melanoma was insufficient.

Finally, we have reported that L-dopa is selectively toxic to pigmented melanoma cells in vitro [11,12]. Furthermore, we have shown that the methyl ester of L-dopa possesses significant antitumor activity against B-16 melanoma *in vivo* and that this activity is enhanced by pretreatment with a dopa decarboxylase inhibitor [13]. Presumably, decarboxylase inhibition as demonstrated here does afford greater *in vivo* cytotoxic concentration.

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