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MELANIN-BINDING RADIOPHARMACEUTICALS

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DISCLAIMER

Melanin Binding Radiopharmaceuticals

INTRODUCTION

The scope of this paper will be limited to an analysis of the factors that are important in the relationship of radiopharmaceuticals to melanin. The problem begins with describing melanin. Its basic structure has not been completely defined. Presently melanin is described as a polymer of ring structures consisting of indole-5,6-quinone units.[1,2] However, differences have been found in the melanins from different sources.[1,3] The significance of these differences to chemical binding has not been examined. Melanins are electron acceptors and charge exchange is considered a major binding force in many reactions. Electrostatic forces seem also to be involved and therefore melanin binding probably includes several mechanisms for affinities as well as combinations.[4]

The complex and as yet undetermined nature of melanin combined with the complex binding mechanisms seemingly available, more than likely accounts for the haphazard approach in the use of radiopharmaceuticals to locate melanin. This paper does not attempt to deal with the differences between melanin-binding vs. melanoma-binding but assumes that there may be a notable variance. Melanin associated protein (melanoprotein) in normal tissues may be different from that in malignant melanoma. Most workers feel that the melanin is the same whether it is in normal or malignant tissue;[5,6,7] however, this is a cytologic and biochemical viewpoint. The physiology and radiopharmaceutical uptake may be very different. This seems highly probable when one realizes that melanin production may vary from normal in malignant melanoma.

The search for melanin-affinic agents naturally led to an examination of melanin precursors and melanogenesis.[8-12] Success with these agents (melanin precursors) has been limited clinically. The melanin precursor tyrosine may be

ineffective because it is also the biochemical precursor of codeine, morphine, papaverine, tyramine, mescaline, pellotine, thebaine, thyroxin, epinephrine, and norepinephrine.[12] The slow metabolic rate of most melanoma may account for the failure and a closer look into these agents is required especially since recent work with melanogenesis stimulators may obviate some of the problems.[14]

The above discussion is further limited by the understanding that there are many other steps involved before the radiopharmaceutical arrives at the melanin containing cell. The blood flow to the area is essential.[15,16] Thus the melanin of the choroid of the eye may, because of its vascular supply have a different uptake of a melanin-affinic agent than malignant melanoma. This might not be due to differences in melanin concentration but rather to differences in blood flow. Other factors include cell membrane characteristics[17] which may have specific affinities and be responsible for the melanin affinity. Also, binding characteristics of the carrier are critical to uptake.[18] Thus the agent must get to the cell, interact with the membrane, be transported into the cell and finally interact with the granules containing melanoprotein. Clearly, the problem of melanin binding radiopharmaceuticals is not a simple one.

Our attempt has been to examine what we think are important factors involved and define needed areas of investigation. The scope of the problem extends beyond localization of malignant melanomas, to treatment of this cancer with melanoma-localizing radiopharmaceuticals that emit therapeutic amounts of radiation. In addition, we are interested in the borated analogs agents for neutron capture therapy.[19,20]

MELANIN

The structure of melanin has not been determined nor has pure natural melanin been isolated. However a considerable body of knowledge exists concern-

ing the physical, chemical and biologic nature of melanin.[1] Melanin is an irregular heteropolymer composed of 5,6-dihydroxyindole and 5,6-dihydroxyindole 2-carboxylic acid units. Melanins that are brown to black are classified as eumelanins and are 1) of high molecular weight, 2) relatively insoluble, 3) polymeric nature and 4) resistant to chemical treatment. Melanins are bound to proteins within cells. The exact nature of the protein(s) and the melanin-protein bond are not known although cysteine units seem to be involved in the linkage. The importance of this bond is that the redox nature of melanin is very different from that of melanoprotein.[21-23] The former is capable of acting as a free electron scavenger. Proteins block the free radical sites of melanin. The importance of the proportion of free radicals in a site may be critical to radiopharmaceutical uptake, especially if trace metal binding is considered to be a mechanism.[24-26]

Melanin exists throughout the human body although there are differences in these melanins.[1] The origin of the melanin in the central nervous system (substantia nigra and locus caeruleus) is thought to be correlated to the tyrosinase independent biochemical pathway. The skin melanin and melanoma melanin are synthesized through the tyrosinase dependent pathway. Melanin also is found in the cells of the adrenal medulla and chromaffin cells. Riley questions the possible significance of the "vestigial" biochemical similarities or potentials due to the common embryonic origin of pigment cells and nerve cells.[30]

Much has been written about the free radical properties of melanin. Mason[24] feels that this is due to the semiquinonoid monomer. It is the free radical properties that allows for electron exchange. Melanin therefore is a good electron acceptor. This is not the case with other proteins.[4]

BINDING MECHANISMS

Melanin is thought to serve as an electron trap,[24,27] while chlorpromazine (CPZ) is an electron donor.[28] In the presence of melanoproteins, CPZ has been found to form a radical ion $(CPZ)^{+}$.[29] It has been suggested that the binding of CPZ to melanin is a result of a completed charge transfer reaction between CPZ and the free radical of melanin.[4,28,29] Studies with various substituted phenothiazines have led to the assertion that the nature of the N side chain or the 2-position substituent does not determine whether or not storage in pigmented tissues occurs, and thus binding to melanin may be a property of the phenothiazine ring.[31]

Melanin is known to be rich in metals ouch as Zn, Cn, Fe and Mn.[3] The strong affinity for metal ions has been ascribed to electrostatic forces between the cations, and anionic sites on the melanin polymer (presumably carboxyl groups).[4] Electrostatic (ionic binding) forces were found to be of major importance in the binding of the organic cation paraquat to melanin.[32]

It has been reported that at physiological pH in dilute aqueous solutions, CPZ exists as a mono-protonated cation.[34] While the possibility that melanin may be able to oxidize CPZ to the positive ion radical was accepted, an alternative binding mechanism was suggested in which both electrostatic and non-electrostatic forces were operative.[4] In fact, three different binding sites and associated constants were found. The non-electrostatic contribution was assigned to possible van der Waals forces occurring at the conjunction of the aromatic rings of CPZ and the aromatic indole-nuclei of melanin.

RADIOPHARMACEUTICALS

Melanin seeking radiopharmaceuticals may be viewed also as potential melanoma seeking agents and as such may be characterized as tumor scanning

agents. These would be delivered systemically and would have to arrive at the site of the melanoma in significant quantity. This would depend on blood flow and on the microcirculation of the tumor.[15,16] Transferrin as a carrier molecule has been shown to be a factor in getting Fe^{++} and Ga^{++} to the melanoma cell membrane.[18,35,36] Once at the melanin cell it must affix to the cell and either remain attached to the cell membrane or be transported into the cell.[17] The receptor sites may be specific immunologically or selective chemically. The importance of the electrical charge relationship must be considered.[37] The cell surface is usually negative; therefore, positively charged agents would accumulate at the cell surface. Also the electrical charge has been shown to increase with malignancy.[38] Once in the cell it may be incorporated into the melanin synthesis or may react with melanin or melanoproteins. These interactions may be biochemical, physiological or immunological. The problem is that there is to date no feature of malignant tumors that cannot be found in normal tissues. Therefore, one is always faced with tumor to background ratios, since it is this ratio that will determine detectability.

We have felt that melanoma because of its melanin content may offer an exception. Quantitatively it was felt that melanoma of the skin and eye usually occur in people with fair skin and that most background organs had low melanin content (the major exception is the choroidal layer of the eye). Larsson[4] has shown that non-metabolic binding forces are involved in metals (electrostatic) and phenothiazines (non-electrostatic) affinity for melanin. He also showed that one class of binding (metal to melanin) would partially inhibit the other (chlorpromazine to melanin). He concluded that more than one binding class existed for chlorpromazine. Bruenger[26] showed that melanin uptake of metal ions occurs rapidly (1 hr) and did not change in the next 24 hours. This is similar

to our investigation with labcled metals in the Greene melanoma.[35] This allows for the use of short-lived nuclides if background tissues wash-out within a reasonable time to allow for adequate tumor to background ratios for scanning. Metabolic uptake seems an obvious method for detection since the ratio of melanin production in malignant melanoma versus normal pigmented tissue should be high enough. Labeled thiouracil, a false precursor, seems worth investigation.[12] DOPA would be another precursor. Blois and Kallman found an increased uptake of 2:1 comparing highly pigmented versus highly pigmented tumors.[8]

The complexity of this problem is reflected in the empirical approach that has been taken. Our data presented in part in the next section will attempt to clarify one aspect of this confusion, that is, the importance of melanin content to radiopharmaceutical uptake.

RESULTS

Melanin content was determined spectrophotometrically. The melanin was deproteinized using a method less harsh than the previously described HCl treatment. Details of this procedure are presented elsewhere.[1]

Table 1 shows that the melanin content in 2 types of melanotic melanoma, average 0.34 and 0.62. This is significantly greater than the melanin content found in amelanotic melanoma. Amelanotic melanoma in other animal models also had low to immeasurable amounts of melanin. Most organs had insignificant amounts. However, hair had significant melanin content. It seems likely that skin and hair are more representative and the combination has a lower content. In addition, this is highly pigmented skin whereas clinically, melanoma occurs predominantly in Caucasians.

The next step was to analyze human melanoma, both primary in skin and metastatic sites (Table 2). Here we see variability that is consistent with clinical observations of the known variability of melanin content in metastatic sites. However, comparing the organ with melanoma to control organs as we can in the case of liver we see a significant difference. We analyzed 4 cases and the % by weight melanin content was: 0.09, 0.28, 0.34, and 0.79. The importance of exact determination of melanin content can be seen in Table 3. The concentration of chlorpromazine (CPZ) varies with the concentration of melanin. Of note is the greater variation at lower concentrations (1.10 and 1.32 μg/g varied by a factor of 6). The possibility of different binding mechanisms depending on (CPZ) concentration has been mentioned by Larsson.[4] The melanin content of animal model melanomas will vary with the age of the tumor, therefore, we suggest reporting melanin concentration along with that of the radiopharmaceutical being investigated.

The biodistribution of twenty-one radiopharmaceuticals has been determined in the Greene melanoma. Table 4 lists only the % uptake and time, however, we have uptake data on most organs and times from 1 hour to 1 week. The details are not pertinent to this paper; however, it is of note that 1) many agents concentrate in melanoma and 2) tumor to background ratios vary. These two factors are of primary concern in determining whether a scan is feasible.

Our clinical experience with melanin-affinic agents has concentrated on iodine-123 labeled 4-(3-dimethylaminopropylamino)-7-iodoquine (^{123}I-DMQ). Other workers have claimed good results in localizing human skin and choroidal (eye) melanoma with this agent.[42-49] We used a modified collimator (dual pinhole)[47] on the gamma camera to obtain "images" of the 2 eyes, one with melanoma and the other normal eye. Our results have been erratic. We have studied 4 patients

with choroidal melanomas (confirmed histologically) that would be considered large (greater than 1000 cu mm) and have positive scans in 3. There is no good explanation for the false negative result in 1 patient. A typical positive scan is seen in the Figure. This "scan" represents a 3% difference in counts between eye with melanoma and normal eye.

COMMENT

From this data one may conclude that melanin seeking agents will have variable uptake and therefore any diagnostic or therapeutic effort will suffer. However it seems improbable that a false positive will occur.

Variable results clinically noted may be due to the decreased rate of melanogenesis in rapidly growing larger melanoma. This was shown in experiments in which the doubling times were correlated to melanogenesis.[40] As doubling time decreases (rapid tumor growth), melanogenesis decreased relative to cells with longer doubling times. Thus larger melanomas may have more melanin but a slower rate of synthesis.

We have determined the biodistribution and uptake by the Greene melanoma in the Syrian golden hamster with 21 radiopharmaceuticals.

Table 3 lists them all with maximum % uptake and the time at which this occurred. Space does not permit further elaboration of this data. It is essential to know maximum tumor to background ratio and the time after injection that this occurs to determine suitability for tumor scanning.

The importance of species variation deserves mention. We found detection of eye melanoma in humans to be quite variable whereas in hamster it was quite easy to obtain a positive scan with a single pinhole.[41] We then looked at brain uptake in man and found it (the "brain scan") to be significant. Dencker[42] points this out in his comparison of cerebrum uptake in monkeys versus rodents.

The former had cerebrum concentration equal to liver. In addition we found a high uptake by the lung, something not found in hamsters but not entirely unsuspected of an amine, such as ^{123}I-4,3DMQ.

Finally, our clinical experience has shown us some of the vagaries of melanoma-seeking radiopharmaceuticals. This reflects the complexity of melanin and melanin-binding and points out the necessity for a more detailed analysis of the mechanisms involved in melanin binding radionuclides.

Table 1

Melanin Content in Animal Tissues

	% by weight*
Greene melanotic melanoma (13)	0.34
Harding-Passey melanotic melanoma (4)	0.62
Harding-Passey amelanotic melanoma	0.02
Hamster: Liver, lung, blood, brain heart, spleen, kidney, intestine, muscle	<0.0025
Hair	0.39
Skin	0.013
Skin and hair	0.13

Table 2

Melanin Content in Human Tissues

Malignant Melanoma	% by Weight	Normal Controls	% by Weight*
Liver with melanoma	0.42	Liver (2)	0.013/0.008
Lymph nodes with melanoma	0.48	Brain (2)	0.016/0.017
Bone with melanoma	0.31	Skin (2)	0.023/0.008
Spleen with melanoma	0.12	Lymph node	
Melanoma-melanotic (4 cases)	0.09-0.79		

Table 3

Melanin Content and Chlorpromazine (CPZ) Uptake

Model	Melanin (% By Weight)	CPZ (µg/g)
Harding Passey (Balb Mouse)	0.68	7.30
Whole Eye (C3H Mouse)	0.40	4.56
Whole Eye Hamster	0.45	3.58
B16 Melanoma C57 Mouse	0.06	1.32
Greene Melanoma Hamster	0.34	1.10
KHDD Melanoma C3H Mouse	0.003	0.10

Table 4

Maximum Uptake in Greene Melanoma

in Syrian Golden Hamsters

Radiopharmaceutical	% Uptake	(Time-hrs)
Indium-111 bleomycin	5.45	(48)
Gallium-67 citrate	4.87	(48)
Indium-111 chloride	2.26	(24)
Phosphorus-32	2.25	(72)
Fluorine-18 deoxyglucose	2.32	(2)
Lead-203 tris	1.61	(6)
Carbon-14 tris	0.24	(18)
Silver-110 tris	0.22	(1)
Silver-110 nitrate	1.08	(48)
Mercury-197 chlormerodrin	1.11	(3)
Iodine-123 quinoline	1.09	(24)
Cobalt-57 bleomycin	0.52	(6)
Thallium-201 chloride	0.48	(6)
Technetium-99 pertechnetate	0.72	(1)
Technetium-99 citrate	0.46	(1)
Technetium-99 phosphate	0.45	(1)
Technetium-99 glucoheptanate	0.32	(1)
Sulfur-35 chlorpromazine	0.41	(1)
Sulfur-35 vitamin-A acid	0.40	(48)
Iodine-123 indocyanine green	0.24	(1)
Tritiated tetracycline	0.26	(3)

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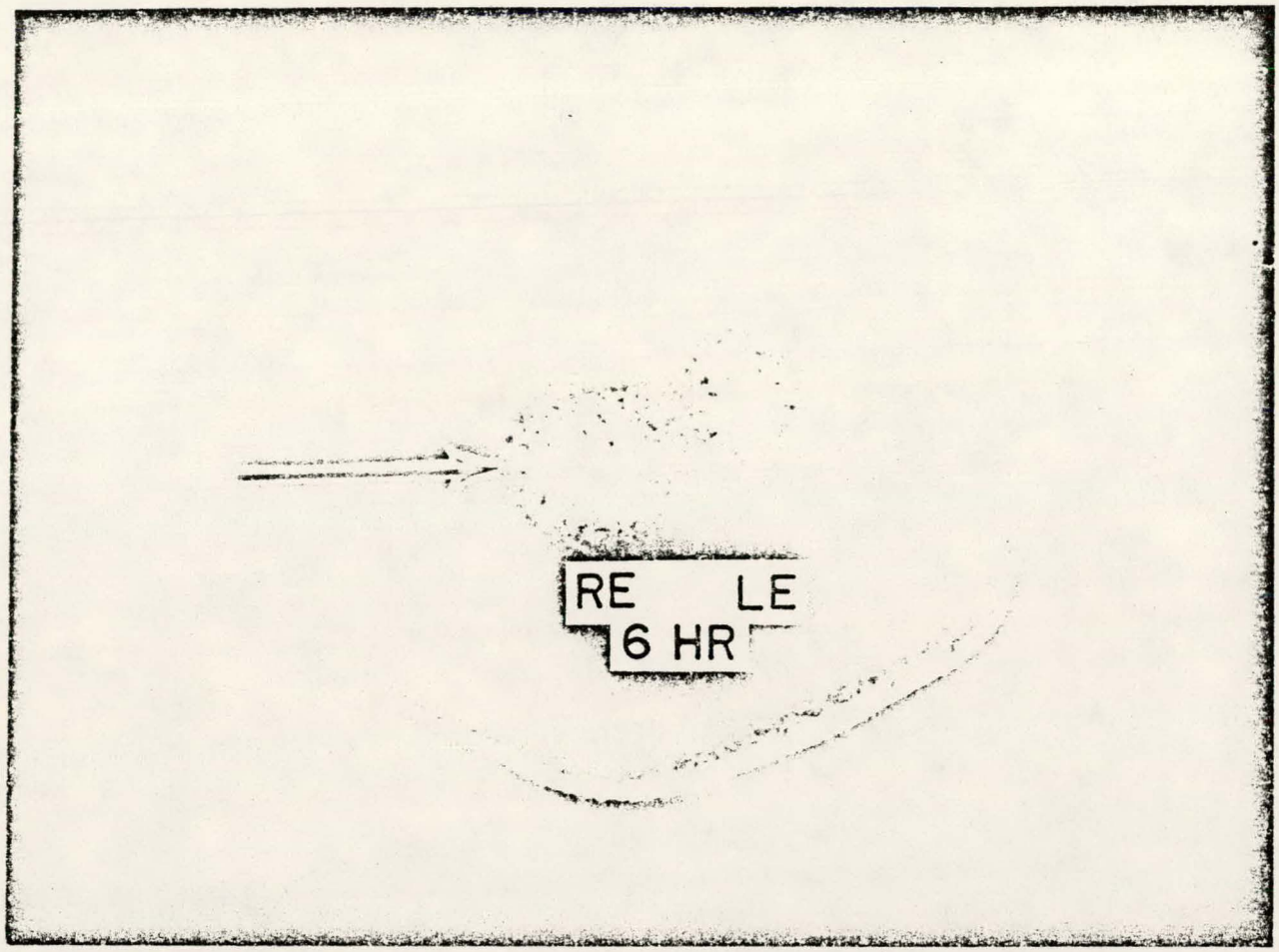
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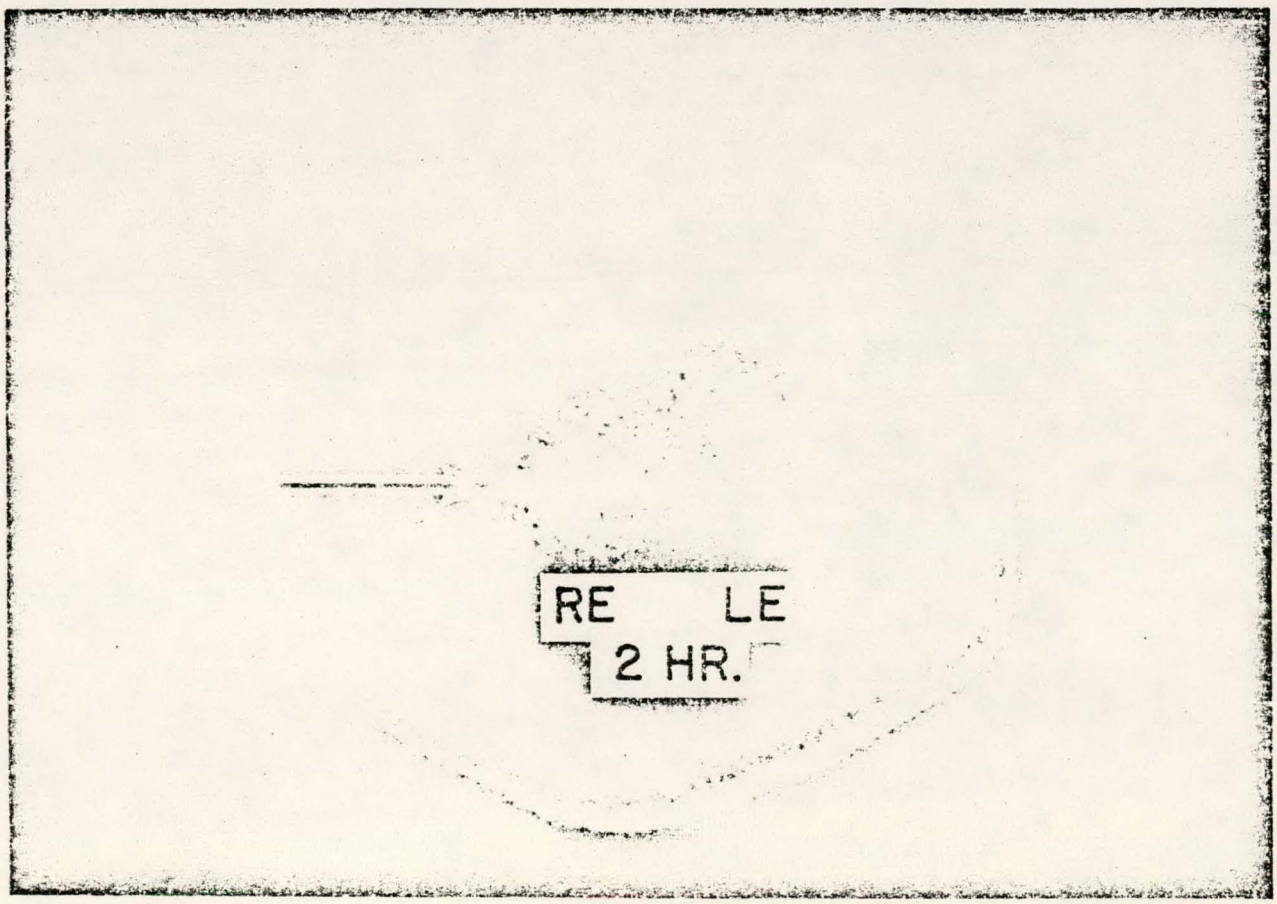
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