

Pretreatment Effects on the Uptake/Retention Kinetics of L-Dopa in Harding-Passey Melanoma

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Malignant melanoma cells possess a unique biochemical pathway that converts L-3,4-dihydroxyphenylalanine (L-dopa) to the biopigment melanin. Selective cytotoxic incorporation of exogenous L-dopa into melanoma cells in vivo may provide a means of designing specific chemotherapeutic agents useful in the treatment of this disease. Using the Harding-Passey murine melanotic tumor model, a preferential uptake of [³H]L-dopa by the tumor was characterized. Following pretreatment of the tumor-bearing mice with nonradioactive L-dopa, a significant enhancement

($p < 0.01$) of [³H]L-dopa incorporation and retention into melanoma for a period of 24 h was observed, when compared with the concomitant tissue distribution and clearance of radioactivity in the control animals. This finding suggests that by initial pretreatment of melanoma with nonradioactive L-dopa, the subsequent selective accumulation of [³H]L-dopa in tumor may provide a useful tool in testing new modalities of therapy in malignant melanoma. *J Invest Dermatol* 86:560-562, 1986

L-3,4-Dihydroxyphenylalanine (L-dopa) is a precursor in the biosynthesis of melanin [1,2]. Malignant melanoma, still a therapeutically resistant tumor, possesses this unique biochemical pathway for the conversion of L-dopa to melanin [3,4]. Several investigators have used this special pathway as a means to design more effective chemotherapeutic agents useful in the treatment of this disease. Wick et al [5] showed a selective cytotoxic incorporation of L-dopa by pigmented melanoma cells in vitro. Blois and Kallman [6] demonstrated a highly selective uptake of radioactive L-dopa into a spontaneously arising murine melanoma. Recently, using the Harding-Passey murine melanoma model, our studies and others were able to show an extensive localization of L-dopa in the tumor and in the adrenal glands [7,8]. In this communication we describe a preferential localization of [³H]L-dopa in the Harding-Passey tumor following pretreatment of tumor-bearing mice with nonradioactive L-dopa, and contrast it to the concomitant tissue distribution and retention of this radioactive agent in the control animals.

MATERIALS AND METHODS

Experimental Procedure CDF₁ mice used in this study were approximately 3 months of age and weighed on average 25 g. Harding-Passey pigmented melanoma tumor cells, prepared from tumor transplant, were serially passaged s.c. in CDF₁ mice. The

cell suspensions were made from excised tumors which were minced and passed through 80-mesh stainless steel screen in alpha-modification of Eagle's minimal essential medium containing 3-(N-morpholino) propane sulfuric acid 4.08 g/liter. Each mouse was inoculated s.c. in the right groin with 1×10^6 tumor cells. Eight days after transplantation of the tumor, when the tumor nodules reached an average diameter of 0.75-1.0 cm, the animals were randomly divided into 2 groups of 8 mice each. The experimental group received a s.c. injection of nonradioactive L-dopa (100 mg/kg) in the opposite groin while the control group received an equivalent volume (0.3 ml) of normal saline s.c. One hour later 20 μ Ci [³H]L-dopa were administered to each mouse i.p. The animals of both groups were sacrificed by etherization and exsanguination by cardiac puncture at 0.5 h, 1 h, 4 h, and 24 h after injection of [³H]L-dopa. The mice were dissected and tissues, including brain, lung, spleen, adrenal glands, kidney, muscles, and tumor, were removed. Each specimen was weighed and burned in a Packard Tri-carb oxidizer. The radioactivity was measured in a liquid scintillation counter and for each tissue the counts were expressed in cpm/mg of wet tissue weight.

Statistical Analysis The objective was to determine any difference in the time course for each organ site between the control and the experimental groups. For each site the uptake and retention of [³H]L-dopa were approximated as an exponential time course. The linear form of this model is:

$$\ln(C_t) = \ln(C_0) - Bt,$$

where C_t is the cpm/mg wet tissue of radioactivity at time t from initial injection of [³H]L-dopa, B is the slope or retention rate, and C_0 is the cpm/mg wet tissue at time 0. Fits of the data by linear regression of both pretreated and control groups are given in Figs 1, 2, and 3A-F for the tumor, adrenal glands, brain, lung, liver, spleen, kidney, and muscle, respectively.

At each site the control and experimental groups were compared by evaluating the colinearity of the regression lines. Standard statistical methods were used with a partial F -test [9]. In essence the 2 pretreatment groups were tested for the same retention rate and the same initial uptake.

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Abbreviations:

AUC: area under curve

L-dopa: L-3,4-dihydroxyphenylalanine

REI: relative enhancement index

RESULTS

Fig 1 illustrates the tumor uptake and retention kinetics of L-dopa after 20 μCi [^3H]L-dopa were injected i. p. into the Harding-Passey melanoma tumor-bearing mice which were grouped by whether or not they had been pretreated with nonradioactive L-dopa. The radioactivity localized was significantly higher in the pretreated tumor than in the control tumor ($p < 0.01$). Whereas the radioactivity retained in the control tumor cleared almost completely at 24 h postinjection, a strong retention was noted in the pretreated tumor over 24 h. This finding indicated that pretreatment of nonradioactive L-dopa could enhance the uptake of subsequently administered [^3H]L-dopa by the tumor, and also could retard the clearance of the radioactive melanin precursor from melanoma.

Fig 2 shows the concomitant uptake and clearance kinetics of L-dopa in the adrenal gland of the Harding-Passey melanoma tumor-bearing mice. Since L-dopa has been a precursor and common intermediary in the biosynthetic pathway of melanin, epinephrine, and norepinephrine [1,2], an expected increase in the time course from the pretreatment with nonradioactive L-dopa was observed in the adrenal gland ($p < 0.05$) [7,8]. However, the slope of the time course for this tissue was unaffected by pretreatment with the nonradioactive melanin precursor (Fig 2). The simultaneous uptake and retention of [^3H]L-dopa in the other vital organs including the brain, lung, liver, spleen, kidney, and muscle were not influenced by this nonradioactive L-dopa pretreatment (Fig 3A-F). Only the tumor and the adrenal gland exhibited a significant statistical difference between the time courses of the control and experimental pretreatment groups (Figs 1, 2).

In addition, the uptake and retention of [^3H]L-dopa at each organ site were computed as the area under curve (AUC) [10], using:

$$\text{AUC} = \int_0^{\infty} C_0 e^{-Bt} dt = C_0/B.$$

For each site the uptake/retention effectiveness between the pretreated and control groups was measured by a relative enhancement index (REI) as follows:

$$\text{REI} = \frac{\text{AUC}_{\text{pretreated}} - \text{AUC}_{\text{control}}}{\text{AUC}_{\text{control}}}.$$

Table I presents the results grouped by site and pretreatment.

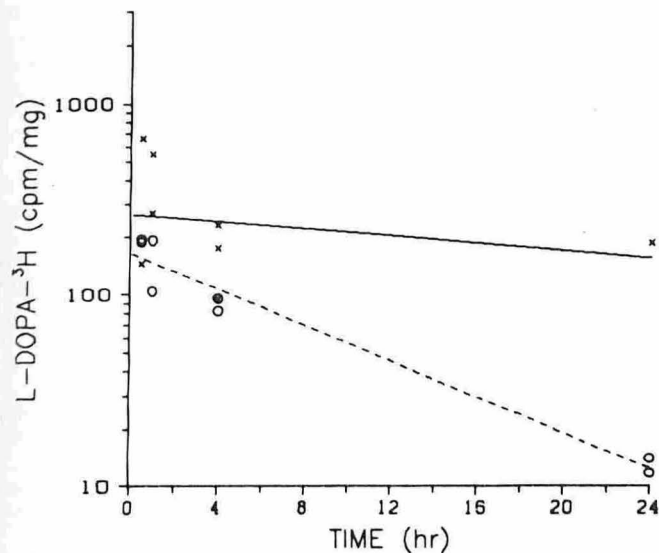


Figure 1. Tumor uptake and retention kinetics of [^3H]L-dopa in pretreated (\times) and control (\circ) Harding-Passey melanoma. Regression lines display the effect of nonradioactive L-dopa pretreatment (solid line) against the control (dashed line) ($p < 0.01$).

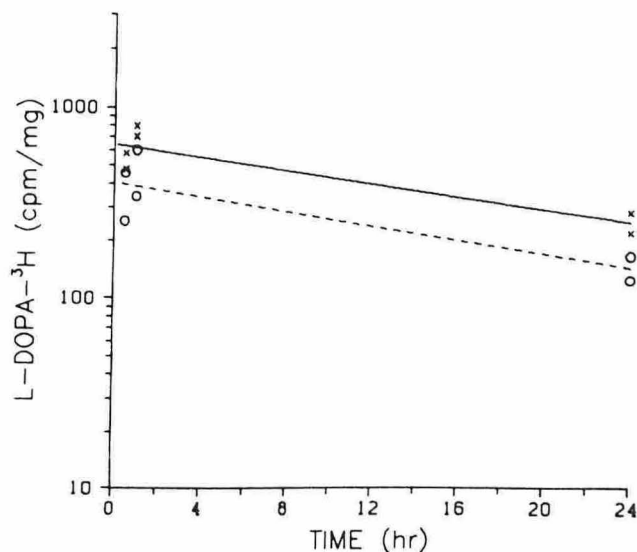


Figure 2. Uptake and clearance kinetics of [^3H]L-dopa in adrenal gland of pretreated (\times) and control (\circ) Harding-Passey melanoma tumor-bearing mice. Regression lines display the effect of nonradioactive L-dopa pretreatment (solid line) against the control (dashed line) ($p < 0.05$).

The pretreated tumor exhibited the largest relative enhancement factor (6.96).

These results suggest that with the selective timed use of L-dopa, a preferential localization of [^3H]L-dopa could be obtained in the Harding-Passey melanotic tumor. Thus, the selective incorporation of [^3H]L-dopa in melanoma by initial pretreatment

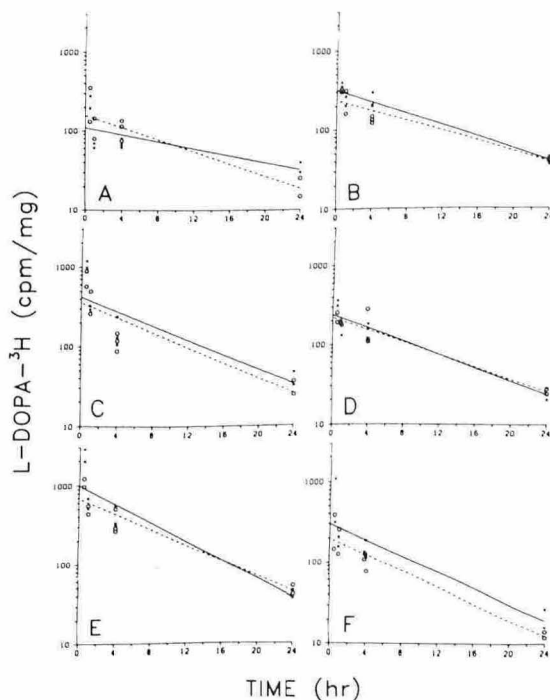


Figure 3. Organ uptake and clearance kinetics of [^3H]L-dopa in pretreated (\times) and control (\circ) Harding-Passey melanoma tumor-bearing mice: (A) Brain, (B) lung, (C) liver, (D) spleen, (E) kidney, and (F) muscle. Regression lines display the effect of nonradioactive L-dopa pretreatment (solid lines) against the control (dashed lines). Each p level not significant.

Table I. Area Under Curve (AUC) Comparison of L-Dopa Pretreatment Effect by Organ Site

Site	AUC (cpm-h/mg)		Relative Enhancement Index
	Pretreated	Control	
Tumor	12,180	1,531	6.96
Adrenal gland	16,460	9,454	0.74
Brain	2,187	1,805	0.21
Lung	3,663	3,144	0.17
Liver	4,000	3,255	0.23
Spleen	2,451	2,423	0.01
Kidney	7,305	5,996	0.22
Muscle	2,628	1,716	0.53

AUC is computed from regression fit for [^3H]L-dopa kinetics. Note the highest relative enhancement index for tumor.

of tumor with nonradioactive L-dopa may provide a means of studying and testing new modalities of therapy in this, one of the most aggressive forms of cancer.

DISCUSSION

In the present study the Harding-Passey pigmented melanoma tumor was chosen as a model because of its similarity to human melanoma with respect to its response to chemotherapy. The Harding-Passey melanoma shows a response rate of 25% to dacarbazine treatment, while other experimental melanoma tumors, e.g., the B16 pigmented melanotic tumor, do not respond as well to dacarbazine [11].

Studies using radioactive melanin precursor as a tool have shown that the concentration of radioactivity found in various animal melanoma tumors may vary [6,7,12]. The available data suggest that this relative difference in increased uptake and concentration of radioactivity in the tumor is related to the rate of melanin synthesis. Blois and Kallman [6] found that the radioactivity concentration was primarily located in the melanin polymer and not in the remainder of the tumor cell. Takahashi and Fitzpatrick [12] found an absence of stable dopa in the hydrolysates of melanosomes from deeply pigmented B16 melanotic tumors, but discovered large concentrations of stable dopa in the Harding-Passey melanoma. This would tend to also support the proposal of Blois and Kallman [6] that the uptake and incorporation of precursors such as dopa are proportional to the rate of melanin synthesis in the tumor.

While most reports have shown that increasing melanization and incorporation of agents that enhance pigmentation can induce selective toxicity to melanoma cells in vitro [3-5], studies by Parsons and Morrison [13] suggest that this toxicity may be closely related to DNA damage. Although the precise mechanism of this DNA damage is not known, the selective preferential uptake of dopa and the resulting toxicity to mammalian cells does correlate well with melanization [5]. In addition, no inhibition by dopa or dopamine agonists and antagonists in amelanotic B16 cells has been observed [14].

Our findings suggest that pretreatment of mice bearing Harding-Passey melanoma tumors with L-dopa does enhance the accumulation of [^3H]L-dopa in the tumor (Fig 1). Conceptually, the available dopa binding sites would have been saturated by pretreated unlabeled dopa, and should result in a subsequent reduction in uptake or accumulation of radiolabeled L-dopa in the tumor. However, the increased uptake of [^3H]L-dopa may suggest an alternate pathway for L-dopa metabolism and melanin formation in melanoma. The cyclic AMP system has been identified as an alternate pathway for melanin synthesis [3,15]. Ivanov et al [16] administered nonradioactive L-dopa to mice bearing Harding-Passey melanotic tumor and found that the cyclic AMP in the tumor tissue was higher ($p < 0.05$) than that in the controls which were treated with [^3H]L-dopa alone. Further, the selective increased uptake of radiolabeled dopa in the Harding-Passey mel-

anoma (Fig 1, Table I) following pretreatment with unlabeled dopa also confirms the same finding of another report of melanoma tumors in hamsters and in albino mice [7]. Alternatively, L-dopa incorporation of melanoma may also be enhanced by dopa decarboxylase inhibition. Wick et al [17] have shown that in the presence of a potent decarboxylase inhibitor, serum level of L-dopa could be elevated, thereby an enhancement of L-dopa incorporation into melanoma was achieved and the diversion of label into adrenal gland was greatly obviated. In our study of tissue levels of L-dopa incorporation, no serum to organ-specific activity was determined. Although the specific mechanism of L-dopa action resulting in such a phenomenon has not yet been resolved, our findings may have an implication for the usefulness of L-dopa in human malignant melanoma therapy [18].

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