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Journal of Pharmaceutical and Biomedical Analysis
17 (1998) 785–792

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

The detection of photodegradation products of irinotecan (CPT-11, Campto®, Camptosar®), in clinical studies, using high-performance liquid chromatography/atmospheric pressure chemical ionisation/mass spectrometry[1]

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Received 3 November 1997; accepted 12 November 1997

Abstract

A method for the detection of the photodegradation products of irinotecan (CPT-11, Campto®, Camptosar®) was developed using high-performance liquid chromatography (HPLC) with fluorescence detection and HPLC/atmospheric pressure chemical ionisation/mass spectrometry (HPLC/APCI/MS). Remnants of infusion solution as well as samples of urine and plasma collected at the end of the infusion of CPT-11 to cancer patients were screened for the five principal known photodegradation products (PDPs) of CPT-11. The concurrent use of standards of the PDPs with ion-extract HPLC/APCI/MS chromatograms enabled the identification of trace quantities of two PDPs in most samples analysed. However, similar analyses of fresh clinical drug solutions revealed that the PDPs were not generated significantly by exposure to light during the infusion period, but were already present in the drug ampoules. Furthermore, this appears to be the source of traces of PDPs detectable in urine and plasma of patients rather than metabolism, per se.

Keywords: Irinotecan; Camptothecin; CPT-11; High-performance liquid chromatography; Atmospheric pressure chemical ionisation mass spectrometry; Photodegradation

1. Introduction

Irinotecan (CPT-11, Campto®, Camptosar®, Fig. 1) is a novel anti-cancer drug recently marketed in the US and Europe with significant activity against a range of malignancies. Recently, it was demonstrated that CPT-11 undergoes facile photodegradation in a pH-dependent manner in aqueous solutions exposed to laboratory lighting

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[1] Presented in part at the 88th Annual Meeting of the American Association for Cancer Research, San Diego, CA, 12–16 April, 1997.

0731-7085/98/$19.00
PII S0731-7085(97)00256-2

[1]. Of some concern was the observation that drug solutions made up in 0.9% saline were among the least stable of those tested. The five principal photodegradation products (PDPs) isolated in these prior studies [1] have unknown toxicological profiles. Therefore, given that therapy with CPT-11 is associated with a number of ill-characterised toxic side-effects, it was apparent that a study on the occurrence of photodegradation products of CPT-11 during routine administration to patients was urgently required.

In addition, some recently postulated metabolites of CPT-11 [2] have structures which correspond to the PDPs isolated in in vitro experiments (Fig. 1). Therefore, a secondary aim of this study was to identify whether these compounds are formed in vivo, or are more likely to be artifacts resulting from the photodegradation of CPT-11 and its principal metabolites in samples exposed to light.

In this paper, we describe the development of highly sensitive and specific high-performance liquid chromatography (HPLC) and HPLC/atmospheric pressure chemical ionisation/mass spectrometry (HPLC/APCI/MS) methods of detection of the PDPs of CPT-11. These were then used in the screening of pharmaceutical and biological samples for evidence of photodegradation.

2. Experimental

2.1. Chemicals

CPT-11 was obtained from Rhône–Poulenc Rorer (Neuilly, France). Water was double-distilled and deionised (Milli-Q, Millipore, Milford, USA) and all other chemicals were of analytical grade or above. All HPLC solvents were filtered and degassed using a 0.45 μm filtration system (Millipore).

2.2. Sample collection

Photodegradation of CPT-11 was studied during administrations ($n = 5$) of CPT-11 (300–350 mg m^{-2}) to subjects as either in- or out-patients at the Institut Bergonié, Bordeaux, France. The study had institutional clearance and patients gave written informed consent. The pharmacy was informed that a drug stability study was in progress but that normal routine procedures should be used throughout. CPT-11 (Campto ®, Rhône–Poulenc Rorer) in 2 ml (40 mg) or 5 ml (100 mg) ampoules was diluted into 250 ml bags of sterile 0.9% saline for administration to patients. The ampoules used to prepare the infusion were collected. At the end of the 60 or 90 min infusion, the infusion set and its contents were salvaged and some of the remaining CPT-11 solution was collected and immediately stored at −20°C in tubes protected with metal foil. Samples of plasma and urine were collected from the patients as soon as possible following the infusion and stored likewise.

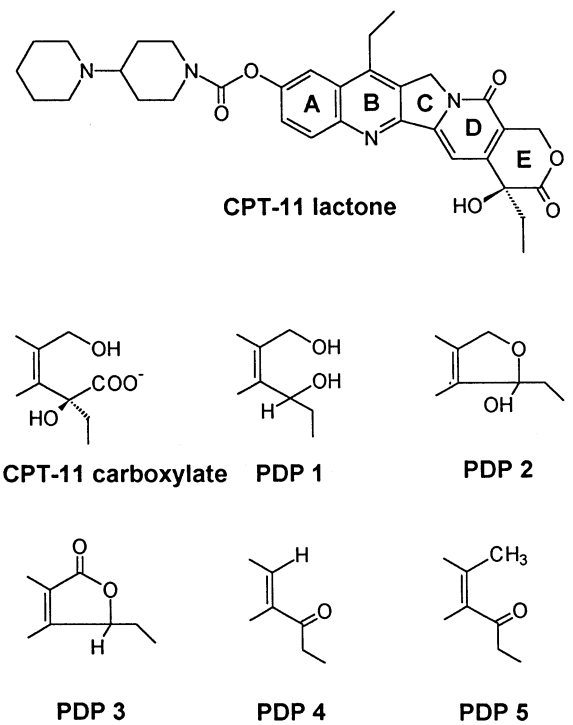

Fig. 1. Structural formulae of CPT-11 in its lactone and carboxylate forms, and the five principal photodegradation products (PDPs).

2.3. Sample preparation

For each analysis of PDPs in infusion set fluid, fresh and equivalent dilutions of the remaining clinical stock in the matching ampoules were carried out in 0.9% saline. Samples (1 ml) were diluted with the addition of 3 ml of 0.05 M citric acid and applied to solid phase extraction columns (Sep-Pak Vac, 3 cc C18, Waters, Milford, USA) which had been conditioned with 5 ml acetonitrile (ACN), followed by 5 ml *dd* H_2O and finally 5 ml 0.05 M citric acid. The loaded columns were washed with 0.05 M citric acid and compounds of interest eluted with 3 ml ACN–0.1 N HCl (95:5 v/v). The eluants were evaporated to dryness under N_2 and the residue reconstitued in mobile phase (50 μl), of which 45 μl was injected into the HPLC/APCI/MS system. Samples were always protected from light. Plasma (1 ml) and urine samples (3 ml) were treated by the addition of an equal volume of a 50:50 mixture (v/v) of cold methanol and ACN and the supernatant ($8000 \times g$) was dried down under N_2. The residue was reconstituted in 1 ml of 0.05 M citric acid and extracted as per the infusion fluid samples.

2.4. HPLC analysis

The HPLC separation of the compounds of interest was carried out using a method published previously [1]. Briefly, a Waters 616 HPLC pump and 600S controller were used to deliver a gradient of mobile phase using 0.15 M ammonium acetate buffer (pH 5.2)-ACN-isopropanol (77:20:3 v/v/v) and 100% ACN at a flow rate of 1.4 ml min^{-1} to a Waters Radial-Pak column (8×150 mm, Nova-Pak phenyl, 4 μm). Samples were injected with an ISS200 sample processor (Perkin–Elmer, Danbury, USA) and the column effluent was monitored with a Hitachi F1000 fluorimeter (Scientific Instruments-Optical Sales, Australia) with excitation and emission wavelengths set at 355 and 515 nm, respectively. Peak data were collected and analysed using Maxima software (Waters).

2.5. HPLC/APCI/MS spectrometry

HPLC/APCI/MS analysis was carried out using the HPLC method described above. In this case however, a fraction of the column outflow (1/6th) was diverted to a Sciex API III triple quadrupole mass spectrometer (Thornhill, Canada) equipped with an APCI interface. The temperature of the interface and the orifice potential were 500°C and 50 V, respectively and the nebuliser gas pressure and auxillary N_2 flow were 4.8 atm and 2 ml min^{-1}, respectively. Total ion count (TIC) data were collected in positive ionisation mode for compounds with mass-to-charge ratios (m/z) ranging from 350 to 800. The mass spectrometer data were analysed with MacSpec software (Perkin–Elmer, Danbury, USA). The delays in the fluorescence detector and mass spectrometer were nearly identical and the traces from both instruments could be overlaid directly to assist in the identification of unknown fluorescent compounds.

3. Results

3.1. HPLC/APCI/MS analysis

The order of elution of the five PDPs and CPT-11 with the HPLC/APCI/MS method is shown in Fig. 2 as a TIC trace along with the ion chromatogram extracts corresponding to their $[M+H]^+$ ions (Fig. 2(B)). As can be seen, the combination of retention time and identification of m/z allows for very specific detection of the PDPs.

3.2. Photodegradation during administration

Analysis of infusion set fluid and freshly diluted clinical stock revealed a number of minor trace contaminants (Fig. 3). Two of these corresponded to PDP3 and PDP5, based on their retention times and m/z (557 and 543, respectively). Although these represented only trace contaminants of CPT-11 ($< 0.2\%$) based on either fluorescence or UV absorption (254 nm), they were present in measurable amounts (Table 1). The paired analy-

Table 1
Dose, infusion parameters and results in peak areas of PDPs and CPT-11 in samples from infusion sets and matching controls ($n = 5$)

Infusion No.	Dose Rate (mg m^{-2}) [Infusion time (min)]	[CPT-11] in infusion (mg ml^{-1})	Infusion set pH	Compound	HPLC Peak areas (Volts $\times 10^7$)	
					Control	Infusion
1	300 [90]	1.88	3.8	CPT-11	>420[a]	>380[a]
				PDP3	7.0	4.5
				PDP5	1.8	0.9
2	300 [90]	2.40	3.8	CPT-11	401[a]	>366[a]
				PDP3	10.8	8.4
				PDP5	3.3	2.4
3	300 [90]	2.40	4.1	CPT-11	>327[a]	>298[a]
				PDP3	8.6	15.8
				PDP5	2.6	2.2
4	350 [60]	2.10	3.8	CPT-11	>382[a]	>339[a]
				PDP3	8.6	6.6
				PDP3	8.6	6.6
5	350 [90]	1.8	3.9	CPT-11	>357[a]	>243[a]
				PDP3	7.6	2.2
				PDP5	2.1	0.4

[a] Detector response was saturated.

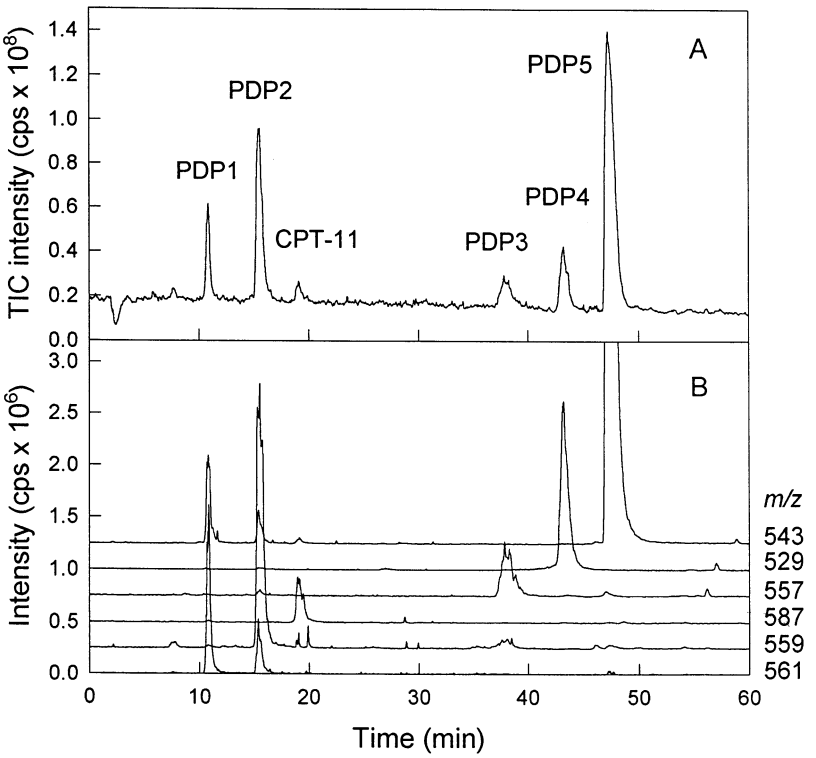

Fig. 2. Total ion count (TIC) trace (A) and ion extract chromatograms from TIC (B) obtained with the HPLC/APCI/MS analysis of the five degradation products and CPT-11.

sis of samples revealed that the concentrations of the two measurable PDPs were not increased in the infusion fluid relative to the freshly prepared control. SN-38 (m/z 393), an active metabolite and synthetic precursor of CPT-11 [3] was barely detectable in the infusion samples. An additional fluorescent impurity eluting prior to CPT-11 (Fig. 3) was found at 13.2 min with m/z of 603.

3.3. Detection of PDPs in plasma and urine

As observed previously [2,4], samples of urine and plasma collected at the end of the period of infusion of CPT-11 are rich in fluorescent species, of which most are presumably metabolites (Fig. 4(A) and 5(A)). The HPLC/APCI/MS methodology enabled us to screen the samples for the presence of PDPs even in the presence of numerous fluorescent metabolites. As can be seen from Fig. 4(B) and 5(B), there were only traces of PDP5 in plasma and PDP3 and PDP5 in urine, respectively. Of the remaining compounds, two could be readily identified as SN-38 and the aminopentanoic metabolite of CPT-11 (APC) [4]. The compounds were identified on the basis of retention time (relative to pure standards, data not shown) and m/z values of 393 and 619, respectively. Most of the remaining compounds would appear to be oxidation products of CPT-11 and produce ions with m/z values of 519 and 603 [2,4] (Fig. 5C).

4. Discussion

Although it has been revealed that CPT-11 photodegradation occurs readily in vitro, we expected the extent of loss of CPT-11 to be relatively small over the period of time of the average infusion period to patients (60–90 min). For this

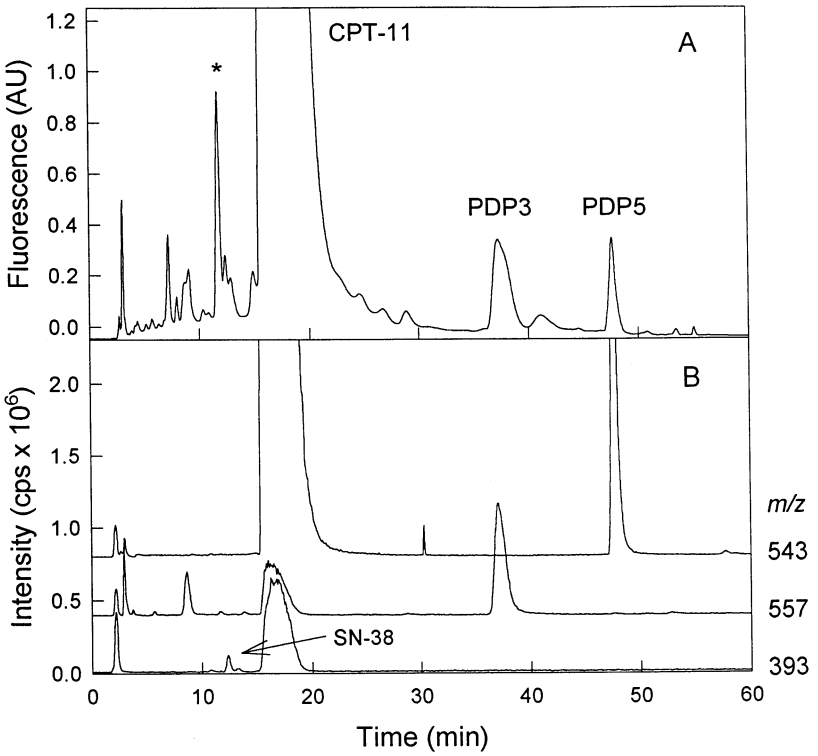

Fig. 3. Fluorescence (A) and ion chromatogram extracts of PDPs from the corresponding TIC trace (B) obtained with the HPLC/APCI/MS analysis of an extract of 1.0 ml infusion set. The peak eluting prior to CPT-11 in (A), indicated by the asterisk, was found to have an m/z of 603. SN-38 (m/z 393), as shown in (B), is present only in trace quantities.

reason, we developed the method presented above as a highly specific and sensitive method for the detection of the generation of photodegradation products of CPT-11. We attempted initially to develop a method using HPLC/electrospray ionisation/MS but were unable to optimise the chromatographic separation of the PDPs with conditions suitable for that type of interface (namely low buffer salt concentration and high organic content). A major advantage with using the APCI interface is that it was possible to use a previously optimised gradient HPLC method with no significant modification [1].

Under the conditions of routine administration there was no evidence of systematic PDP production when the analyses of the infusion fluid were compared to fresh dilutions of the corresponding clinical stock. The variability in the areas observed is likely to be due to the fact that we could not exactly reproduce the composition of the clinical infusion.

The lack of appreciable photodegradation in this study in comparison to our previous in vitro study [1] is likely to have been due to a number of factors, including short exposure time, moderate light intensity and self-quenching of concentrated solutions. In addition, the photodegradation of CPT-11 is pronounced in solutions with pH >6.0 [1]. Infusion solutions of CPT-11 are prepared using an acid-buffered clinical stock to prevent lactonolysis [5,6]. Because the patients studied here were receiving 300–350 mg m^{-2} of CPT-11 in 250 ml pouches of saline, the infusion solutions were relatively concentrated and acidic (Table 1). Therefore, a major protective effect is likely to have been afforded by the resulting low pH of the infusion solution.

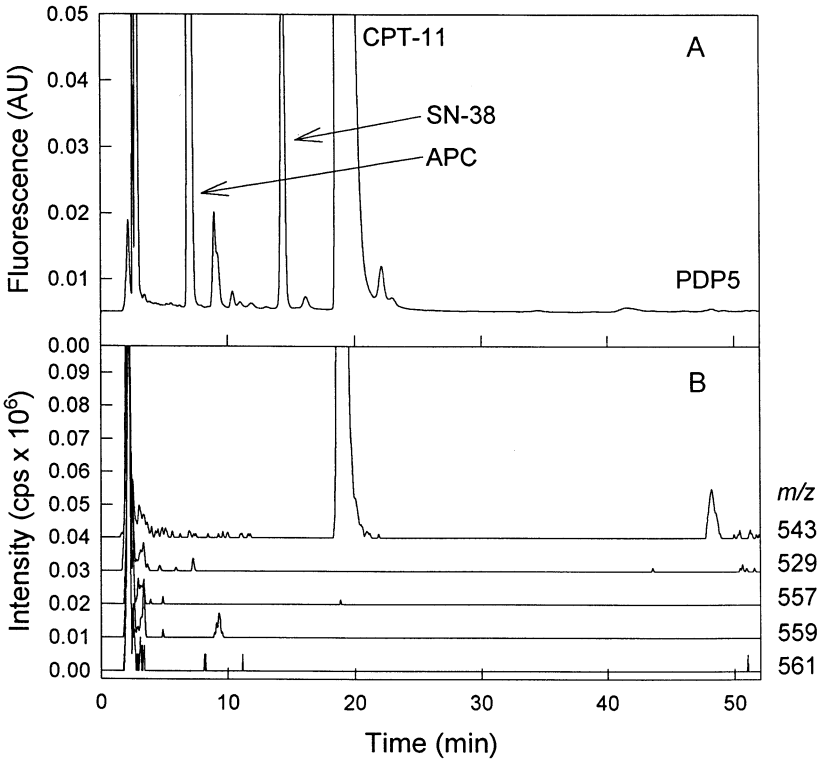

Fig. 4. Fluorescence (A) and ion chromatogram extracts of PDPs from the corresponding TIC trace (B) obtained with the HPLC/APCI/MS analysis of an extract of 1.0 ml plasma. PDPs were largely absent with the exception of trace amounts of PDP-5.

Despite the lack of photodegradation encountered in the present study, institutions using lower doses of CPT-11 (125 mg m^{-2} is the currently recommended dose in the US), larger pouch volumes or prolonged infusion protocols should consider protecting the drug solution from exposure to light. This may be particularly important in situations where there is a strong likelihood of exposure of the infusion bag to direct or even glass-filtered sunlight. Unfortunately, the exact impact of this can not be deduced from the current study in which most lighting was artificial.

Finally, traces of PDP3 and PDP5 could be detected in samples of plasma and urine collected from patients soon after the infusion period. Given that they were also present in the infusion fluid, it is unlikely that they represent metabolites of CPT-11, although this cannot be completely excluded.

5. Conclusions

We developed an analytical method using HPLC/APCI/MS to screen pharmaceutical and biological samples for the presence of the photodegradation products of CPT-11. Although two PDPs could be easily detected in many samples, we found these to be present in the starting clinical solution as trace contaminants. We conclude that there was no significant photodegradation of CPT-11 during its administration to these patients and that the presence of PDPs in plasma and urine is consistent with their inadvertent administration rather than metabolism in vivo.

Acknowledgements

H.M. Dodds and L.P. Rivory are the grateful recipients of Dora Lush (National Health and

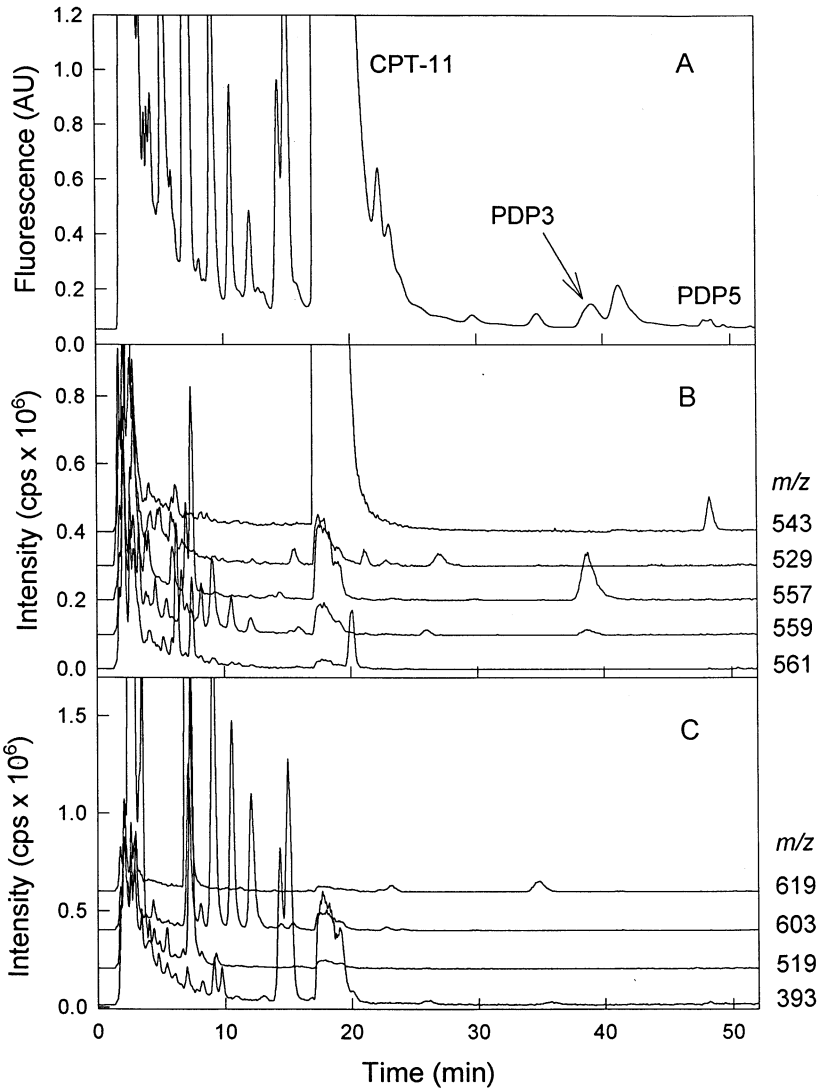

Fig. 5. Fluorescence (A), ion chromatogram extracts of PDPs (B) and metabolites (C) from the corresponding TIC trace obtained with the HPLC/APCI/MS analysis of an extract of 3.0 ml urine.

Medical Research Council) and Lions Kidney and Medical Research Fellowships, respectively. This study was also supported in part by the Princess Alexandra Hospital Research Foundation.

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