^{125}I-LABELED CHLOROQUINE ANALOG IN THE DIAGNOSIS OF OCULAR MELANOMAS

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A definite diagnosis of intraocular melanoma can be made only by enucleation of the eye since the choroid cannot be biopsied. Many benign disorders with minor visual impairment can simulate intraocular melanoma (e.g., choroidal nevus, melanocytoma, hemorrhage, and hemangioma). Ferry has shown that 19% of enucleated eyes with a clinical diagnosis of malignant melanoma do not contain a tumor (*1*).

Blind eyes with opaque media precluding ophthalmoscopy harbor melanoma with disturbing frequency (*2*). A reliable nonoptical, nonsurgical diagnostic aid specific for the ocular malignant melanoma would be very helpful. We wish to report the development of such a diagnostic aid.

A ^{125}I-radioiodinated analog of chloroquine (4,3-dimethylaminopropylamino-7-iodoquinoline), hereafter called NM-113, has been demonstrated to concentrate in human malignant melanomas in 7–70 times greater amounts than in skin, muscle, and fat (*3,4*) because of its specific affinity for melanin (*5*). The radioactivity in the eye from this compound is therefore concentrated principally in the choroid (*6*). Following ^{125}I-NM-113, melanin-containing tissue present in ocular melanomas should add counts to the radioactivity detected from the normal choroid. These increased counts obtained over the tumored eye would aid in the specific diagnosis of ocular melanomas without enucleation. This report describes our experience with ^{125}I-NM-113 in the diagnosis of ocular melanomas.

METHODS

Two millicuries of ^{125}I-NM-113 containing 6–55 mg of chloroquine analog were given orally to 28 patients. Complete ophthalmologic examination preceded administration of the tracer dose.

Radioactivity in each of the two eyes was compared by external counting using a specially designed 1-in. single-hole lead collimator on a 5-in. NaI(Tl) crystal photoscanner with an attached scaler. A minimum of three 5-min counts were made at each examination. Radioactivity determinations by external counting were made 1–5 times on each patient from 2–50 days after the tracer dose. Results were expressed as the average percent difference between the mean counting rates over two eyes using the formula

$$\frac{A - B}{A + B/2} \times 100.$$

Tissues for histology, autoradiography, and radioactivity distribution studies were obtained on enucleated eyes.

The patients were divided into three groups.

Group I consisted of 11 "control" patients who were being investigated for extraocular malignant melanomas.

Group II consisted of 11 patients with ocular lesions other than malignant melanoma.

Group III consisted of six patients subsequently proved to have intraocular malignant melanomas. One patient (OT) had tissue distribution studies only.

RESULTS

Patients without ocular pathology. The 11 nonocular dermal melanoma control patients had a mean percent difference in external counting rate between the eyes of 7.6% after 14 days following the dose, with an upper limit of 18% with 2 s.d. (Fig. 1).

After administration of the compound, external counting generally revealed an increase in the total ^{125}I radioactivity in the eyes during the first 14–21 days, and then a gradual decrease in counting rate at a rate of approximately 0.5–1.5%/day corrected for physical decay. Our studies confirm previous chemical studies showing that chloroquine is rapidly

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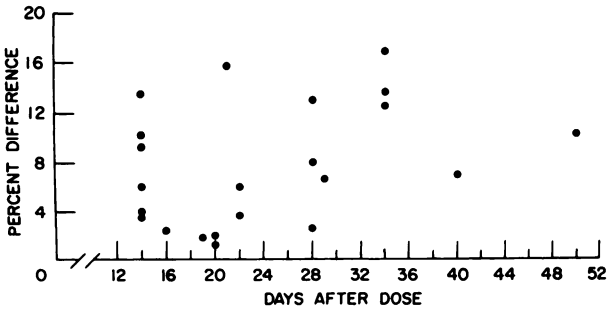

FIG. 1. Percent difference in counting rate between eyes in control patients.

bound to melanin of the choroid and released very slowly (7); therefore the effective half-life was assumed to be the physical half-life.

The radiation dose to the choroid from 2 mCi of ^{125}I-chloroquine analog is approximately 46 rads. Total-body radiation dose, estimated from tissue distribution and excretion studies, was approximately 1 rad from 1 mCi of ^{125}I-NM-113 administered orally (3).

Ocular lesions other than malignant melanoma. Nonmelanoma lesions in the eyes produced an insignificant difference in counting rate between the eyes (Table 1). A patient with breast carcinoma metastatic to the eye showed a percent difference of only 3% at 13 days after the dose. Degenerative and inflammatory disease (some with increased pigmentation) were consistently in the "normal" range of percent difference.

Ocular melanomas. Here all deviations from zero percent difference in counting rate over the two eyes showed a higher counting rate over the eye containing the ocular melanoma.

TABLE 1. PATIENTS WITH SUSPECTED MELANOMA LESIONS OF THE EYE (GROUP I)

Patient	Clinical or pathological diagnosis	Maximum percent difference after 14 days
	Nonmelanoma Lesions	
MP	Kuhnt-Junius degenerative disease	—8%
CB	Metastatic breast carcinoma	3%
SB	Coloboma	15%
JP	Chorioretinitis	10%
FR	Retinal detachment	12%
EA	Retinal vein occlusion	5%
WG	Pigmented nevus	2%
CML	Pigmented nevus	0%
EB	Iris pigmentation (normal variant)	2%
SG	Nonspecific inflammation	17%
LT	Pigmented scar	14.8%
	Ocular Melanomas	
MB	Malignant melanoma, OD	48%
EN	Choroidal melanoma, OS	24%
DO	Conjunctival melanoma, OD	69%
OT	Choroidal melanoma, OS	—
MK	Choroidal melanoma, OS	—
ES	Melanoma of ciliary body and peripheral choroid, OD	—

Table 1 gives the final diagnosis in six patients. Figure 2 shows the deviations of their percent difference in counting rates with time. One patient (DO) had a conjunctival melanoma, and the remaining five had intraocular melanomas. One of these five (OT) had tissue distribution studies on the

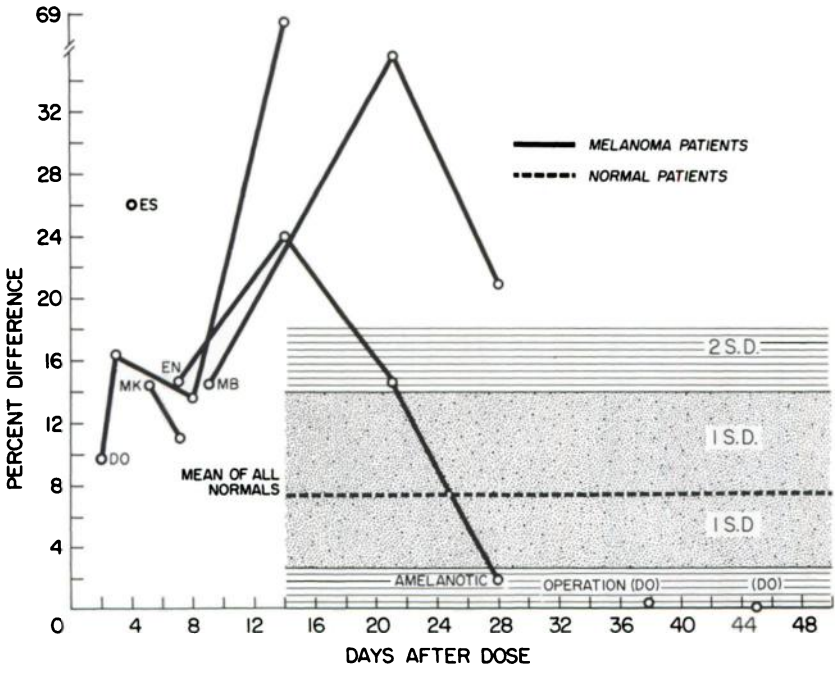

FIG. 2. Mean percent difference in counting rates between the eyes in patients with ocular melanomas and in patients with nonocular melanomas.

tissues obtained at enucleation, but no external counts were made before enucleation.

Two patients (MB and DO) showed a maximum percent difference of 48 and 69%, respectively. Patient DO had a conjunctival melanoma measuring approximately 0.43 cm^3 which was excised 38 days after the dose before enucleation. The percent difference between the eyes after excision of the melanoma was 0%.

The percent difference between the eyes in MB and DO was highly significant despite a high choroid-to-tumor ratio of deposited dose per gram of tissue (8 and 37, respectively). This significant difference demonstrated the contribution of the melanoma to the total detected radioactivity.

Although EN with an "amelanotic" melanoma had a 24% difference at 14 days, this difference had fallen to less than 15% by 21 days. The tumor was described as amelanotic by histopathologic examination, but it did contain melanin in low concentration. Patients ES and MK unfortunately could not be evaluated past the fourth and eighth days, respectively, because they had enucleations. Radioassay of the various ocular tissues obtained at operation in the patients with subsequent histological evidence of intraocular malignant melanoma showed concentration of the radioactivity to be highest in the choroid, next highest in the tumor, and negligible or low in the other ocular tissues (Table 2). Autoradiography confirmed these tissue distribution studies.

TABLE 2. TISSUE DISTRIBUTION (% DOSE × 10^{-3}/gm) OF ^{125}I-NM-113

Patient	Oral dose (μCi/mg)	Tumor	Choroid	Retina	Sclera	Cornea	Lens
DO	1,498 26	0.9	32.0	0.3	0.2	—	—
MK	1,987 11	4.2	4.3	0.1	0.2	0.0	0.0
ES	2,462 12	1.9	8.7	0.3	0.1	0.1	—
MB	2,194 19	1.8	15.2	0.1	0.1	0.1	—
EN	1,481 20	2.7	8.0	—	—	0.3	—
OT	1,894 22	4.2	21.2	0.0	0.1	—	—
	Avg	2.6	14.9	0.2	0.1	0.2	0.0

TABLE 3. CONCENTRATION OF ^{125}I-NM-113 IN TISSUES OF ENUCLEATED EYES (μCi/gm)

Patient	Choroid	Tumor	Ratio (choroid: tumor)	Tumor size (cm^3)	Color of tumor
MK	0.085	0.084	1.0:1	0.3	Amelanotic
ES	0.214	0.047	4.6:1	0.45	Melanotic
MB	0.334	0.040	8.4:1	0.24	Pale gray (moderately amelanotic)
EN	0.119	0.040	2.9:1	0.5	Amelanotic
DO	0.879	0.084	36.6:1	0.43	Melanotic
OT	0.402	0.084	4.8:1	1.32	Melanotic

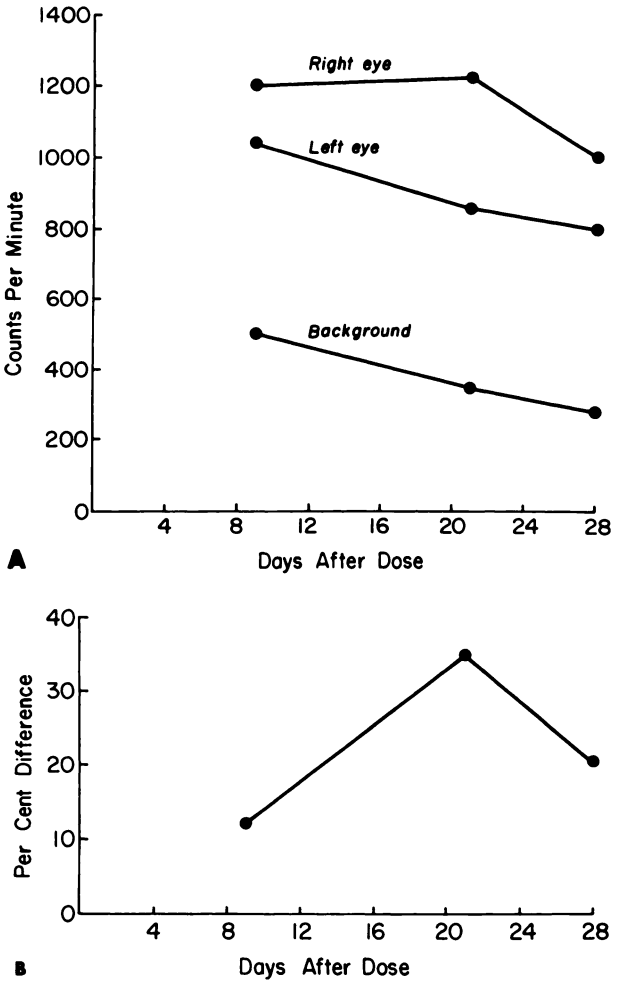

FIG. 3. A shows serial counting rates over eyes in Patient EN studied over 28-day period. B shows percent difference in counting rates between eyes of Patient EN over 28-day period.

The choroid-to-tumor ratio (Table 3) in μCi/gm of tissue in enucleated eyes averaged 6:1 but varied from 1:1 to 8:1 except in DO (37:1) who had a conjunctival melanoma. No clear-cut correlation was found between the pigmentary characteristics of the tumors and the relative isotope concentration in choroid-to-tumor ratios.

Case example. Patient EN, a 41-year-old man, had noted blurring of vision in the right eye for 3 months before examination. Funduscopic examination revealed a protuberant gray mass located nasally in the right eye elevating the retina, with some small hemorrhagic areas on the mass. The initial clinical

diagnosis was malignant melanoma. He received 2 mCi of ^{125}I-NM-113 and had external eye counts at 9, 21, and 28 days after the dose. Figure 3A shows a graph of the results of external counts in the two eyes. The percent differences are shown in Fig. 3B. Thirty-eight days after administration of the tracer dose, the right eye was enucleated. Grossly the tumor was gray-yellow in color (Fig. 4), measuring 0.24 cm^3. Concentrations of radioactivity in ocular tissues showed the highest concentration in the choroid, being over 20 times more than in the retina (MB in Tables 2 and 3). The choroid contained eight times the concentration of radioactivity (μCi/gm) of that in the ocular melanoma. This was presumably due to the relatively amelanotic histologic character of the tumor. Histologic sections (Fig. 5A) showed the presence of malignant melanoma (Spindle Cell "B" type), with scattered melanin granules throughout the tumor (Fig. 5A).

Autoradiography showed scattered grains throughout the tumor above that of background grain count, with some increased concentration over pigmented cells, some nonpigmented cells (Fig. 5B), and some in the choroid.

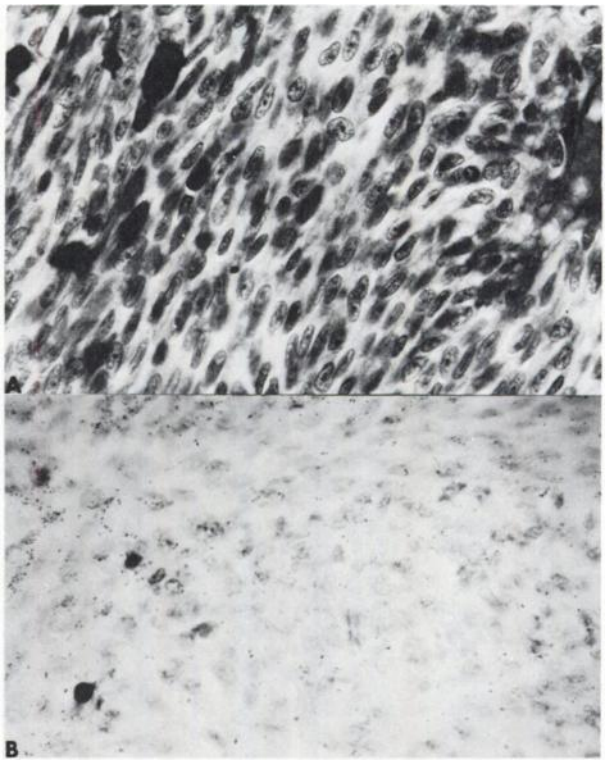

FIG. 5. A is histologic section of spindle cell "B" type malignant melanoma from Patient EN. Scattered deeply pigmented cells are seen (stain, H & E; × 430). B is autoradioradiography of tumor from EN showing scattered radioactivity throughout tumor with higher concentrations over pigmented cells and some relatively nonpigmented cells (stain, toluidine blue; × 100).

DISCUSSION

Various tests have been devised to aid in the preoperative diagnosis of intraocular lesions. The ^{32}P test is not specific for the ocular melanoma, and it has shown both false-positive and false-negative results. It is difficult to perform for the detection of posteriorly located lesions (*8*). Recent improvements of the ^{32}P-localization test suggest greater accuracy than previously reported (*9*). The ^{197}Hg-chlormerodrin uptake test is nonspecific and is particularly abnormal in the presence of retinal hemorrhage (*10*). Iodine-125-diidofluorescein has shown a high percentage of false-negative results (*11*).

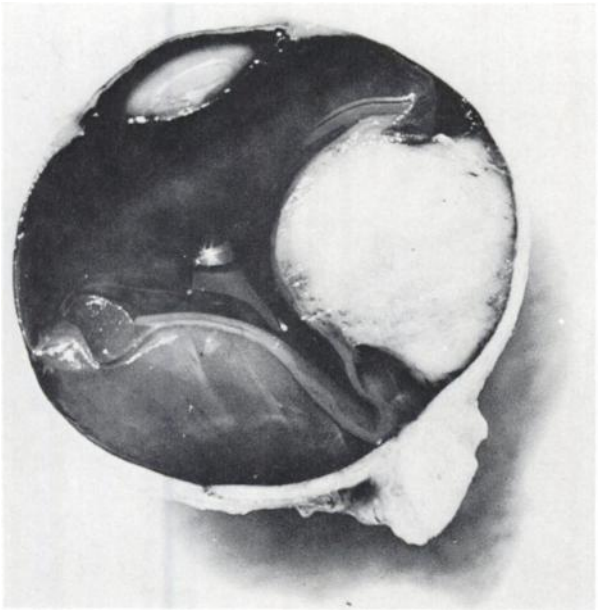

FIG. 4. Hemisection of right eye of Patient EN showing large gray-yellow tumor protruding into eye. Jelled subretinal liquid beneath detached retina is also present as gray area below tumor.

The diagnosis of dermal malignant melanomas with our original radioiodinated chloroquine analog (*4*), and the work of Blois (*12*) with radioiodinated chloroquine, prompted our study of ocular malignant melanomas with ^{125}I-NM-113.

Our results in patients with intraocular malignant melanomas demonstrate that (A) melanotic melanomas show a significantly higher than normal counting rate over the tumored eye 14 days or more after the dose; (B) amelanotic melanomas may concentrate the tracer equally well before 14 days but apparently lose the tracer at a faster rate; (C) nonmelanoma lesions show a counting rate within the normal range.

The observation that "amelanotic" lesions concentrate the tracer is probably related to the fact that "amelanotic" lesions usually contain melanin histopathologically, but in lower concentrations than are found in the melanotic melanoma.

We have no explanation for the observation that benign ocular pigmented lesions exhibited a normal percent difference between the eyes. This observation fits with our previous observations, however, on benign pigmented skin lesions (moles) (*4*). We have not yet studied large benign pigmented intraocular lesions such as benign melanocytomas.

Placement of the external probe is critical for reproducibility. Lateral displacement of the probe by as little as 5 mm results in a significant alteration in counting rate (5%). Thus the development of a probe which could be consistently and reproducibly placed over the globe would be a great improvement. External counts directly over the eyes were 15–20% higher than over the surrounding periorbital areas. Closer placement of the crystal to the eye would result in a better target (eye)-to-nontarget (retro-orbital structures) count ratio, presumably allowing more accurate determinations. Preliminary studies with a new 2 × 2-cm NaI(Tl) crystal detector without collimation applied closely to the eye have produced a 4–5-fold improvement in counting efficiency, allowing variable geometric factors to be greatly reduced.

Preliminary evaluation of a small semiconductor avalanche detector proved to be inefficient in this application.

The tissue concentration studies demonstrated at best a 1:1 choroid-to-tumor ratio in one case, with the others having a lower differential uptake in the tumor (Table 3). Despite these findings, significantly different *external* counting rates between the eyes were obtained in MB and DO with melanotic melanoma as shown in Fig. 2. The higher external counting rates would be expected because of the additional counts furnished by the melanoma.

The diagnostic dose used in this study would not be expected to produce radiation damage to the retina. Radiation dosimetry shows that the choroidal dose is approximately 46 rads from 2 mCi of orally administered ^{125}I-NM-113. Harmful permanent effects on the retina from external radiation have been seen with over 3,250–4,500 rads (*13*).

This study suggests that the diagnosis of ocular malignant melanoma, under the experimental conditions described, can presently be made with a 95% confidence when the mean difference in counting rate between the two eyes is 18% after 14 days with the higher counting rate over the eye with the lesion.

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