The Relationship of the Eye Uptake of N-Isopropyl-*p*-[^{123}I]Iodoamphetamine to Melanin Production

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Eye uptake has been a potential concern with N-isopropyl-*p*-[^{123}I]iodoamphetamine (I-123 IMP) because it has been observed in certain animal species. We have investigated the cause of the eye uptake and its relationship to melanin synthesis. In a 1-yr-old cynomolgus monkey, high concentration of the tracer was seen in the eyes regardless of the type of anesthesia (pentobarbital or ketamine) or the oral administration of Lugol's solution. The eye uptake at 24 hr after injection of I-123 IMP was equally high in an 8-yr-old rhesus monkey. The ratio of radioactivity in the eye of black compared with white albino mice was 10:1 at 30 min, 18:1 at 2 hr and 36:1 at 24 hr after injection of I- 123 IMP. No eye uptake above soft-tissue background was seen in five patients at 2, 24, and 48 hr after injection. I-123 IMP is avidly incorporated into melanocytes actively producing melanin, but substantially less in melanocytes where production of melanin has ceased as in the human eye.

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N-isopropyl-*p*-[^{123}I]iodoamphetamine (IMP) has a number of attributes that make it an attractive choice for cerebral perfusion imaging (*1,2*). After intravenous injection it is lipophilic, passing efficiently into the cerebral parenchyma. Once inside the brain, it is either bound to nonspecific sites or is converted to a metabolite that is retained in the brain. Washout from the brain is slow, permitting substantial time for static imaging of cerebral perfusion. Its initial distribution is proportional to cerebral blood flow over a wide range of flows (*3*). The tracer has been coupled with emission computed tomography to evaluate patients with a wide range of neurologic disorders, including cerebral vascular disease and epilepsy (*4–9*).

Eye uptake has been noted incidentally in a number of animal models (*2,10*). Since eye uptake would significantly affect patient radiation dose, we have studied this phenomenon in a number of animal and cell models as well as in human volunteers. Our purpose was to determine the cause of the eye uptake in these animals, to establish its relationship to melanin synthesis, and to determine the degree of uptake in humans.

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METHODS

N-isopropyl-*p*-[I-123]iodoamphetamine (I-123 IMP) was prepared commercially* using a technique previously described (*3,4*). Radiochemical purity was checked with thin-layer chromatography using silica gel-60 plates with methanol chloroform/glacial acetic acid, (15:85:1, v/v/v) as eluent. Specific activity of the I-123 IMP was 10 mCi/mg. The proportion of iodine-124 present was checked by counting the radiotracer with a Ge(Li) detector and multichannel analyzer.

A 1-yr-old female *Macaca fascicularis* (cynomolgus monkey) was studied to determine the effect of anesthesia and Lugol's solution on eye uptake. During the first phase of the study, anesthesia was maintained with intraperitoneal pentobarbital (62.5 mg on Day 1 and 40 mg on Day 2). After the animal was anesthetized, 1 mCi (0.1 mg) I-123 IMP was injected intravenously. Sixty

minutes later, anterior and lateral images of the head were obtained using a large-field-of-view Anger camera with a medium-energy collimator. The pulse-height analyzer was peaked symmetrically over the 159-keV photopeak of I-123 using a 15% energy window. One million counts were acquired in each projection, collecting and storing the data on a special-purpose digital computer. Twenty-four hours later, the animal was reanesthetized and imaging was repeated in the three projections, collecting 100,000 counts per image. One week later, the second phase of the study was performed using ketamine as the anesthetic and using the intramuscular route for its administration (110 mg on Day 1 and 60 mg on Day 2). Following anesthesia, imaging was performed as described above. One week later, the third phase of the study was performed after pretreating the animal with Lugol's solution and again using pentobarbital as the anesthetic (60 mg on Day 1 and 20 mg on Day 2). Imaging was performed as described above. For data acquired during all phases of the study, regions of interest were drawn manually over the eye and the brain on the 60-min and 24-hr lateral images. Counts/min-pixel were compared for the brain and the eye on the 60-min and 24-hr images.

The study was repeated using a male *Macaca mulatta* (rhesus) monkey, 8 yr old, to determine the effect of age and species on the eye uptake. Intramuscular ketamine was used to induce anesthesia, 300 mg on Day 1 and 200 mg on Day 2. The eye-to-brain ratio for activity/pixel-mCi was determined from the 60-min and 24-hr images.

To determine the chemical form of the radiotracer after eye uptake, we used I-125 IMP. The specific activity was 6.3 mCi/mg and the purity was greater than 99%. Twenty-four hours after intravenous injection of 13.0 mCi I-125 IMP, the monkey (*M. fascicularis*) was killed. The left eye and the pigmented layer of the right eye (choroid, pigmented epithelium, and retina) were homogenized separately with a ground-glass homogenizer in the presence of 1 *N* NaOH. Each sample was extracted with ethyl ether until no more activity could be extracted. The ether layer was separated and acidified with 0.3 *N* HCl and the activity extracted into the HCl layer; this was then basified with sodium hydroxide and the activity extracted back to ether. About 50 μg of unlabeled IMP and *p*-iodoamphetamine were added to the final ether extract. After the solvent was reduced to 0.5 ml by N_2 purging, the extracted radioactivity was analyzed by TLC on silica gel with methanol:chloroform:glacial acetic acid (15:85:1) as solvent.

To determine the relationship between melanocyte concentration and I-123 IMP eye uptake, CD-1 albino Swiss mice and C-57 black mice were injected intravenously with I-123 IMP. Six albino and six black mice were killed at the following times after injection: 30 min, 1, 4, and 24 hr. The eyes, skin/fur and brain were excised, weighed, and counted in a gamma well counter. The percent injected dose per gram was determined for each of the tissues and the ratio between tracer concentration in black and albino mice was determined.

The origin and maintenance of the S-91 murine melanoma cells used in this study have been described (*11*). Briefly, the cells were maintained in McCoy's 5A medium supplemented with 10% fetal calf serum, glutamine, and antibiotics. For each experiment, cells were inoculated at 0.5×10^6 cells/per 35-mm petri dish and incubated at 37°C or at 4°C for 48 hr. Following incubation, the cells were washed, and 100 μl of I-123 IMP was added. Following exposure, the medium was removed and the plates were washed three times with phosphate-buffered saline. The cells were removed mechanically and centrifuged at 500 *g* for 15 min. The supernatant was discarded, and the radioactivity in the cell pellet determined.

Five Caucasian human volunteers ranging in age from 18 to 40 were studied for eye uptake. These patients varied in eye color (blue 2, brown 2, hazel 1). They received between 2 and 6 mCi of I-123 IMP intravenously 3 hr after oral administration of Lugol's solution. Imaging was performed at 2, 24, and 48 hr after injection. Images were obtained with an Anger camera in the anterior and lateral projections for 300,000 counts using a 20% window bracketing the 159-keV photopeak of I-123 and data stored on a computer. Using an electronic cursor, areas of interest were drawn around the region of the brain, the eyes, and the facial background to determine the counts/pixel in these structures.

RESULTS

Images obtained 60 min after injection of I-123 IMP in both the rhesus and cynomolgus monkeys showed uptake primarily in the brain, lungs, and liver. After 24 hr, uptake was distributed more evenly throughout the body, but with particular concentration in the eyes and thyroid. The images were similar with pentobarbital, ketamine, and pentobarbital plus Logol's solution, except that thyroid activity was reduced after Lugol's solution (Fig. 1). The ratios between the count rate/pixel-mCi in the regions of interest overlying the eye and the brain were also similar at 24 hr (Table 1).

Fifty-seven percent of the activity in the eye and eye pigment of the macaque killed 24 hr after intravenous injection of I-125 IMP was in the form *p*-iodoamphetamine. Unaltered N-isopropyl *p*-iodoamphetamine accounted for 37% and 35% of the activity in the whole eye and eye pigments, respectively. About 1% to 2% of the activity was left in each extraction step, and it remained unidentified. The I-123 IMP concentration was higher in the eyes of the pigmented (black) mice compared with the nonpigmented (albino) mice at all time points (Table 2). The ratio between the concentration

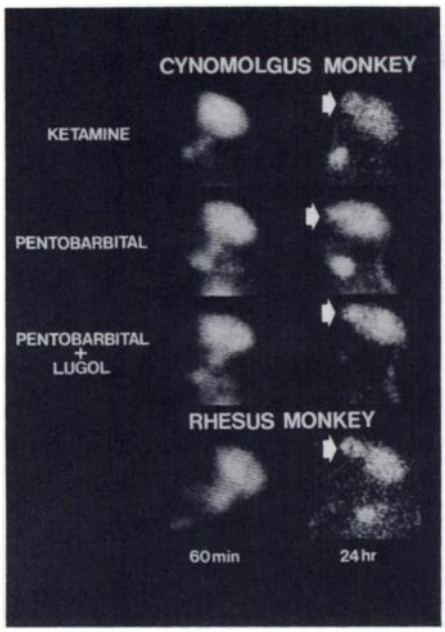

FIG. 1. Uptakes of I-123 IMP at 60 min and 24 hr after injection. Note eye uptake at 24 hr in all cases (arrow).

of tracer in the pigmented and nonpigmented mice was 10:1 at 30 min after injection, 18:1 at 2 hrs, and 26:1 at 24 hr. The greater concentration of the tracer in skin/fur in the black mice was less dramatic than in the eyes. There was no significant difference in the concentration in the brain between the albino and black mice.

TABLE 1. RATIO OF EYE-TO-BRAIN ACTIVITY PER PIXEL DETERMINED FROM ANGER-CAMERA DATA

	60 min after injection of I-123 IMP	24 hr after injection of I-123 IMP
Macaca fascicularis (cynomolgus) (1 yr old)		
i.p. pentobarbital	3.1:1	1.1:1
i.m. ketamine	2.2	1.3
i.p. pentobarbital + Lugol's	2.3	1.3
Macaca mulatta (rhesus) (8 yr old)		
i.m. ketamine	2.8	1.3

TABLE 3. TIME COURSE OF I-123 IMP UPTAKE INTO MOUSE MELANOMA CELLS IN CULTURE

	0	5 min	15 min	30 min	45 min
Pigmented	42%	76%	88%	93%	100%
Nonpigmented	51%	71%	83%	84%	100%

There was greater uptake of I-123 IMP into both the Cloudman heavily pigmented and the hypopigmented mouse melanoma cells, relative to nonmelanocyte control CHO cells (Chinese hamster ovary). The small degree of uptake in the B line parallels the small residual degree of melanization remaining in these cells (*12*). The ratio was substantially higher for pigmented cells (13.25:1) compared with the hypopigmented (4.1:1). The time course for uptake of I-123 IMP into mouse melanoma cells was rapid for both murine lines (Table 3). Temperature variation had a minimal effect upon uptake. Incubating the cells at 4°C rather than 37°C reduced uptake from 28.9 to 16.9K counts/10^6 pigmented cells, and from 6.6 to 4.0K counts/10^6 hypopigmented cells. These results suggest that the mechanism of uptake is relatively independent of energy or enzyme requirements.

In the five human studies, no eye uptake could be observed in concentrations above soft-tissue background at 2, 24, and 48 hr after injection. The ratio between the counts per pixel over the eye and over the soft-tissue background averaged 0.94 at 24 hr (range 0.9–1.0) and 0.95 at 48 hr (range 0.9–1.0)).

DISCUSSION

I-123 IMP has been reported to concentrate avidly in the pigmented portion of the eye in dogs, rodents, and monkeys anesthetized with pentobarbital (*2,10*), but not in monkeys anesthetized with ketamine (*2*) or in man (*13*). In the cynomolgus monkey anesthetized with pentobarbital, we have previously shown that the concentration of I-123 IMP steadily increases in the eye until reaching maximum activity (0.2% of the injected dose) at 24 hr after injection, and falling slowly thereafter (*10*). Sixty-five percent of the activity is in the pigmented tissue specimen (retina, choroid, and epi-

TABLE 2. PERCENT INJECTED DOSE PER GRAM OF I-123 IMP IN ALBINO (A) COMPARED WITH BLACK (B) MICE (±s.e.m.) (n = 6 FOR EACH GROUP)

	30 min		1 hr		4 hr		24 hr	
	A	B	A	B	A	B	A	B
Eye	1.0 ± 0.7	20 ± 5	1.7 ± 0.4	13 ± 4	1.7 ± 9	20 ± 3	0.6 ± 2	20 ± 3
Skin/fur	1.2 ± 0.1	4.3 ± 2.6	1.2 ± 0.2	1.9 ± 0.9	1.6 ± 0.8	2.0 ± 7	0.2 ± 0.1	1.0 ± 0.7
Brain	7.4 ± 0.7	10 ± 1	7 ± 0.6	9 ± 1	4 ± 1	5 ± 0.5	0.3 ± 0.2	0.4 ± 0.1

thelial pigment), with 20% in the optic nerve and 15% in the sclera.

In this study, we show that the eye uptake of I-123 IMP is not affected by the type of anesthesia or by the age or species of the monkey. Avid eye uptake was observed 24 hr after injection with either ketamine or pentobarbital anesthesia, and in the young cynomolgus monkey as well as the older rhesus monkey. The form of the tracer fixed to the eye 24 hr after injection is almost exclusively N-isopropyl *p*-iodoamphetamine and *p*-iodoamphetamine. We cannot tell from this study whether the former is dealkylated in the eye or elsewhere.

The high concentration of I-123 IMP in the eyes of most animal species is most likely due to a marked affinity of this tracer for melanin, with slow release from pigmented tissues. Our observations of a markedly greater concentration of the tracer in black relative to albino mice mirror the findings seen with chloroquine analogs, which have been used successfully in the diagnosis and treatment of occular and dermal melanomas (*14*,*15*). Like the chloroquine analogs, there is a significantly greater concentration in pigmented compared with nonpigmented melanomas.

A number of receptor-specific radioligands, including C-11 nicotine and C-11 etorphine, are avidly sequestered in the eyes of baboons and are retained there long after activity in the brain has cleared (*16*). While uptake of all of these tracers could be explained by receptor binding, with higher concentrations of receptor sites in heavily pigmented animals, it is more likely that I-123 IMP uptake is due to nonspecific binding of melanin, since I-123 IMP is not observed in the pigmented eyes of the humans we studied. I-123 IMP is not directly involved in the synthetic pathway of melanin, since its uptake is not blocked when synthesis of melanin is blocked.

The most interesting question raised by our studies is the failure to observe significant concentrations of I-123 IMP in the human eye. Until recently it has been assumed that melanin synthesis ceases by birth or shortly thereafter in most animal species (*17*). Several recent studies have brought this hypothesis into question (*17*,*18*). For example, cysteinyldopa has been observed in the mature bovine eye (*18*). Cysteinyldopa is thought to be a specific marker of melanin turnover, and its presence is indicative of active melanin synthesis in the adult eye. Active melanin synthesis would explain I-123 IMP uptake in monkey and rodent eyes. The absence of I-123 IMP uptake in our human subjects' eyes raises the interesting hypothesis that, unlike all pigmented animal species up to and including the monkey, melanin synthesis does not occur in the adult Caucasian eye. Our studies need to be extended to other races and to the pediatric age group before a generalized statement can be made concerning uptake and retention in the human eye.

The difference in I-123 IMP concentration in the human and in the monkey eye means that caution must be used in extrapolating radiation doses to the human eye from animal data. While the biodistribution data derived from animal data are accurate because in vitro methods can be used for radioassay, significant species variability—even when primates are used—may lead to substantial errors in dosimetry estimates that predict potential clinical applications in the human.

The marked avidity of I-123 IMP for pigmented melanoma cells suggests that this tracer may be a preferable alternative to labeled chloroquines for the diagnosis and evaluation of occular and dermal melanomas, although with some of the same limitations. Detection of metastases in the lungs and liver may be difficult due to high concentrations of the tracer in these organs. Nonpigmented and necrotic metastatic foci may be difficult to visualize because of lower concentrations of the tracer. Nevertheless, the future commercial availability of I-123 IMP for cerebral perfusion studies should provide sufficient reason to explore its potential for the detection and evaluation of melanoma. Finally, with the advent of chemotherapy directed at the pigmented phenotype, it may become even more important to develop radiologic techniques to assess the degree of differentiation (*19*).

FOOTNOTE

* Medi-Physics, Incorporated, Emeryville, CA.

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