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Uptake in melanoma cells of *N*-(2-diethylaminoethyl)-2-iodobenzamide (BZA_2), an imaging agent for melanoma staging: relation to pigmentation

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Abstract

N-(2-diethylaminoethyl)-2-iodobenzamide (BZA_2) has been singled out as the most efficacious melanoma scintigraphy imaging agent. Our work was designed to assess the mechanisms of the specific affinity of the radioiodinated iodobenzamide for melanoma tissue. We studied the cellular uptake and retention of [^{125}I]-BZA_2 on various cell lines. In vitro, cellular [^{125}I]-BZA_2 uptake was related to the pigmentation status of the cells: higher in pigmented melanoma cell lines (M4 Beu, IPC 227, B 16) than in a nonpigmented one (M3 Dau) and nonmelanoma cell lines (MCF 7 and L 929). Two mechanisms were assessed: binding of the tracer to melanin or to sigma receptors of melanoma cells. First, the uptake of [^{125}I]-BZA_2 after melanogenesis stimulation by α-melanocyte-stimulating hormone and L-tyrosine increased in the B 16 melanoma cell line both in vitro and in vivo according to melanin concentration. Moreover, the binding of [^{125}I]-BZA_2 to synthetic melanin was dependent on melanin concentration and could be saturated. Second, no competition was evidenced on M4 Beu cells between [^{125}I]-BZA_2 and haloperidol, a sigma ligand, at concentrations $\leq 10^{-6}$ M. We show that the specificity and sensibility of BZA_2 as a melanoma scintigraphic imaging agent are mostly due to interactions with melanic pigments.

Keywords: Iodobenzamide; Melanoma cells; Melanoma radiopharmaceutical; Melanin binding

1. Introduction

Melanoma is a highly malignant tumor, and its incidence is continuously rising worldwide [1,2]. Melanoma displays a high spreading potential, rapidly becoming one of the most aggressive and chemoresistant tumors [3]. However, while the treatment of melanoma metastases is still problematic, there is hope that early detection of metastases by a single specific examination as whole-body scintigraphy imaging will improve patient management, in particular for solitary sites where surgical treatment may be indicated [2]. Melanoma is a pigmented tumor, and if the level of pigmentation may vary, the entirely nonpigmented form is very uncommon [4,5]. This characteristic gives to melanin a target role to which radiopharmaceuticals may be specifically addressed.

The last decade has seen the development of iodobenzamides, a class of compounds that display a noteworthy affinity for melanoma tissue. Radioiodinated iodobenzamides have been proposed as potent imaging agents for the detection of melanoma metastases and for the specific diagnosis of ocular melanoma as opposed to nonmalignant (ocular naevi) or nonmelanocytic tumors [6]. *N*-(2-diethylaminoethyl)-4-iodobenzamide (BZA) was the first selected compound to be tested in a Phase II clinical trial on 110 patients for these indications. The results were positive with a sensitivity of 81% and a specificity of 100%. The only finding suggesting that further improvements might be required was the time of approximately 20 h after tracer administration needed to show the best tumor definition [7] Various authors have evaluated other members of the same

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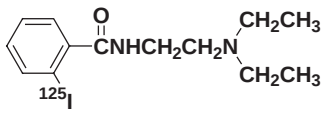

Fig. 1. Chemical structure of [^{125}I]-BZA$_2$.

series in preclinical experiments, some of which have been studied in clinical applications, also with good results [8–10]. To identify a tracer with better pharmacokinetic behavior, several derivatives have been studied in preclinical models [11,12]. Owing to its experimental pharmacokinetic profile in mice, which promised better imaging contrast, a new compound, *N*-(2-diethylaminoethyl)-2-iodobenzamide (BZA$_2$), was evaluated in a Phase II clinical trial conducted on 40 patients with either a primary ocular lesion or metastases from cutaneous or ocular melanoma [13]. For 25 patients with histologically proven cutaneous melanoma, after a follow-up of more than 1 year, the overall results of [^{123}I]-BZA$_2$ scintigraphy on a per-patient basis showed a sensitivity of 100% and a specificity of 95% [14]. In this trial, [^{123}I]-BZA$_2$ allowed the imaging of all the proven secondary lesions. The first experimental and clinical results obtained with BZA were thought to derive from the binding of the radiotracer to melanin [6,7], a hypothesis confirmed by studies on several experimental models [15]. Another hypothesis has been put forward, however some authors consider that the scintigraphic results may be due to the binding of iodobenzamide to sigma receptors of the melanoma cell membrane [16].

This work is mainly an in vitro approach to the [^{125}I]-BZA$_2$ (Fig. 1) uptake process in melanoma cells. A comparative study of uptake kinetics in several cell lines, mainly melanoma cells, human and murine, pigmented or nonpigmented, is presented. To evaluate the two hypotheses for the mechanism of the iodobenzamide uptake in melanoma cells, the study was performed in different experimental conditions: (1) binding of [^{125}I]-BZA$_2$ to synthetic melanin or uptake in melanoma cells in varying conditions of melanogenesis in vitro or in vivo in mice bearing melanoma and (2) competition of [^{125}I]-BZA$_2$ with a sigma-receptor ligand (haloperidol) on melanoma cells ‘‘or in vivo’’ because at revision, results in vivo have been added.

2. Materials and methods

2.1. Cell lines

Several cell lines were used: two human cutaneous melanoma cell lines established from metastasis biopsy specimens by J. F. Doré (INSERM U 453, Lyon, France), M4 Beu and M3 Dau, pigmented and nonpigmented, respectively; IPC 227, human pigmented ocular melanoma provided by C. Aubert (INSERM U 119, Marseille, France); B 16, murine melanoma cell line (ICIG, Villejuif, France); MCF 7, human breast carcinoma (Novartis, Bâle, Switzerland); and a nontumoral cell line, L 929 murine fibroblast (Department of Hydrology, Faculty of Pharmacy, Clermont-Ferrand, France).

All the cells were maintained as monolayers in 80-cm^2 culture flasks in Eagle’s minimum essential medium (MEM) GlutaMAX (Life Technologies, Cergy-Pontoise, France) supplemented with 10% fetal calf serum (SVF, Boeringher, Mannheim, France), vitamin solution (MEM vitamins, 100×), nonessential amino acids (100×), sodium pyruvate (1 mM) and gentamicin (4 mg/L; Life Technologies). Cells were cultured at 37°C in a saturated water atmosphere: 95% air/5% CO_2 (water-jacketed incubator, Forma Scientific). The cells were adherent in monolayers and, when confluent, were harvested from the cell culture flasks with trypsin EDTA (5×, Life Technologies) and seeded farther apart. For the experimental procedure, cells were used at close passage for different preparations of a same line.

2.2. Radiotracer

Radioiodination of benzamide was performed by a simple isotopic exchange reaction with sodium [^{125}I] iodide using already published procedures [11] with no copper salt catalyst. The labeled hydrochloride was confirmed by thin layer chromatography and high-performance liquid chromatography to be identical to the nonradioactive standard. The radiochemical yield was 93–98% and the specific activity was in the range of 9.9–12.7 GBq/mmol.

2.3. Uptake kinetics of [^{125}I]-BZA$_2$ in several cell lines

Cells were plated in culture dishes of 35-mm diameter: 10^5 cells/dish for L 929 and B 16; 2×10^5 cells/dish for M4 Beu, IPC 227 and M3 Dau; and 2.5×10^5 cells/dish for MCF 7 in 2 ml of culture medium. These concentrations ensured that the culture was in a proliferative phase when used 2 days later.

For the experimentation, the culture medium was removed, cells were washed with medium without SVF and incubated with 5 μM of [^{125}I]-BZA$_2$ in 2 ml of medium without SVF to avoid parasitic binding of the radiotracer to the serum proteins. The incubation was maintained for 1, 5, 10 and 30 min and 4 and 24 h (four dishes at a time for one experiment; two different experiments for each cell line). At the end of the incubation time, the medium was quickly removed and cells were washed twice with cold physiological serum and harvested in Dulbecco’s phosphate-buffered saline (PBS, Life Technologies) with a cell scraper. The activity of the samples was counted (Minaxi γ 5530, Packard, Rungis, France). The protein concentration was measured using Bradford’s colorimetric method with a Coomassie blue G250 solution (Pierce, Oud-Bejerland, The Netherlands) and bovine serum albumin as