Molecular Pharmacological Differences between Carminomycin and Its Analog, Carminomycin-11-methyl Ether, and Adriamycin

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ABSTRACT

The effects of carminomycin and its 11-methyl ether analog were characterized and compared with those of Adriamycin in several systems both in vivo and in vitro. When comparing maximum effective doses, carminomycin was found to be approximately 10- to 20-fold more potent in vivo than was Adriamycin against mouse L1210 leukemia, and the latter agent was approximately equipotent with carminomycin-11-methyl ether. Similarly, 50% inhibitory concentrations of 0.09, 0.39, and 0.53 μM were obtained for carminomycin, Adriamycin, and carminomycin-11-methyl ether, respectively, using in vitro colony survival studies against Novikoff hepatoma ascites cells. This ranking was not repeated for the other systems tested. The 50% inhibitory concentrations of Adriamycin for whole cellular nucleic acid syntheses were 2- to 3-fold lower than those of carminomycin and over 10- to 15-fold lower than those of carminomycin-11-methyl ether. The apparent binding constants obtained for calf thymus DNA and salmon sperm DNA for Adriamycin were 3.67 × 10^6 and 11.68 × 10^6 M⁻¹, respectively. Those obtained for carminomycin were 0.26 × 10^6 and 0.15 × 10^6 M⁻¹, respectively, and no detectable binding was observed for carminomycin-11-methyl ether. These findings were confirmed by analysis of the effects of these anthracyclines on superhelical PM-2 DNA by agarose gel electrophoresis. Increasing concentrations of Adriamycin of up to 200 μM progressively decreased the superhelicity of PM-2 DNA in a manner typical of an intercalative binding agent, and concentrations 2- to 5-fold and 20- to 50-fold higher for carminomycin and its analog, respectively, were required to obtain equivalent results. These results demonstrate that DNA-binding and nucleic acid synthesis-inhibitory effects do not correlate with the antitumor action of carminomycin and its 11-methyl ether analog. This suggests the importance of other subcellular targets, possibly distinct from those of Adriamycin, which may be important in the cytotoxicity of carminomycin and its 11-methyl ether analog.

INTRODUCTION

CMM is an anthracycline antitumor antibiotic isolated from the mycelia of Actinomadura carminata (8, 22). Clinically, CMM has been shown to have a spectrum of activity similar to that of ADM (8). CMM is structurally similar to ADM, as indicated in Chart 1. Recently, the synthesis of CMM-OMe, shown in Chart 1, has been described (19). Previous studies have shown that ADM (7, 9, 23, 28, 40, 43) and CMM (9) inhibited DNA and RNA syntheses as well as nucleolar preribosomal RNA synthesis (9) at approximately equivalent concentrations with no significant effect on protein synthesis. Additionally, the nucleolar morphological alterations seen with CMM closely resemble the changes seen with ADM (8, 11, 29). Thus, it has been assumed that the mechanisms of action of CMM and ADM are similar.

The present study investigates the effects of CMM-OMe on macromolecular syntheses, cell viability, and in vivo tumor growth and compares these effects with those of CMM and ADM. The DNA-binding characteristics of the 3 drugs are also compared.

MATERIALS AND METHODS

Materials. Radioactive uridine and thymidine were obtained from Schwarz/Mann, Orangeburg, N. Y. Calf thymus DNA, ethidium bromide (2,7-diamo-10-ethyl-9-phenylphenanthridinum bromide), and salmon sperm DNA were purchased from Sigma Chemical Co., St. Louis, Mo. All spectrophotometric determinations were made on the Zeiss PMQ-3 spectrophotometer using 1-cm-path length quartz cuvets. The anthracycline antibiotics ADM and CMM were generously supplied by Bristol Laboratories, Syracuse, N. Y. CMM-OMe was prepared as reported previously (19) and was generously supplied by Drs. J. M. Essery and T. W. Doyle. Aqueous stock solutions of anthracyclines were prepared by wetting the drug crystals with dimethyl sulfoxide followed by addition of water or aqueous buffer. Stock drug solutions were stored at −20° and used within 2 to 4 weeks. The molar extinction coefficients, determined in methanol, for ADM, CMM, and CMM-OMe were 12,200 M⁻¹ cm⁻¹ at 477 nm, 8,200 M⁻¹ cm⁻¹ at 492 nm, and 5,500 M⁻¹ cm⁻¹ at 443 nm, respectively. The molar extinction coefficients for calf thymus DNA and salmon sperm DNA were 6,600 M⁻¹ cm⁻¹ at 260 nm for both. Spectrophotometric measurements were made on an Aminco Bowman 4-8106 spectrophotofluorometer using a 1-cm quartz cuvet. The agarose (Agarose ME) used in electrophoretic studies was obtained from Miles Laboratories Inc., Elkhart, Ind. Gels were prepared by dissolving agarose, at a final concentration of 1% (w/v), in electrophoresis buffer at 100°.

Cell Culturing. Cultured NHAC, type N.S.-73, were grown in Roswell Park Memorial Institute Type 1640 medium (RPMI-1640 supplemented with 10% fetal calf serum, 2 mM glutamine, penicillin (100 units/ml), and streptomycin A (100 μg/ml). NHAC were maintained in monolayer cultures, as previously
Effect of DNA. Fluorescence spectra of each of the anthracyclines tested were determined by exiting a 1-ml solution of 5 μM drug at the visible absorption maximum of each drug and varying the emission wavelength in the lower-energy region of each spectrum. Quenching of drug fluorescence by DNA was measured by adding μl volumes of concentrated DNA solutions to the cuvet. The dilution effect caused by addition of DNA-binding buffer to each tube, followed immediately by mixing and incubation in a 25°C water bath for 1 hr. To eliminate fluorescence due to DNA, parallel control titrations consisting of increasing concentrations of DNA in 1.0 ml of DNA-binding buffer were run for each experiment.

The DNA binding of anthracycline antibiotics was measured by spectrofluorometry in a manner analogous to that of previous studies (10, 13, 34, 36, 48). This was done by titrating fixed concentrations of anthracycline (3, 4, 5, or 10 μM) with increasing concentrations of DNA, thereby varying the DNA/drug ratios from 0 to 200. The DNA/drug ratio of 200 is taken as the end point in this titration assay with the drug considered totally bound. Increasing concentrations of DNA in a total of 0.90 ml of DNA-binding buffer were added to a series of acid-cleaned glass tubes. The binding reaction was initiated by addition of 0.10 ml of anthracycline working stock solution in DNA-binding buffer to each tube, followed immediately by mixing and incubation in a 25°C water bath for 1 hr. To eliminate fluorescence due to DNA, parallel control titrations consisting of increasing concentrations of DNA in 1.00 ml of DNA-binding buffer were run for each experiment.

The DNA binding data were analyzed by the Scatchard method (39). The Scatchard variables of r (mol of ligand bound/nucleotide) and C (the molar concentration of free antibiotic) were calculated from the fluorescence data according to the method of Peacocke and Skerrett (33). Binding parameters were determined from plots of C/r versus r, where Kapp (apparent association constant) is the negative slope and naf,p (the apparent number of binding sites per nucleotide) is the intercept of the curve with the x-axis.

**PM-2 DNA Preparation.** Covalently closed circular PM-2 DNA was isolated as previously described (38, 42). Purification was effected by centrifugation on ethidium bromide-containing cesium chloride gradients, followed by extensive dialysis against 0.15 M NaCl. Each batch of PM-2 DNA was analyzed for integrity and purity by agarose gel electrophoresis. Only samples which contained greater than 80% ccc-DNA were used in the present study.

**Anthracycline-PM-2 DNA-binding Reactions.** The reaction conditions used were identical to those used for the other DNA's, except that the total reaction volume was 0.05 ml. Triplicate experiments were run from which a minimum of duplicate agarose gel electrophoretic analyses were performed. Reactions were run for 1 hr followed by the addition of an equal volume of 75% glycerol/0.2% bromophenol blue solution and chilling on ice.

**Agarose Gel Electrophoresis.** The procedure used was as previously reported (41) except that electrophoresis was conducted in 40 mW Tris-HCl/5 mM sodium acetate/1 mM EDTA, pH 7.8, at 5 V/cm for 10 hr at room temperature, using a horizontal slab gel apparatus (Aquebogue Machine Shop, Aquebogue, N. Y.). Under these conditions, the order of anodal migration of DNA was: superhelical ccc-DNA; partially relaxed ccc-DNA; completely relaxed ccc-DNA; partially relaxed ccc-DNA; completely relaxed ccc-DNA.

**RESULTS**

**Antitumor Effects.** The 3 anthracyclines ADM, CMM, and CMM-OMe were tested against in vivo mouse L1210 leukemia, and the results are shown in Table 1. When comparing maximum effective doses, it is clear that CMM (0.8 mg/kg) was approximately 20-fold more potent than was ADM (15 to 18...
Table 1

<table>
<thead>
<tr>
<th>Material</th>
<th>Dose (mg/kg i.p.)</th>
<th>MST* (days)</th>
<th>Effective MST (%T/C)</th>
<th>Av. wt change</th>
<th>Survivors on Day 5</th>
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<tr>
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<td>207</td>
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<td>6/6</td>
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*MST, median survival time.

Effective MST: % T/C = (MST treated/(MST control) x 100.

In other experiments, the minimal effective dose of ADM was approximately 0.8 mg/kg.

In vivo antitumor activity of ADM, CMM, and CMM-OMe on L1210 leukemia

Tumor inoculum was 10⁶ ascites cells implanted i.p. Host was female C57BL/6 × DBA/2 F₁ mice. Drug was administered i.p. on Day 1 only. A % T/C of 125 was considered significant antitumor activity.

Similar results were obtained in vitro, using colony survival techniques. Chart 2 shows the results of the 3 anthracyclines tested against cultured NHAC in vitro. The results, plotted as the log of the survival fraction versus drug concentration, indicate that the order of activities of the 3 anthracyclines in this assay system was CMM > ADM = CMM-OMe. The IC5₀ values for cell viability inhibition were estimated by probit analysis of the results shown in Chart 2 with values of 0.09, 0.39, and 0.53 μM being obtained for CMM, ADM, and CMM-OMe, respectively. Thus, CMM was 4- to 5-fold more potent than ADM and 5- to 6-fold more potent than CMM-OMe in vitro against NHAC.

Effects on Macromolecular Synthesis. Chart 3 shows a probit analysis of the effects of the 3 anthracyclines on whole cellular DNA and RNA syntheses in cultured NHAC, using a filter assay method as described previously (9). The IC5₀ values obtained from Chart 3A for the incorporation of [³H]thymidine into acid-insoluble material were 8.52, 10.14, and >80 μM for ADM, CMM, and CMM-OMe, respectively. The IC5₀ values obtained for the incorporation of [³H]uridine into acid insoluble material were 2.91, 4.87, and >25 μM for ADM, CMM, and CMM-OMe, respectively. Thus, when comparing IC5₀ values, nucleic acid synthesis-inhibitory activities did not correlate with the cytotoxicity or antitumor potency of these 3 anthracyclines.

DNA-binding Characteristics. To determine the DNA-binding characteristics of these anthracyclines, DNA binding stud-
Chart 3. Effects of ADM, CMM, and CMM-OMe on the incorporation of \([3H]\)thymidine and \([3H]\)uridine into acid-insoluble material. Results were obtained using a filter assay method, as previously reported (9). Means of a minimum of triplicate determinations are shown, and the curves represent the results of duplicate or triplicate experiments. A, incorporation of \([3H]\)thymidine into acid-insoluble material. The best-fit lines were constructed, and the IC\(_{50}\) values obtained for ADM, CMM, and CMM-OMe were 8.52, 10.14, and >80 \(\mu\)M, respectively. \(p\) values of <0.001, <0.001, and <0.01, respectively, were obtained for ADM, CMM, and CMM-OMe. B, incorporation of \([3H]\)uridine into acid-insoluble material. The best-fit lines were constructed, and the IC\(_{50}\) values obtained for ADM, CMM, and CMM-OMe were 2.91, 4.87, and >25 \(\mu\)M, respectively. \(p\) values obtained for ADM, CMM, and CMM-OMe were <0.05, <0.001, and <0.05, respectively.

Chart 5 shows the results of the fluorescence titration studies using all 3 anthracyclines in the presence of increasing concentrations of DNA. The curves obtained for ADM and CMM are similar, although higher DNA concentrations were required to achieve maximum fluorescence quenching for CMM. However, for CMM-OMe, even at the highest DNA/drug ratio of 200, only 62% of the original fluorescence was quenched (versus >95% for ADM and CMM).

Chart 6 shows the Scatchard plots of the results shown in Chart 5. The \(K_D\) values, which are the negative slopes of the Scatchard curves, for ADM and CMM are \(3.67 \times 10^6\) and \(0.26 \times 10^6\) M\(^{-1}\), respectively. No binding was detected with CMM-OMe. Thus, the order of DNA-binding ability of these agents is ADM > CMM > CMM-OMe. Similar trends were seen with salmon sperm DNA, and the results of the DNA binding studies are summarized in Table 2.

Effects on Superhelical PM-2 DNA. It has been shown that upon interaction of PM-2 superhelical ccc-DNA (Form I) with intercalating agents, marked structural alterations can be demonstrated (18, 31, 37, 47). Fig. 1 shows the results of PM-2 DNA binding reactions with ADM, CMM, and CMM-OMe analyzed by agarose gel electrophoresis. A single agarose slab gel
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Chart 4. Fluorescence spectral changes of CMM (A) and CMM-OMe (B) upon interaction with salmon sperm DNA. Spectra were taken on 5 μM anthracycline solutions in 0.05 M sodium phosphate buffer (pH 6.2)/0.05 M NaCl/0.001 M EDTA. Spectra were obtained by using the specific excitation wavelengths of 490 and 445 nm, respectively, for CMM and its analog and recording drug fluorescence at wavelengths in the low-energy region of the spectrum (longer wavelengths). Increasing concentrations of DNA were obtained by addition of μ volumes of a concentrated solution of salmon sperm DNA in the above buffer.

Chart 5. Fluorescence quenching of anthracyclines in the presence of increasing concentrations of calf thymus DNA. To a series of glass tubes containing increasing concentrations of nucleic acid in DNA-binding buffer, 0.05 M sodium phosphate buffer (pH 6.2), 0.05 M NaCl, and 0.001 M EDTA, a fixed concentration of each anthracycline was added, the mixture was incubated at 25° for 1 hr, and fluorescence measurements were taken using the fluorescence parameters indicated in "Materials and Methods." The results of duplicate experiments, each of which contained duplicate or triplicate values at each DNA concentration, are shown.

is shown, on which was electrophoresed samples of all 3 anthracyclines. All lanes contain a constant amount, 1 μg, of PM-2 DNA. Lanes A through H contain increasing concentrations of ADM (0, 5, 10, 25, 50, 75, 100, and 200 μM). Lanes I through P contain identical concentrations of CMM and Lanes Q through X contain CMM-OMe corresponding to concentrations of 0, 5, 10, 25, 50, 100, 200, and 500 μM. As shown in Lanes A to H, increasing concentrations of ADM progressively decreased the electrophoretic mobility of Form I DNA, producing a diffuse pattern at concentrations > 10 μM. This diffuse pattern represents a gradient of Form I DNA molecules with different levels of relaxation. Similar results were obtained with CMM (Lanes I to P), although higher drug concentrations (> 25 μM) were required to produce diffuse patterns. This suggests differences in the DNA-binding ability of the 2 drugs. For CMM-OMe, no effect on the electrophoretic mobility of Form I DNA was seen at concentrations up to 100 μM, and diffuse patterns were produced only at concentrations greater than 200 μM. Thus, these results suggest that the order of the DNA-binding ability for the 3 anthracyclines is ADM > CMM.
synthesis-inhibitory potencies (see Chart 3). This also represents the order of nucleic acid duplicate or triplicate values at each DNA concentration. Furthermore, the nucleolar morphological changes seen with ADM than those of other anthracyclines (9, 23, 40, 46), in tissue culture cells (7, 9, 23, 28), and in vitro cell-free systems (2, 30). Its effects on nucleolar preribosomal template activity (2, 30) and an altered sensitivity to nucleases perturbation of normal nucleic acid metabolism. The existing literature has suggested a similar mechanism of action for CMM.

Although ADM was clearly more effective than were CMM and CMM-OMe against mouse L1210 leukemia, when administered in single doses (compare the maximum % T/C values in Table 1), CMM was significantly more potent than ADM when comparing maximum effective doses. Maximum effective doses were compared because of the reproducibility of these values, in contrast to the high degree of variability observed when comparing minimum effective doses. The maximum %T/C value for CMM required a 20-fold lower dose than that of ADM. Accordingly, ADM was approximately equipotent with CMM-OMe. The fact that a different ranking of these agents was obtained when the minimum effective doses were compared, indicating nonparallel dose-response relationships, is suggestive of differing underlying mechanisms of cytotoxicity.

The ordering of the antitumor potencies obtained in vivo was repeated in vitro against NHAC, when comparing IC50 values (see Chart 2). Previous studies have demonstrated similar sensitivities of these 2 cell lines to anthracycline antitumor antibiotics (14). The differences in the slopes of the dose-response curves in vitro suggest differing mechanisms for these compounds as indicated previously. While CMM was more potent than ADM both in vivo and in vitro, further toxicological studies are required to determine whether CMM is superior to ADM as a chemotherapeutic agent.

Further evidence that CMM and CMM-OMe differ mechanistically from ADM derives from studies on their inhibition of macromolecular syntheses. Studies reported here and in a previous publication (9) have shown that ADM and CMM inhibit DNA synthesis at concentrations approximately equal to those necessary to inhibit whole cellular RNA and nucleolar RNA syntheses. However, the ratios of the IC50 values for DNA synthesis to that for cell viability inhibition are 21, 112, and >160 for ADM, CMM, and CMM-OMe, respectively. Thus, the cytotoxicity of CMM and CMM-OMe is difficult to explain on the basis of nucleic acid synthesis inhibition. Clearly, CMM and CMM-OMe are mechanistically different from ADM.

The DNA-binding characteristics of these compounds were examined by using the 3 naturally occurring DNA’s, calf thymus DNA, salmon sperm DNA, and supercoiled PM-2 DNA. While concentrations of ADM as low as 10 to 25 μM were able to

![Chart 6. Scatchard plots of the binding data for the interaction of ADM, CMM, and CMM-OMe with calf thymus DNA. The Scatchard parameters r, C, and r/C were calculated from the data presented in Chart 5.](chart6.png)

**Table 2**

<table>
<thead>
<tr>
<th>Anthracycline</th>
<th>Calf thymus DNA</th>
<th>Salmon sperm DNA</th>
</tr>
</thead>
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<td></td>
<td>K&lt;sub&gt;app&lt;/sub&gt; (x 10&lt;sup&gt;6&lt;/sup&gt; M&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>n&lt;sub&gt;app&lt;/sub&gt;</td>
</tr>
<tr>
<td>ADM</td>
<td>3.67</td>
<td>0.15</td>
</tr>
<tr>
<td>CMM</td>
<td>0.26</td>
<td>0.50</td>
</tr>
<tr>
<td>CMM-OMe</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Values are obtained by linear regression analyses of composite Scatchard curves obtained from 2 or more separate experiments, each of which contained duplicate or triplicate values at each DNA concentration.
* Values are obtained as for K<sub>app</sub>.
* ND, not detectable.

DISCUSSION

The results of a number of studies have demonstrated that ADM rapidly inhibits both RNA and DNA syntheses in vivo (12, 23, 40, 46), in tissue culture cells (7, 9, 23, 28), and in vitro cell-free systems (2, 30). Its effects on nucleolar preribosomal RNA synthesis have also been reported (9). Fewer reports exist for the effects of CMM on nucleic acid syntheses; however, the results obtained have suggested a similarity of action for the 2 agents (8, 9). Furthermore, the nucleolar morphological alterations seen with CMM more closely resemble those changes seen with ADM than those of other anthracyclines (9, 11, 29).

Many studies examining the anthracycline-DNA interaction specificities have been reported (13, 15, 16, 21, 31, 35, 45, 48). A major portion of this interaction involves an intercalative mechanism (25) as demonstrated by a variety of studies (21, 31, 35, 45, 47). The results of this interaction are a decreased template activity (2, 30) and an altered sensitivity to nuclease activity (20, 45). Thus, the mechanism of action of ADM is thought to be contributed to largely by DNA binding and a subsequent perturbation of normal nucleic acid metabolism. The existing
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Fig. 1. Agarose gel electrophoretic separations of anthracycline-PM-2 DNA reaction products. Reactions were performed as indicated in "Materials and Methods," and agarose gel electrophoresis was conducted as previously reported (31, 41). Direction of electrophoresis is from top to bottom with the fastest migrating band being the superhelical PM-2 ccc-DNA and the slowest migrating band (faintly visible) being the relaxed form of DNA. Lanes A through H correspond to increasing ADM concentrations of 0, 5, 10, 25, 50, 75, 100, and 200 μM, respectively. Lanes I through P correspond to identical concentrations of CMM. Lanes Q through X correspond to increasing CMM-OMe concentrations of 0, 5, 10, 25, 50, 100, 200 and 500 μM, respectively. Electrophoresis was at 5 V/cm for 10 hr at room temperature. Gels were stained with ethidium bromide. 0.5 μg/ml. induce conformational alterations in superhelical PM-2 DNA, concentrations 2- to 5-fold higher for CMM and 10- to 50-fold higher for CMM-OMe were required to obtain equivalent results (Fig. 1). Scatchard analyses of the interactions of these agents with calf thymus DNA and salmon sperm DNA revealed a similar ordering of the DNA-binding abilities (see Table 2). Therefore, a correlation exists between the DNA-binding abilities and the nucleic acid synthesis-inhibitory activities of these anthracyclines. Thus, CMM and CMM-OMe are much more potent antitumor agents than can be accounted for on the basis of their DNA-binding characteristics.

The present study raises interesting questions regarding the subcellular targets of the antitumor action of CMM. Several sites of action of ADM have been described, including the cytoplasmic membrane (23, 32) and mitochondria (1, 3, 4, 24, 44). While the majority of cell-associated ADM was associated with the cell nucleus (23, 40), recent findings indicate that cell-associated CMM is cytoplasmically localized (17). Studies have shown that ADM affects mitochondria in a number of ways, including superoxide generation (44), perturbation of the electron transport system (3, 4, 24), and inhibition of the synthesis of cytochromes a and a3 (1). Kishi et al. (24) have demonstrated that ADM inhibits bovine heart mitochondrial succinoxidase and NADH oxidase systems and that this inhibition can be prevented by addition of several forms of CoQ. It was further shown that daunomycinone was significantly less inhibitory than was adriamycinone, indicating that the hydroxyl group of the latter may be important for receptor binding. Since CMM is also a close structural analog of ADM, contains a hydroxyl group in place of the methoxy group at position 4, and appears to be localized in the cytoplasm (17), it is possible that CMM may bind preferentially to the putative mitochondrial receptor. The result would be a preferential perturbation of mitochondrial energy metabolism by CMM. The fact that CMM-OMe has markedly reduced DNA-binding activity but retains significant antitumor activity suggests that the 11-methyl ether function may sterically hinder nucleic acid binding while its interaction with the putative mitochondrial receptor may not be altered significantly.

Alternatively, CMM and its analog may act at the cell membrane as suggested for ADM (23, 32). However, preliminary studies on membrane synthesis have failed to demonstrate significant activity for CMM or CMM-OMe. Moreover, neither CMM nor CMM-OMe was shown to produce significantly greater degradation of PM-2 DNA than was shown by Lown et al. (27) for ADM. Thus, the possible mechanism(s) of action of these 2 agents remain speculative.

That minor structural modifications result in significant changes in the molecular pharmacology of anthracyclines has become increasingly evident and suggests that there are multiple mechanistic classes of anthracyclines.

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REFERENCES


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