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Quantitative Mapping of 4′-Iododeoxyrubicin in Metastatic Squamous Cell Carcinoma by Secondary Ion Mass Spectrometry (SIMS) Microscopy[1]

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ABSTRACT

Secondary ion mass spectrometry microscopy enables quantitative mapping of chemical elements in tissue sections. It was used for the detection of ^{127}I contained in 4′-iododeoxyrubicin (IDX). Metastatic cutaneous squamous cell carcinoma from 7 patients participating in a phase I study (IDX dose, 80 mg/m^2, 10-min i.v. infusion) were biopsied 10 min after drug administration and compared to 3 controls who did not receive any treatment (one squamous cell carcinoma and 2 gastric carcinomas). Biopsy specimens were fixed and embedded in methacrylate resin. Then, serial semithin sections (3 µm) were analyzed simultaneously with ionic and optical microscopes in order to identify the histological structures given by the ^{31}P distribution in which ^{127}I in IDX was mapped. The iodine signal was undetected in controls and found mainly in the nuclei of tumor cells of the treated patients. Its concentration, measured in at least 30 nuclei of each specimen, was undectable in 8% of the nuclei and 91% of them were within 1 and 16 ng/mg. The mean concentration of each specimen ranged from 5 to 23 ng/mg. This study demonstrates the capacity of ion microscopy to localize a cytotoxic drug (IDX) in a human biopsy specimen without the need for radioactive labeling and enables the evaluation of drug penetration in cancer cells which is critical for its activity.

INTRODUCTION

The antitumor activity of most drugs used in cancer chemotherapy is due to their specific DNA-damaging properties which interfere with the DNA replication and transcription processes. Doxorubicin, an anthracycline currently used in clinical practice, is known to intercalate with DNA, which leads to the impairment of DNA template function for DNA and RNA synthesis (1). Furthermore, doxorubicin metabolism generates free radicals which appear to be responsible for cardiac toxicity. For this reason, a considerable amount of effort has been devoted to identifying new analogues with enhanced antitumor activity and reduced cardiotoxicity (2–4). One of them, IDX[3] was obtained by replacing the C′4 hydroxy radical in the sugar part of the doxorubicin molecule by ^{127}I (2).

Although it is a well-known fact that penetration of antitumor drugs into the cells of neoplastic tissue is critical for their activity, the *in vivo* distribution between normal and tumor cells remains enigmatic. Recently, SIMS microscopy, the first available method capable of mapping chemical elements in tissue sections (5–7), was used to detect and quantify ^{127}I in thyroid follicles of human tissue sections (8). The aim of this study was to localize and quantify the ^{127}I in IDX found in cell nuclei of biopsies obtained from patients treated with this drug.

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[3] The abbreviations used are: IDX, 4′-iododeoxyrubicin; SSC, squamous cell carcinoma; MSCC, metastatic squamous cell carcinoma; SIMS, secondary ion mass spectrometry.

MATERIALS AND METHODS

Protocol. This study was performed during the phase I trial of IDX (Farmitalia, Carlo Erba, Milan, Italy). Oral consent was obtained from 7 patients (7 men; age range, 46–63 years) with metastatic squamous cell carcinoma (MSCC) (Table 1). Their cutaneous metastases were biopsied 10 min after i.v. administration of the drug (IDX level, 80 mg/m^2, for a 10-min infusion). Control tissues were obtained from 3 surgical specimens of untreated patients: one with primary SCC (oropharynx) and 2 with gastric carcinomas.

Tissue fragments were cut into small pieces and fixed in a solution containing 1 g/liter glutaraldehyde and 20 g/liter paraformaldehyde in cacodylate buffer (0.1 mol/liter, pH 7.4). After ethanol dehydration, the pieces were embedded in methacrylate resin (Historesin; Pharmacia, Upsalla, Sweden). This method preserves the localization of iodine bound to macromolecules (9). Serial semithin sections were deposited on glass slides for histological examination (1.5-µm sections) and on ultrapure gold holders for ion analysis (3-µm sections).

IDX Mapping. The ^{127}I in IDX was mapped by SIMS microscopy. A primary ion beam was focused onto the surface of the resin-embedded tissue section. Under ion bombardment the atoms of the most superficial molecular layers (1–5 nm) of the specimen were progressively sputtered, some of them as ions. These secondary ions, characteristic of the atomic composition of the analyzed area, were focused, energy filtered, and separated by a mass spectrometer. An analytical image of the selected element was displayed on a fluorescent screen; the selected ion beam intensity was also measured with an electron multiplier. The instrument used in this study was the Cameca IMS 3F fitted with a Cs^+ primary ion source which increases the sensitivity and the detection limits of halogens.

Element Mapping. Direct electronegative ion imaging was performed with a 10-keV Cs^+ primary beam current of low intensity (20–30 nA) on areas of 60–150 µm. A mass resolution (> 2000) was used in order to eliminate interferences between cluster ions and the specific ion studied (10). A sensitive video camera linked to an image-processing system allowed high-speed signal integration, which was averaged with an improved signal to noise ratio (11).

Element Quantification. Secondary ion beam intensity measurement with the electron multiplier was used for ^{127}I quantification of IDX within cell nuclei. In SIMS there is a direct relationship between the secondary ion beam current and the local elemental concentration of the specimen. An internal reference such as carbon and an iodine standard curve established with an iodized resin (12) was used, and the results are expressed in ^{127}I ng/mg of tissue within the analyzed area. The specific detectable minimum (resin background + 3 SD) is 0.3 ng/mg.

Cell nuclei were selected with an adapted aperture (8-µm diameter). The analysis (mean of 10 measurements of the same nucleus) was performed on at least 30 nuclei/tissue specimen.

RESULTS

IDX Imaging. Iodine was never detected in the biopsies of untreated patients, while it was always found in the specimens of IDX-treated patients. Fig. 1 shows the images of serial sections of MSCC with optical (*A*) and ionic (*B* and *C*) microscopes. The main advantage of SIMS microscopy is its ability to preserve elemental mapping in relation to the histological structure. The latter is defined by the distribution of ^{31}P associated with cellular nuclear DNA and the phosphorylated cy-

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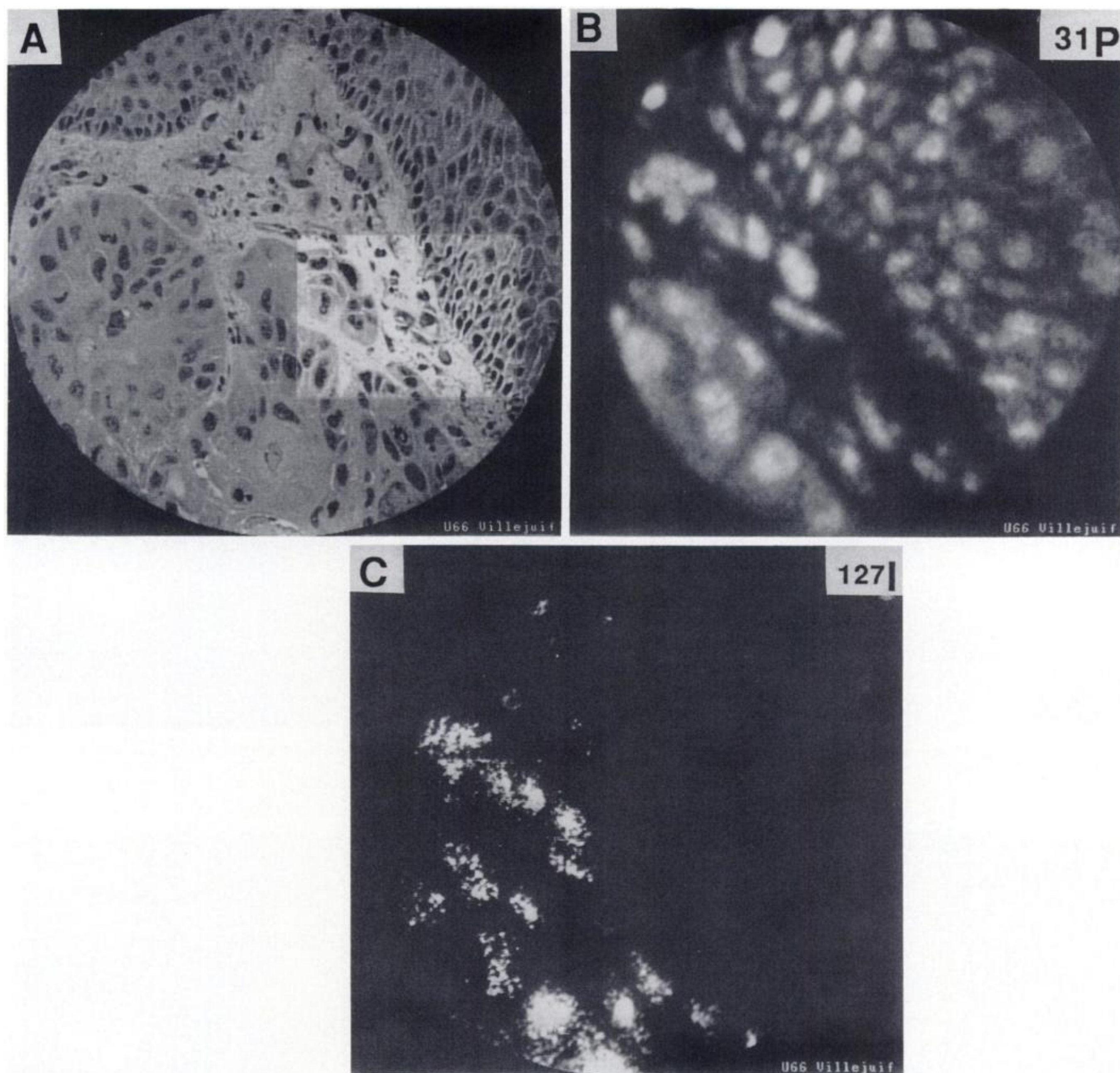

Fig. 1. Optical and ion images obtained from two serial cross-sections of MSCC. In the section observed with the optical microscope (*A*), the normal and neoplastic cutaneous tissue can be easily identified. The white area corresponds to that observed with SIMS microscopy (*B* and *C*). The histological structure of the tissue is given by ^{31}P distribution; ^{127}I in IDX is mainly detected in the tumor part of the tissue. Image field: *A*, 400 μm; *B*, 60 μm.

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toplasmic molecules. Normal and neoplastic tissue can be identified on optical serial sections and tested for ^{127}I. The intensity of ^{127}I emission appears to be stronger in tumor cells than in normal cutaneous epithelial cells. Fig. 2 shows that the nuclei of tumor cells (*A–C*) were able to concentrate IDX, and it is noteworthy that ^{127}I can be visualized in the nucleus of a mitotic cell (*D–F*), suggesting that the drug also can be bound at this step of the cell cycle. Furthermore, Fig. 3 shows that the drug is released from the blood vessels and reaches its targets: the nuclei of both endothelial and tumor cells.

IDX Quantification. Image integration time and photographic processing were optimized for the quality of the image. Because the ^{127}I image did not reflect its concentration accurately, quantitative evaluation was performed on the phosphorus image of nuclei. The overall results of ^{127}I concentration measurements in tumor cells are summarized in Fig. 4. IDX intranuclear concentration was undetectable in only 8% of the 252 nuclei measured; 90% of the values were within 1–16 ng/mg. Table 1 shows the mean ^{127}I concentration of each MSCC specimen; it ranged from 5 to 23 ng/mg. The individual values were very scattered in 3 patients (factor 100–400 between extreme values).

DISCUSSION

This study demonstrates the localization of an iodinated anthracycline (IDX) in human biopsies obtained 10 min after its infusion and confirms the preliminary results obtained with a fluorinated drug in gastric carcinoma (13). The detection which is now possible without radioactive labeling is based on the presence of a halogen atom (^{127}I or ^{19}F) inside the molecule

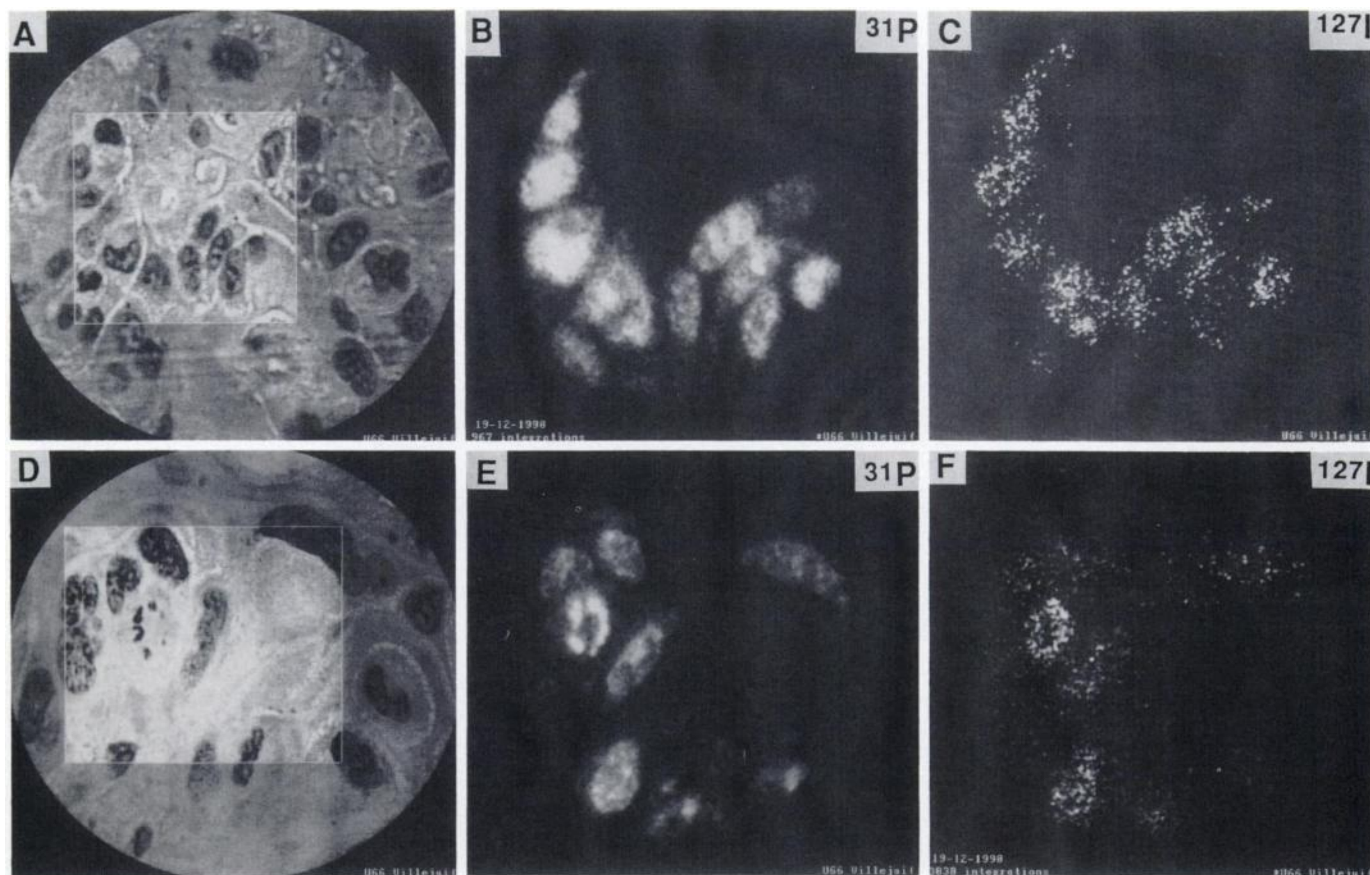

Fig. 2. IDX intranuclear localization. *A* and *D*, optical images; *B* and *E*, ^{31}P distribution. ^{127}I is mainly demonstrated inside mitotic (*F*) and nonmitotic nuclei (*C*). Image field: *A*, 150 μm; *B–F*, 60 μm.

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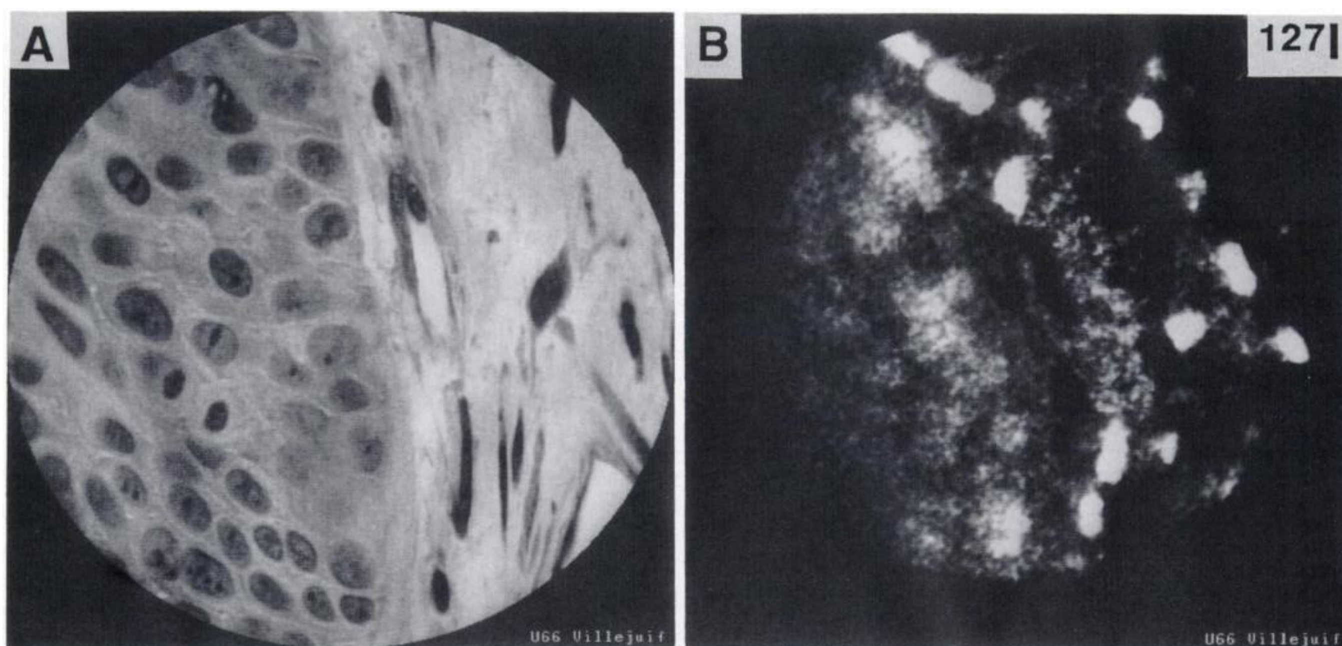

Fig. 3. IDX distribution from blood vessels to tumor. Nuclei of both endothelial and tumor cells are able to concentrate IDX during chemotherapy. *A*, optical image; *B*, ^{127}I image of the serial section. Image field, 60 μm.

which can be evidenced in histological structures by SIMS microscopy. The chemical fixation of biopsied tissue eliminates diffusible elements (9). The iodine images and countings are therefore only representative of iodine bound to macromolecules, *i.e.*, ^{127}I in IDX or its metabolite. It is noteworthy that the ^{127}I signal was mainly found in tumor cell nuclei; this suggests that the images are evidence of drug-DNA interaction.

Experimental data obtained from different human cell lines

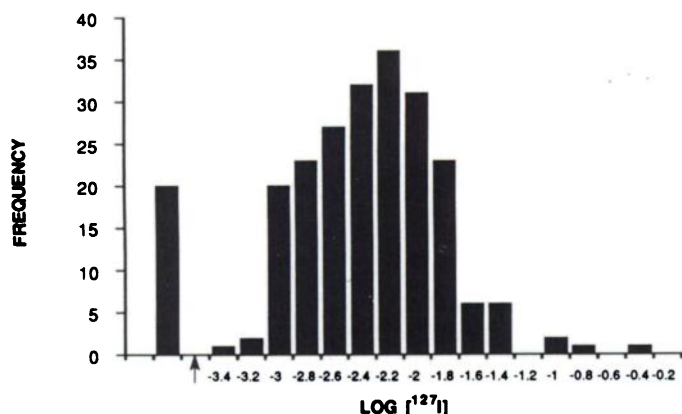

Fig. 4. Histogram of IDX concentrations in tumor cell nuclei. For each MSCC specimen, the local ^{127}I concentration was measured in at least 30 tumor cell nuclei selected on ^{31}P image with adapted apertures. Each reported value is the mean of 10 consecutive measurements on the same point. The results obtained from the 7 specimens (Table 1) are pooled. *Arrow,* detectable minimum.

Table 1 *IDX concentrations*

Patients	Age (yr)	Primary tumor (SCC)	^{127}IDX (ng/mg)[a]		
			No.	Mean ± SE	Extreme values
1	63	Oropharynx	35	16 ± 6	1–179
2	62	Larynx	33	5 ± 1	1–20
3	61	Skin	36	9 ± 1	1–37
4	58	Mouth floor	30	11 ± 1	4–33
5	47	Urethra	30	20 ± 7	0–119
6	46	Oropharynx	38	9 ± 2	0–45
7	63	Oropharynx	30	23 ± 13	1–400

[a] Calculated with the detectable values.

indicate that the measurement of the IDX concentration in the cell nuclei appears to be an effective measure of growth inhibition due to the anthracycline (14). One of the major advantages of SIMS microscopy is its capacity to quantitatively measure elements such as ^{127}I in IDX on histological specimens without cell disruption. Our data show that the mean of IDX concentration varies by a factor of approximately 5 in the different specimens, with a large scattering of the values in 3 of them (Table 1). Although it is difficult to draw conclusions of the clinical significance based on the measurements performed in 7 patients with advanced disease, our data raise the question of interest of quantitative drug mapping with regard to clinical response. This information would complement that given by high-performance liquid chromatography plasma evaluation.

Numerous mechanisms of chemoresistance of tumor cells have been evoked (15). One of them and perhaps one of the most important might be the absence of drug penetration which can now be explored at the cellular level through SIMS microscopy *in vivo* and in clinical studies. Our methodological approach combines the results of the ionic and photonic microscope. Thus, it is possible to correlate in a given tissue inadequate drug concentration with the expression of the multidrug resistance gene revealed by immunohistochemistry and/or by *in situ* hybridization. Because cryopreparation techniques (16) make it possible for the different microscopic imaging methods to use serial sections of the same embedded specimens (17), we should be able to achieve a better evaluation of successful treatment.

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