Photodistribution of Blue-Gray Hyperpigmentation After Amiodarone Treatment

Molecular Characterization of Amiodarone in the Skin

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Background: For decades, the photodistributed blue-gray skin hyperpigmentation observed after amiodarone therapy was presumably attributed to dermal lipofuscinosis. Using electron microscopy and high-performance liquid chromatography, we identified amiodarone deposits in the hyperpigmented skin sample from a patient treated with this antiarrhythmic agent. Our findings therefore indicate that the hypothesis relating the blue-gray hyperpigmentation to lipofuscin should be challenged.

Observations: A 64-year-old man, skin phototype III, presented with asymptomatic skin hyperpigmentation that had been slowly developing on sun-exposed areas since April 2004. He had been taking amiodarone for 4 years (cumulative dose, 277 g). Electron microscopy did not show lipofuscin pigments in his skin. Conversely, abundant electron-dense membrane-bound granule deposits were observed in most of the dermal cells (fibroblasts, macrophages, pericytes, Schwann cells, and endothelial cells), especially in photoexposed skin. High-performance liquid chromatography confirmed that the skin deposits were composed of amiodarone. These results demonstrate that amiodarone hyperpigmentation is related to drug deposition on photoexposed skin.

Conclusion: Amiodarone-related hyperpigmentation should be considered a skin storage disease that is secondary to drug deposition.

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REPORT OF A CASE

A 64-year-old man, skin phototype III, presented with progressive, asymptomatic skin hyperpigmentation that had been slowly developing on sun-exposed areas since April 2004. His medical history was remarkable for myocardial infarction, ventricular arrhythmia, and heart failure. He had no known history of drug allergy. For several years, he had been treated with acebutolol (200 mg/d), lisinopril (10 mg/d), simvastatin (20 mg/d), and lysine acetylsalicylate (75 mg/d). He had also taken amiodarone (Cordarone) at a dosage of two 200-mg tablets per day, 5 days per week, from 2001 until July 2005. The cumulative dose was estimated to be 277 g over 4 1/2 years. His physical examination revealed photodistributed, blue-gray hyperpigmentation on sun-exposed areas.
pigmentation of the face and ears, sparing the area under the nose, all eyelids, nasolabial folds, wrinkles, and submental and postauricular areas (Figure 1). Phototests revealed a polychromatic minimal erythema dose at 600 mJ/cm² (reference value, ≥400 mJ/cm²). The results of the UV-A phototest (13 J/cm²) were negative after 24 hours, with mild hyperpigmentation. Histologic examination of a pigmented skin specimen revealed numerous macrophages accumulated around superficial dermal vessels. The cytoplasm of these cells showed brownish deposits that were positive on periodic acid–Schiff and Fontana stains (Figures 2, 3, and 4).

Electron microscopy of a nonpigmented skin sample showed the presence of a few homogeneous strongly electron-dense granules confined to the upper dermis, whereas a pigmented skin sample revealed numerous similar granules within the thickness of the dermis. These deposits were localized mainly in fibroblasts as well as in other cells, particularly macrophages, endothelial cells, and Schwann cells (Figure 5). At high magnification, they appeared to be surrounded by a membrane. There were no abnormal deposits within the epidermis, hair follicles, or sebaceous glands. No lipofuscin deposits were observed in the pigmented skin sample from our patient.

**TECHNIQUE**

The first step of the molecular identification of amiodarone deposits in the skin involved the extraction of amiodarone from a skin biopsy specimen. A skin biopsy (punch, 4 mm; 8 mg) was performed on the pigmented skin of the face to demonstrate the presence of drug deposits by extracting the active molecule of amiodarone. Accordingly, the skin specimen was homogenized with 10 mL of methanol and kept at 5°C for 24 hours. The homogenate was then crushed and filtered. The filtrate was evaporated to dry-
Figure 5. Electron microscopy of photoexposed pigmented skin. Electron-dense granules are observed within (1) a fibroblast of the deep dermis at low magnification (A [original magnification ×15000]); (2) a fibroblast of the deep dermis at high magnification (the arrow indicates the membrane surrounding a granule) (B [original magnification ×21000]); (3) an endothelial cell (double arrow) and a pericyte (single arrow) of a capillary of the superficial dermis (C [original magnification ×5200]); and (4) a Schwann cell surrounding amylentic nerve fibers (arrows) (D [original magnification ×15 000]).

ness, dissolved in 2 mL of diethylether, and acidified with 500 µL of 1M hydrochloride. It was shaken for 15 minutes and centrifuged for 10 minutes at 3000 rounds per minute. This last step resulted in an upper organic layer (containing amiodarone) and a lower aqueous layer. The organic layer was dried with anhydrous sodium sulfate and then evaporated to dryness.

The second step involved sample analysis. After skin extraction, the concentrate was reconstituted in methanol and then analyzed by means of high-performance liquid chromatography (HPLC) (isocratic mode, 75% methanol and 25% ammonium formate [25 mM]; column Sunfire C18, 3.5 µm 2.1 × 50.0 mm). To obtain a standard molecule, commercial amiodarone was isolated and purified from the amiodarone tablets and then sequentially added to the extracted sample, with a new HPLC analysis performed after each addition.

High-performance liquid chromatography of the skin sample showed 4 peaks corresponding to 4 retention times: 0.707 minutes, 0.868 minutes, 1.447 minutes, and 8.207 minutes (Figure 6). After each sequential addition of the commercial amiodarone, HPLC analysis revealed a peak increase at 8.207 minutes. The UV spectrum determined at 8.207 minutes showed a perfect match at each peak (Figure 6).

Amiodarone hydrochloride (2-butyl-3-benzofuranyl 4-[2-(diethylamino)-ethoxy]-3,5-diiodophenyl ketone hydrochloride) is an iodinated compound that is widely used in the treatment of cardiac arrhythmias and is known to cause photosensitivity and cutaneous hyperpigmentation. Although amiodarone photosensitivity is quite common and occurs in more than 50% of treated patients, blue-gray cutaneous hyperpigmentation occurs in fewer than 10%. The clinical features of the photosensitivity response represent a phototoxic reaction to both amiodarone and its major metabolite, mono-N-desethylamiodarone. Also, it has been shown that amiodarone therapy might induce photoallergy in guinea pigs. However, the phototoxic effect of the drug has generally been masked by its phototoxic potential. Phototoxic reactions can be experimentally elicited with UV-A; the UV-A minimal erythema dose is significantly reduced after 12 months of treatment. The photoactivating wavelengths are primarily found in the long-wave UV-A spectrum between 350 and 380 nm. However, phototests may show acute reactions to UV-A and UV-B and significant delayed reactions to UV-A and/or UV-B. Zinc oxide–containing preparations appear to be the most effective agents for reducing cutaneous photosensitivity. Under the regimens commonly used, photosensitivity can be expected to occur after 4 months of continuous treatment and a minimal cumulative dose of 40 g. It appears to be unrelated to the skin type. Photosensitivity gradually decreases and returns to normal between 4 and 12 months after discontinuation of amiodarone therapy. However, it can sometimes last for several years after drug withdrawal.

Amiodarone hyperpigmentation develops mainly in patients with skin type I. It occurs after an average of 20 months of continuous treatment and a minimal cumulative dose of 160 g. The slow rate of elimination of amiodarone and the high uptake by fat-associated tissues may explain the delayed spontaneous disappearance of cutaneous photosensitivity and the late resolution of the blue-gray discoloration. In 1 patient, massive amiodarone-induced hyperpigmentation was found to be reversible 33 months after the use of the drug was discontinued. However, in cosmetically stigmatizing hyperpigmentations, treatment with a Q-switched ruby laser has shown impressive results.

In our case, photosensitivity toward amiodarone or another drug was ruled out because phototests showed a normal polychromatic minimal erythema dose and a negative UV-A phototest result. Therefore, this case corresponds clinically, histologically, and ultrastructurally to typical amiodarone-photodistributed blue-gray hyperpigmentation, which occurred after 52 months of continuous treatment and a cumulative dose of 277 g.

Previous electron microscopy studies of amiodarone-pigmented skin demonstrated 6 distinctive morphological types of intracytoplasmic inclusions in many dermal cell types. The pathogenesis may be related to the action of the drug on cell membranes, local metabolic damage, and accumulation of the drug on the lysosomes, with acceleration of the physiological aging cell...
process. In a previous report, the presence of high concentrations of iodine, which was observed on electron probe analysis, suggested that the cutaneous deposits are made up of amiodarone itself or a metabolite. Our results confirm this hypothesis. After the extraction procedure that was performed on the hyperpigmented skin of our patient's face, HPLC of the skin sample showed 4 peaks corresponding to 4 retention times: 0.707 minutes, 0.868 minutes, 1.447 minutes, and 8.207 minutes. Later on, after each sequential addition of commercial amiodarone (extracted from Cordarone tablets), HPLC revealed a clear increase of the peak at 8.207 minutes. This finding suggests that the molecule corresponding to the retention time of 8.207 minutes and the commercial amiodarone that was added are the same compound. To be more precise, the UV absorption spectrum of each peak was determined at 8.207 minutes. These UV absorption spectra were perfectly identical (Figure 6). Therefore, in this case, the molecule extracted from the skin, which showed a peak at 8.207 minutes, is amiodarone. We were not able to identify the nature of the other 3 molecules corresponding to yellow and not blue fluorescence. Conversely, electron microscopic examination of the sun-exposed skin of patients without amiodarone discoloration shows pigment deposits similar to those already described in patients with amiodarone hyperpigmentation. Lipofuscin is a naturally occurring autofluorescent lipopigment that accumulates in aging cells as a normal part of senescence; it is called the wear-and-tear or aging pigment. Because this material exhibits fluorescence, it has been described by its spectral properties, with an excitation between 320 and 480 nm and an emission wavelength between 460 and 630 nm, with a peak at 580 nm corresponding to yellow and not blue fluorescence. Conversely, electron microscopic examination of the sun-exposed skin of patients without amiodarone hyperpigmentation shows pigment deposits similar to those already described in patients with amiodarone hyperpigmentation in exposed and nonexposed skin.
comparative study with nonpigmented, photoprotected skin would need to be carried out to find out whether the other 3 unidentified molecules (0.707 minutes, 0.868 minutes, and 1.447 minutes) on HPLC are photoproducts of amiodarone.

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REFERENCES