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Biodistribution of two ^{131}I–IMBA preparations, differently labelled, in mice with experimental B16 melanoma tumours

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Abstract

BACKGROUND:Numerous reports indicate that some iodinated compounds of benzamide derivatives display a strong affinity to the cells of melanoma. In the present report, a compound [N-(2-diethylaminoethyl)-3-iodo-4metyoxybenzamide (^{131}I-IMBA)] has been prepared by two different labelling methods.
Biodistribution of the injected compound was followed in mice with experimentally induced B16 melanoma tumours, and tumour/tissue ratios were studied as a function of time post administration.
MATERIAL AND METHODS: The iodinated ^{131}I-IMBA was obtained by means of ^{131}I exchange for nonradioactive iodine atoms (method I) and by means of ^{131}I substitution for a metalorganic group (method II). The last preparation was purified by chloroform extraction. The chemical purity was assessed by means of ascending thin layer chromatography (TLC).
The biodistribution of ^{131}I-IMBA in C57 Black mice was studied in animals with experimentally induced B16 mice melanoma tumours.
RESULTS: The mean labelling efficiency exceeded 95 and 80 % for methods I and II, respectively, at radiochemical purity > 95% in both cases.
^{131}I-IMBA was vividly cumulated by melanoma tumours in mice. At 24-hours post ^{131}I-IMBA administration the values of tumour/ /non-tumour ratios for the compound labelled by method II reached the following values: tumour/liver 10 ± 3, tumour/lung 15 ± 12, tumour/blood 153 ± 39, tumour/intestines 176 ± 26, tumour/kidneys 270 ± 107, and tumour/muscle 448 ± 82. These values exceeded, by an order of magnitude, the corresponding ratios for the same compound labelled by method I.
CONCLUSIONS: High values of tumour/non-tumour ratios indicate that ^{131}I-IMBA could be a promising radiopharmaceutical for clinical diagnosis (staging) of melanomas in humans.
Key words: melanoma, aminoalkyl-iodobenzamides, radioiodinated IMBA

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Introduction

Melanoma is a malignant neoplasm originating from the pigment-containing cells which synthesize melanin. This neoplasm has become a serious public health problem in most countries because of its dramatically increasing incidence accompanied by high mortality. In Europe, the melanoma is the seventeenth most frequent cancer in males and the eighth in females. Among the aetiological factors involved, exposure to ultraviolet light during sunbathing is the most pronounced. The endogenic factors, including light carnation of the skin and genetic predisposition, play the key role [1]. Melanoma is a particularly aggressive neoplasm with high and early capacity for metastasis to practically all organs. Even after early ex-

cision the metastases may appear later after a long period of spurious eradication of the disease. The most frequent metastases are to regional lymphatic nodes and skin; other affected organs are usually the brain, lungs, intestinal tract, and kidneys. Whereas early excision of the original focus of melanoma still offers hope for radical eradication, the metastases carry a bad prognosis. Mean 5-year survival for patients with metastases to regional lymph nodes oscillate between 20 and 50% [2, 3]. Thus, early detection of melanoma may reduce lethality and offers the possibility of effective treatment.

Nuclear medicine provides diagnostic possibilities for melanoma detection and staging which exceed the efficacy of traditional imaging procedures (radiology, ultrasound). The main limitations of the latter involve difficulties in differentiation of benign changes from malignant foci of melanoma and of post therapeutic changes from relapse of melanoma. Functional imaging based on the use of specific radiopharmaceuticals for tumour detection, combined with anatomic imaging, often leads to more effective identification of malignant foci [4].

Over the years there have been numerous attempts to use various compounds labelled with radioactive nuclides for the diagnosis of melanoma (^{67}Ga-citrate, ^{99m}Tc-MIBI, ^{131}I-methylthyrosine, and analogues of melanotropine-alfa MSH) [5–10] . However, none of these possibilities offered sufficiently high diagnostic efficacy and therefore did not become useful clinical tools.

Nonetheless, a highly effective procedure was introduced into early staging of melanoma in the form of sentinel lymph node detection by means of injection of colour or/and radioactively labelled colloids, injected into the vicinity of the primary melanoma focus. This procedure enables, by means of histological inspection, a diagnosis whether the node contains malignant metastatic cells or not. This procedure has become a diagnostic standard and dictates the selection of treatment alternatives [11–13].

Another technology has also acquired an important position in staging of melanoma: namely positron emission tomography (PET) [14, 15]. However, this highly potent technique also has some limitations.

Firstly, ^{18}F-fluorodezoxyglucose (^{18}FDG) is not a specific radiopharmaceutical for melanoma, which is taken up by muscles, inflammatory changes, and last but not least — the central nervous system [15] in which melanoma metastases often occurs. The latter often carries the most serious prognosis for longer survival.

In addition, PET technology is still not commonly available in many countries and is an expensive diagnostic tool. Therefore, looking for new conventional radiopharmaceuticals, specific for melanoma, if possible, and allowing for use of whole body scintigraphy and SPECT procedures, seems justified.

In recent years several studies have demonstrated that there are some compounds in the group of N-alkilated derivatives of benzamides, which when iodinated with radioactive iodine (^{123}I, ^{131}I) facilitate detection of melanoma foci [16–20]. The most promising member of this group is N-(2-diethylaminoethyl)-3-iodo-4metoxybenzamide, known under the acronym of IMBA. After IV administration, the compound leaves the body very fast; however, it is taken up vividly by the melanoma foci and therefore offers the highest tumour/non-tumour ratios among the derivatives of the group [21, 22].

In the present study, the preparation of IMBA, iodinated with ^{131}I, was obtained by means of two exchange methods: with non-radioactive iodine and with metalorganic substitute. The first method required performing the exchange reaction at a high temperature, and the resulting product contained copper ions, which played the role of catalyst. In addition, the final product also contained a rather high amount of non-radioactive IMBA, used for labelling with ^{131}I. The second method, free of these disadvantages, utilized a metalorganic precursor for the labelling.

Both final products were used for investigation of biodistribution of the labelled ^{131}I-IMBA in mice carrying the experimentally induced B16 melanoma tumours. From measured concentrations of ^{131}I activity in the tumours and several remaining organs (tissues), the tumour/non-tumour ratios of activity concentrations were calculated.

Material and methods

Radiolabelling

Method I

To obtain the IMBA preparation labelled with ^{131}I, a method was applied based upon exchange in the liquid phase of nonradioactive iodine atoms with radioactive ones, as reported by Berthomier [23]. The reaction took place at a temperature of 140°C in the presence of copper ions, which acted as the catalyst. For the labelling, a carrier (free iodine 131) was used, manufactured by IEA OR POLATOM (RI-10 — Na^{131}I delivered in carbonate buffer; radionuclide purity > 99.9%). The carrier IMBA to be labelled was prepared in the Department of Pharmaceutical Chemistry and Drug Analysis of the Medical University of Lodz. The radiochemical purity of the labelled preparation was evaluated by means of ascending thin layer chromatography on glass plates covered with silica gel (silica gel glass plate, Merck). The moving phase was a mixture of chloroform and methanol with a volume ratio of 1:7. Analysis of impurities was performed by means of autoradiography. The position of ^{131}I-IMBA on the chromatogram was confirmed under UV light, using the "cold" IMBA as the standard. The autoradiogram served as the basis for cutting the chromatographic plate into appropriate fragments, and the activity of the components was measured by automatic gamma counter (Perkin-Elmer).

Method II

The ^{131}I-labelled IMBA preparation was obtained by means of electrophilic substitution reaction using molecular iodine in situ, a metalorganic derivative of IMBA (N-(2-diethylaminoethyl)-3-tributyltin-4-metoxybenzamid) in an aqueous medium with chloramine T as the oxidizer. The procedure was previously developed by C.S. John and her group for labelling other benzamide derivatives (IPAB, PIMBA; 24, 25). The precursor, tributhyltin derivative, was obtained from the Department of Pharmaceutical Chemistry and Drug Analysis of the Medical University of Lodz. The preparation was labelled with carrier-free ^{131}I manufactured by IEA OR POLATOM, Swierk.

The labelled compound was purified by extraction with chloroform. The dry remnant, after removal of the chloroform with a stream of argon, was dissolved in physiological saline. The radiochemical purity of the product was determined by means of ascending thin layer chromatography (TLC) on plates covered with silica gel with

a fluorescent label (Silica Gel 60 F254, Merck).,A mixture of chloroform and methanol in the ratio 1:7 was used as a developing medium. Under these conditions the Rf for IMBA was 0.20–0.25, and the Rf for ^{131}I- was 0.90–0.95.

Animal biodistribution studies

Before undertaking the investigation, acceptance was applied for, and granted by, the Ethic Commission for Experiments on Animals. In the experiments, female mice of the C57 Black strain were used.

The B16 melanoma cells were obtained from the L. Hirszfeld Institute of Immunology and Experimental Therapy (Polish Academy of Sciences, Wroclaw). The suspension of B16 melanoma cells (ab. 0.5×10^6 in 0.1 ml saline) was injected into mice subcutaneously in the interscapular region. After 14 days there was a palpable tumour. Some animals died at this stage.

In further studies, the mice were used with a tumour of ~ 10 mm diameter, in which ^{131}I-IMBA of activity 2–3 MBq at a volume of 0.05 ml was injected intravenously (tail vein). The animals were sacrificed after 4 and 24 hours after injection of the benzamide.

In all animals, the whole body retention was measured and subsequently the tumour, blood, kidney, liver, lungs, and muscle fragments were sampled. In these samples, an activity concentration of ^{131}I-IMBA per gram was determined. On the basis of these measurements, the activity ratios of tumour/non-tumour were calculated for all samples. To avoid experimental errors resulting from extra venous injection of ^{131}I-IMBA, the activity of the tails of the animals was measured, and individuals with extravasations of ^{131}I-IMBA at injection were rejected from further analyses. Eventually, the number of mice evaluated which were sacrificed at 4 and 24 hours amounted to 4 and 5, respectively, for those given ^{131}I-IMBA obtained by method II. For those injected with the preparation obtained by method I, the number of animals sacrificed at 4 and 24 hours equalled 4.

Results

By labelling IMBA via exchange of non-radioactive iodine for radioactive atoms (method I), we obtained a labelled ^{131}I-IMBA with an efficiency and radiochemical purity of ab. 95%. Using the alternative method (method II - exchange of tributhyltin for ^{131}I) the yield exceeded 80%. The purification by extraction with chloroform led to a radiochemical purity > 95%. The advantages of the second method included the elimination of cuprous ions from the final preparation and of nonprocessed carrier IMBA substance (nonactive). There was also no need to carry out the labelling at a high temperature.

The preparation injected into the mice displayed a high accumulation in the tumour and fast elimination from the body. The mean whole body retention of 49% at 4 hours post injection dropped to 6% after 24 hours.

After 24 hours the majority of activity was contained in the tumour. This led to high tumour/non-tumour indices of the activity. The values of these ratios for the studied organs and tissues in the animals given ^{131}I-IMBA obtained by method I and method II are presented in Tables 1 and 2, respectively. The ratios increase substantially with time post-administration. The increase over the time interval from 4 to 24 hours was several fold for method I, and more than an order of magnitude when the activity of ^{131}I-IMBA was obtained by method II. In the latter case, the highest tumour/ /non-tumour ratios of activity were seen in the muscles and kidneys, where they reached values of 448 ± 82 and 270 ± 107, respectively. Values exceeding 100 were also obtained for the intestines and for blood. Figure 1 presents the scintigraphic image of a mouse with a hot focus corresponding to the experimental tumour.

Table 1. The tumour/non-tumour ratios (mean ± SD) after 4 and 24 hours post administration of ^{131}I-IMBA, labelled by method I

Tumour/non-tumour ratio	Time post administration	
	4 hours (n = 4)	24 hours (n = 4)
Tumour/liver	1.7 ± 1.0	1.1 ± 0.3
Tumour/lungs	6.6 ± 3.6	11.9 ± 3.7
Tumour/blood	7.2 ± 3.4	23.4 ± 4.6
Tumour/kidneys	4.0 ± 2.0	4.5 ± 1.3
Tumour/muscle	24.3 ± 11.8	44.1 ± 21.7

Table 2. The tumour/non-tumour ratios (mean ± SD) after 4 and 24 hours post administration of ^{131}I-IMBA, labelled by method II

Tumour/non-tumour ratio	Time post administration	
	4 hours (n = 4)	24 hours (n = 4)
Tumour/liver	2 ± 1	10 ± 3
Tumour/lungs	4 ± 3	15 ± 12
Tumour/blood	20 ± 18	153 ± 39
Tumour/intestines	4 ± 5	176 ± 26
Tumour/kidneys	10 ± 11	270 ± 107
Tumour/muscle	30 ± 16	448 ± 82
Whole body retention (%)	49 ± 15	6 ± 3

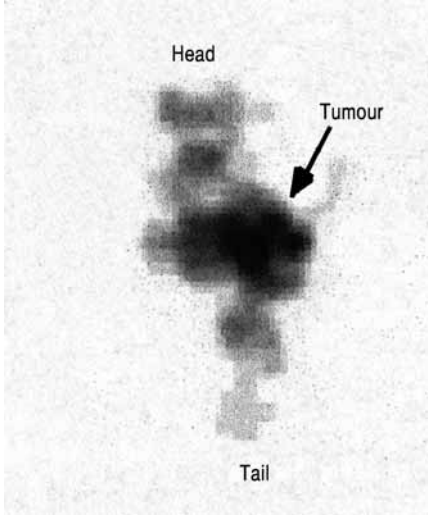

Figure 1. Uptake of ^{131}I-IMBA by experimental B16 melanoma in mouse.

Discussion

The reported study on uptake of ^{131}I-IMBA by experimental melanoma tumours and tissues of mice demonstrated an avid uptake of the compound by the tumour and rapid excretion from the body. The tumour/non-tumour ratios reached particularly high values at 24 hours post IV administration of ^{131}I-IMBA. Similar observations were reported by other authors [21, 22].

The tumour/non-tumour ratios were systematically different for animals injected with the studied benzamide derivative prepared by the two methods reported above. The relative concentrations reached by ^{131}I-IMBA in melanoma and other tissues displayed higher tumour/non-tumour ratios when the substance injected was obtained by method II; they exceeded those seen after preparation of 131I-IMBA by method I by approximately an order of magnitude.

It seems that this difference can be linked to the much lower specific activity of the ^{131}I-IMBA obtained by method I. The highest ratios seen were those for tumour/muscles, both after 4 and 24 hours post administration. The concentration ratio for tumour/lungs reached similar values for groups of animals injected with ^{131}I-IMBA prepared by the two methods (11.9 for method I and 15 for method II, at 24 hours post injection). In animal experiments, Edreira and Pozzi obtained the corresponding ratio of 40 (Table 3); at the same time these authors claimed that the lowering of this ratio could result, perhaps, from intensive uptake by micrometastases.

When comparing the results reported by Edreira [21], we found (for the preparation obtained by method II) higher tumour/blood (by a factor of 3) and tumour/kidney ratios (~ 7 times).

The high tumour/non-tumour ratios for ^{131}I-IMBA reported in this study and by other teams [21, 22, 26] indicate that this compound could be used for scintigraphic detection of melanoma foci (both primary and metastases) in humans. This is also confirmed by the preliminary study on a few patients made by Nicholl et al. [22] in which ^{123}I-IMBA provided high contrast between melanomatous foci and non-tumour tissues at just 4 hours post administration. However, later there were many more melanoma foci visible [22]. It may be concluded, therefore, that the optimal time of imaging after

Table 3. Comparison of mean tumour/non-tumour ratios at 24 hours post administration of ^{131}I-IMBA

Tumour/non-tumour ratio	Method I	Method II	Edreira [21]
Tumour/liver	1.1	10	19.98
Tumour/lungs	11.9	15	39.97
Tumour/blood	23.4	153	51.94
Tumour/kidneys	4.5	270	34.88
Tumour/muscle	44.1	448	–

^{131}I-IMBA administration remains an open question. Perhaps imaging at 24 hours post injection of the radiopharmaceutical should be considered. This applies particularly to the search for metastases in the lungs and liver.

Conclusion

^{131}I-IMBA preparation is avidly taken up by experimental B16 melanoma. The results reported in this paper strongly suggest that the substance could be used for the diagnosis and staging of melanoma in humans.

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