Upsala J Med Sci 113 (2): 171–180, 2008

Relative Proportions of Serum Carbamazepine and Its Pharmacologically Active 10, 11-Epoxy Derivative: Effect of Polytherapy and Renal Insufficiency

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Abstract

Background: The proposed action mechanism and pharmacological activity of carbamazepine (CBZ) and its major metabolite, carbamazepine-10,11-epoxide (CBZE), are the same. The aim of our study was the investigation of the effect of concomitant antiepileptic treatment and renal insufficiency on the relative proportions of serum CBZ and CBZE.

Methods: Serum trough steady-state CBZ and CBZE concentrations were determined by high-performance liquid chromatography (HPLC) in 140 epileptic patients treated with CBZ in monotherapy (n=100) and polytherapy with phenytoin, phenobarbital and valproate (n=40). The levels of CBZ were also determined using the Dade Behring enzyme multiplied immunoassay technique (EMIT). The glomerular filtration rate (GFR) was estimated from serum cystatin C using the Dade Behring nephelometric immunoassay.

Results: The CBZE/CBZ and CBZ+CBZE/CBZEMIT ratios were significantly increased in 7 cases (3 in monotherapy and 4 in polytherapy) with GFR<60 mL/min/1.73m^2 in relation to the patients treated in monotherapy or polytherapy having normal or mildly decreased renal function ($p<0.001$).

Conclusions: In patients with moderate to severe renal insufficiency the relative proportion of CBZE with respect to the parent drug is significantly increased. In these cases, the CBZ concentrations obtained using the EMIT, or other immunoassays having low CBZE cross-reactivity, may have an inadequate diagnostic efficiency.

Introduction

Carbamazepine (CBZ) is one of the most widely prescribed drugs for the treatment of childhood and adult epilepsies. In humans only 2% of the dose is excreted as unchanged drug, and the biotransformation pattern of CBZ is varied and complex (1). The principal pathway of CBZ metabolism is oxidation to the pharmacologically active carbamazepine-10,11-epoxide (CBZE), and then hydration to inactive *trans*-carbamazepine-10,11-diol (CBZD) (1,2). The metabolisation of CBZ to CBZE is catalysed by the cytochrome P450 (CYP) isoforms CYP3A4 and CYP2C8, and the hydrolysis of CBZE to CBZD is catalysed by a microsomal epoxide hydrolase (3). The corresponding glucuronide derivatives, and also unconjugated CBZ, CBZE and CBZD, have been characterized in urine from patients treated with CBZ (2). The

Key words: carbamazepine; carbamazepine-10,11-epoxide; renal insufficiency; EMIT; HPLC; therapeutic monitoring.

Received 16 January 2008
Accepted 28 January 2008

renal clearances of CBZ and CBZE, about 1 mL/min and 8 mL/min respectively, are urine flow dependent, and CBZD clearance is greater than that of creatinine and relatively independent of urine flow (4). These low CBZ and CBZE clearance values and their urine flow dependence indicate that glomerular filtration is the main mechanism of renal elimination for both compounds (4). Concomitant administration of enzyme-inducing anticonvulsant drugs (phenobarbital and phenytoin) or valproic acid, albeit through different mechanisms, significantly increase the relative proportion of CBZE in serum (5–7); however, to our knowledge, the possible effect of renal insufficiency on the relative proportions of CBZ and CBZE has not been still well studied.

Drug metabolites with pharmacological activity are candidates for monitoring, although in clinical practice the metabolites that are routinely measured are those for which a convenient method is available (8). Determination of CBZ is usually carried out using different commercial immunoassays, and the active CBZE metabolite is not routinely monitored together with CBZ as its measurement requires a high-performance liquid chromatography (HPLC) method (8). The cross-reactivity against CBZE of the different immunoassays currently used for routine CBZ determination varies considerably (9–13), and the enzyme multiplied immunoassay technique (EMIT) has a low cross-reactivity with the CBZE metabolite (11,14–16). The main aim of our study was to investigate the comparative effect of concomitant antiepileptic treatment and renal insufficiency on the relative proportions of CBZ and its epoxy derivative in serum, and its possible implications on the CBZ determination using EMIT.

Patients and methods

A group of 140 epileptic patients (73 males and 67 females) with a mean age of 33.8±2.0 years (range 3–88 years), and treated with CBZ in monotherapy (n=100) and polytherapy (n=40), was studied. In the cases of polytherapy, the concomitantly administered antiepileptic drugs were phenobarbital (n=9), phenytoin (n=14) and valproic acid (n=18). Daily CBZ administration was always given in multiple doses, and blood samples were taken at least after a 2 month period without any modification of the dosage, and immediately before the first corresponding daily dose. As a result, the CBZ and CBZE serum concentrations correspond to the steady-state trough levels. The study was carried out according to the good practice rules for the investigation in humans of the Conselleria de Sanidade-Xunta de Galicia, and all patients provided their written informed consent to participate.

Serum levels of CBZ and CBZE were determined by HPLC using an Agilent 1100 Series Chromatographic System. The assays were carried out using an isocratic system with UV detection at 204 nm, a detection limit of approximately 0.5 mg/L for CBZ and CBZE, and intra-and inter-assay variation coefficients of less than 5%. Likewise, serum concentrations of CBZ were determined using the EMIT 2000 carbamazepine assay in a V-Twin analyzer (Dade Behring). Serum samples

were stored for less than 7 days at -25 ºC, conditions that provide adequate stability for the parent drug and its epoxide derivative (17). Total CBZ clearance (Clt) was calculated using the expression (18): Clt=F(Dose/τ)/Css, where F is the bioavailability (0.8), τ the dosing interval, and Css the steady-state trough serum concentration. Cystatin C was determined using the Dade Behring particle-enhanced nephelometric immunoassay on the BN ProSpec nephelometer system, and the glomerular filtration rate (GFR) was estimated from serum cystatin C according to Hoek et al (19). In order to estimate the GFR from serum creatinine, the six-variable Modification of Diet in Renal Disease (MDRD) formula (20) was used. Serum albumin, creatinine and urea concentrations, were determined in an Advia 1650 analyzer (Siemens Medical Solutions).

The Microsoft Excel package (v 5.0) was used to statistically analyse the data, and the Kolmogorov-Smirnov test was applied to check for normality. Pearson's correlation coefficient was used when the data had Gaussian distributions; otherwise, Spearman's correlation coefficient was used. The regression study was made using the Passing-Bablock method, and the ma68 value was used as error of the estimate. According to the consensus validation criteria of analytical methods for quantitative determination of drugs and their metabolites in a biological matrix (21,22), the acceptance criterion for EMIT accuracy was a deviation of no more than 15% from the nominal (HPLC) value. The results were expressed as mean±SEM (median).

Results

Seven of the 140 patients studied (3 in monotherapy and 4 in polytherapy), with a mean (±SEM) age of 78.3±2.7 years (range: 76–88 years), presented an estimated GFR from serum cystatin C <60 mL/min/1.73m^2, which in accordance with the National Kidney Foundation guidelines indicates a moderate to severe kidney function impairment (22). In these 7 cases, the mean GFR value estimated from serum cystatin C (19) was 41.97±6.29 mL/min/1.73m^2 (range 19.3–57.5 mL/min/1.73m^2), and from serum creatinine using the six-variable MDRD formula (20) was 43.15±8.40 mL/min/1.73m^2 (range 12.8–61.0 mL/min/1.73 m^2), with a correlation coefficient between them of r=0.963 (p<0.005).

As shown in Figure 1A, the relative proportion of CBZE with respect to CBZ was significantly higher in the patients who had GFR values of less than 60 mL/min/1.73m^2, independently of whether they were treated in mono- or polytherapy. Furthermore, in these patients the CBZ+CBZE/CBZ$_{EMIT}$ ratios were significantly higher than in the patients with better renal function (Figure 1B); however, the CBZ$_{HPLC}$/CBZ$_{EMIT}$ ratio does not appear to be significantly altered by the patients' GFR, as indicated in Figure 1C. A highly significant correlation was found for the total number of patients studied between the CBZE/CBZ and CBZ+CBZE/CBZ$_{EMIT}$ ratios (r=0.774, p<0.001). These results are in accordance with the low cross-reactivity of the EMIT against to CBZE previously described (11,14–16).

Figure 2 shows the regression and correlation found between the results obtained by EMIT for CBZ and the results obtained by HPLC for CBZ (Figure 2A) and CBZ+CBZE (Figure 2B). The difference between the means (medians) obtained by EMIT and HPLC for the CBZ concentration, 8.20±0.24 mg/L (8.0 mg/L) and 7.66±0.23 mg/L (7.30 mg/L) respectively, is acceptable in accordance with the validation criterion used (21,22). The degree of renal insufficiency does not appear to affect the dispersion between the values for CBZ obtained by EMIT and HPLC (Figure 2A), although on considering the values of CBZ+CBZE, the deviation is higher in cases with a GFR<60 mL/min/1.73m^2 (Figure 2B).

The results obtained for the concentrations of CBZ and CBZE in the patients with GFR greater than 60 mL/min/1.73m^2 (n=133) are shown in Table 1. In accordance with our previously published results (10,13), the mean CBZE/CBZ ratios in the patients with concomitant valproic acid (0.40±0.04) or phenobarbital and phenytoin (0.41±0.04) were analogous. The difference between the means (medians) of the concentrations obtained for CBZ by EMIT and HPLC is acceptable in accordance with the validation criterion used (21,22); however, the sum CBZ+CBZE has a difference higher than 15% with respect to the parent drug levels obtained using EMIT or HPLC. As would be expected, the CBZE/CBZ ratio and CBZ Clt were significantly higher in the patients treated in poytherapy ($p<0.001$), and a highly significant correlation was found between both pharmacokinetic variables ($r=0.420$, $p<0.001$). In the 7 patients having GFR <60 mL/min/1.73m^2, the levels of CBZ and CBZE were respectively 5.91±1.03 mg/L (5.2 mg/L) and 5.29±1.11 mg/L (6.1 mg/L), with a CBZE/CBZ ratio of 0.94±0.18 (0.91) significantly greater than those obtained for the patient groups with normal renal function treated in mono or polytherapy ($p<0.001$).

Discussion

In our patients, the estimation of the GFR was carried out using serum cystatin C according Hoek et al (19). Cystatin C has been proposed as a good GFR marker, as this low-molecular protein is produced at a constant rate regulated by a "house-

Table 1. Serum levels of carbamazepine, carbamazepine-10,11-epoxide, and total clearance of carbamazepine in the patients with glomerular filtration rate greater than 60mL/min/1.73 m^2

	n	CBZEMIT (mg/L)	CBZHPLC (mg/L)	CBZE (mg/L)	CBZE/ CBZ	Clt CBZ (mL/min)
Monotherapy	96	8.61±0.26 (8.40)	8.10±0.25 (7.80)	2.06±0.10 (1.90)	0.25±0.01 (0.24)	50.83±1.67 (50.16)
Polytherapy	37	6.95±0.51 (6.80)	6.40±0.50 (6.30)	2.30±0.14 (2.30)	0.40±0.03 (0.40)	87.33±8.00 (85.50)

The results are expressed as mean±SEM(median)

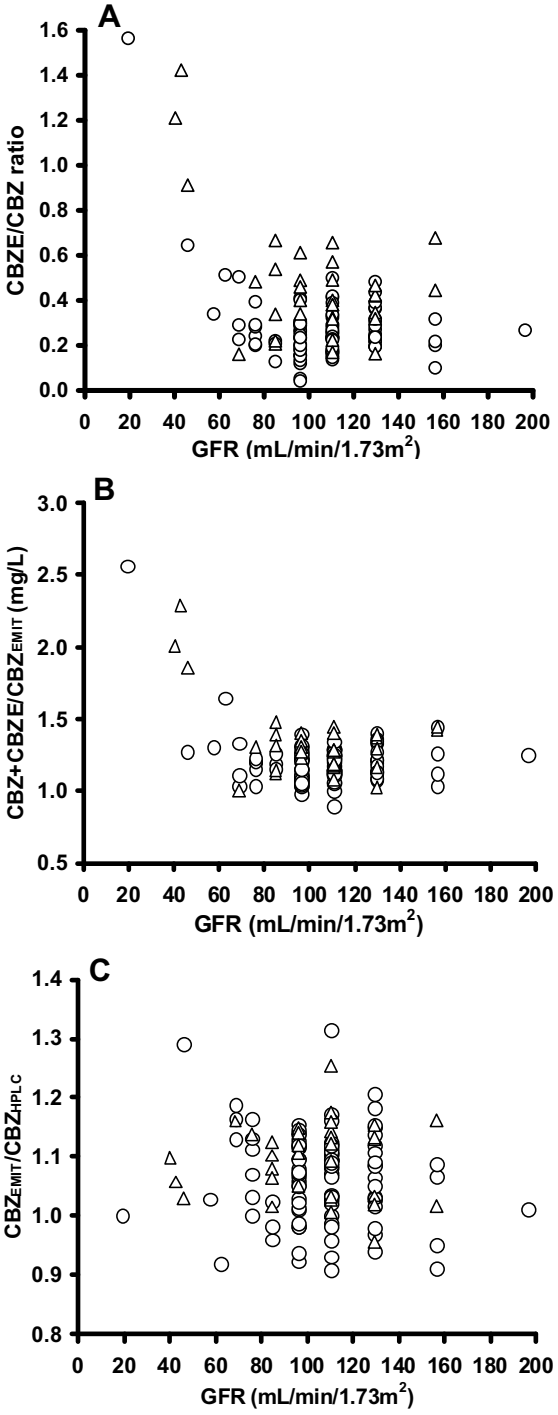

Figure 1. Relationship of the CBZE/CBZ, CBZ+CBZE/CBZEMIT, and CBZEMIT/CBZHPLC ratios with the GFR in epileptic patients treated with CBZ in monotherapy (circles) and polytherapy (triangles).

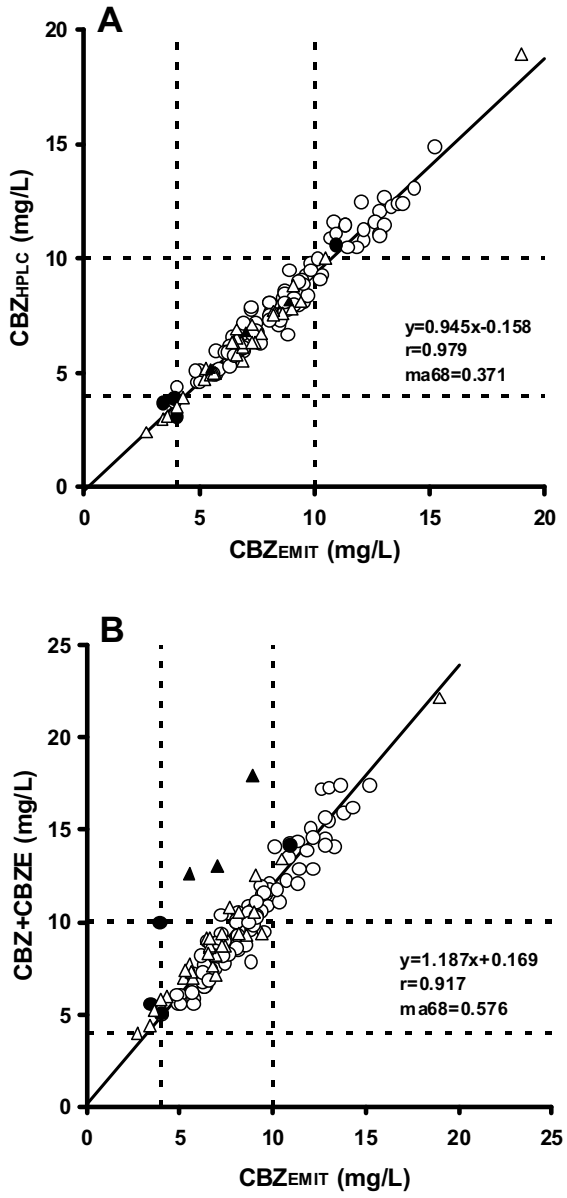

Figure 2. Correlation and regression between the obtained CBZ by EMIT and CBZ (A) and CBZ+CBZE (B) using HPLC in epileptic patients treated with CBZ in monotherapy (circles) and polytherapy (triangles), with GFR lower (closed) and higher (open) than 60 mL/min/1.73m2. The dashed lines correspond to the CBZ therapeutic range limits (4-10 mg/L).

keeping" gene expressed in all human nucleated cells (23), and freely filtered at the glomerulus (19). The recent results of Pirttilä et al, demonstrate an increased cystatin C expression in astrocytes and neurons during human chronic epilepsy (24); however, this unregulated expression of cystatin C does not appear to possess a systemic condition, as in our epileptic patients with GFR <60 mL/min/1.73 m^2, the estimation of GFR values from serum cystatin C (19) and creatinine using the six-variable MDRD equation (20) led to analogous results.

Our results show that in the patients with GFR<60mL/min/1.73m^2 there is an exponential increase of the CBZE/CBZ ratio, in parallel with the CBZ+CBZE/CBZEMIT ratio, as renal function decreases, without the CBZEMIT/CBZHPLC ratio being modified in any significant way (Figure 1). The increase of the relative proportion of CBZE with respect to the parent drug in renal insufficiency is significantly higher than that produced by the concomitant administration of phenobarbital, phenytoin and valproic acid. In the 5 patients having GFR <50 mL/min/1.73 m^2, serum concentrations of the CBZE metabolite may be similar, or even greater, than those of CBZ, in contrast to the patients, both in mono or polytherapy, with normal or mildly decreased renal function in which the CBZE/CBZ ratio was always <0.7 (Figure 1A).

The CBZE metabolite has been shown to posses similar pharmacological activity and proposed action mechanism as that of the CBZ (25), and consequently, the monitoring of the parent drug and its epoxy derivative may be desirable (11). In patients with severely impaired renal function, we should expect that serum levels of CBZ obtained using EMIT may be clinically unacceptable, because therapeutic serum concentrations of CBZ would present neurological toxicity as CBZE adds to the therapeutic effect of CBZ (Figure 2B).

In the patients having normal or mildly decreased renal function, the CBZ results obtained by EMIT present a lower deviation with respect to the sum CBZ+CBZE (Figure 2B). In these patients, CBZ+CBZE would be estimated from the CBZEMIT values using the linear regression equations: CBZ+CBZE=1.23CBZEMIT-0.49 (ma68=0.48mg/L,r=0.961)formonotherapy,andCBZ+CBZE=1.14CBZEMIT+1.02 (ma68=0.48, r=0.969) for polytherapy, with a diagnostic efficiency of 93% for the correct classification of the results as subtherapeutic, therapeutic and supratherapeutic levels (data not shown).

In conclusion, an increased proportion of the CBZE metabolite in relation to the parent drug was obtained in serum from patients with renal insufficiency, which was significantly greater than that produced by concomitant phenobarbital, phenytoin and valproic acid administration. Consequently, on the contrary to the case of phenytoin (26), the CBZ monitoring using immunoassays may be clinically unsuitable in patients with severely impaired renal function. In these cases, the CBZ determination using the Dade Dimension immunoassay, which has the highest CBZE cross-reactivity of all the most frequently used commercial immunoassays (10,11), should be evaluated.

Acknowledgements

One of the authors (MJTC) received a grant from Dade Behring obtained through the Fundación Investigación, Desarrollo, Innovación, Complexo Hospitalario Universitario de Santiago (IDICHUS).

References

1. Amore BM, Kalhorm TK, Skiles GL et al (1997) Characterization of carbamazepine metabolism in a mouse model of carbamazepine teratogeneity. Drug Metab Dispos ; 25: 953–62.
2. Maggs JL, Pirmohamed M, Kitteringham NR, Park BK (1997) Characterization of the metabolites of carbamazepine in patient urine by liquid chromatography/mass spectrometry. Drug Metab Dispos; 25: 275–80.
3. Reith DM, Appleton DB, Hooper W, Eadie MJ (2000) The effect of body size on the metabolic clearance of carbamazepine. Biopharm Drug Dispos; 21: 103–11.
4. Vree TB, Janssen TJ, Hekster YA, Termond EF, van de Dries Ac, Wijnands WJ (1986) Clinical pharmacokinetics of carbamzepine and its epoxy and hydroxy metabolites in humans after an overdose. Ther Drug Monit; 8: 297–304.
5. Potter JM, Donnelly A (1998) Carbamazepine-10,11-epoxyde in therapeutic monitoring. Ther Drug Monit; 20: 652–57.
6. Ramsay RE, McManus DQ, Guterman A et al (1990) Carbamazepine metabolism in humans: effect of concurrent anticonvulsant therapy. Ther Drug Monit; 12: 235–41.
7. Liu H, Delgado MR (1994) Improved therapeutic monitoring in epileptic children using carbamazepine polytherapy. Ther Drug Monit; 16: 132–38.
8. Warner A, Provitera M, Bates D (1988) Standards of laboratory practice: antiepileptic drug monitoring. Clin Chem; 44: 1085–95.
9. Frank EL, Schwarz EL, Juenke J, Annesley TM, Roberts WL (2002) Performance characteristics of four immunoassays for antiepileptic drugs on the Immulite 2000 automated analyser. Am J Clin Pathol; 118: 124–31.
10. Hermida J, Tutor JC (2003) How suitable are currently used carbamazepine immunoassays for quantifying carbamazepine-10,11-epoxide in serum samples?. Ther Drug Monit; 25: 384–88.
11. Shen S, Elin RJ, Soldin SJ (2001) Characterization of cross reactivity by carbamazepine-10,11-epoxide with carbamazepine assays. Clin Biochem; 34: 157–58.
12. Willimowska J, Gomollka E, Pasich A, Jenner B, Szpak D (2005) Comparison of fluorescence polarization immunoassay and high performance liquid chromatography for determination of carbamazepine concentration in blood of poisoned patients. Przegl Lek; 62: 595–98.
13. Hermida J, Bóveda MD, Vadillo FJ, Tutor JC (2002) Comparison between the Cobas Integra immunoassay and high-performance liquid chromatography for therapeutic monitoring of carbamazepine. Clin Biochem; 35: 251–24.
14. Parant F, Bossu H, Gagnieu MC, Lardet G, Moulsma M (2003) Cross-reactivity assessment of carbamazepine-10,11-epoxide, and 10-hydroxy-carbazepine in two automated carbamazepine immunoassys: PETINIA and EMIT 2000. Ther Drug Monit; 25: 41–45.
15. Lancelin F, Cattelotte J, Boyer C et al (2003) Influence de la carbamazépine-10,11-epoxide dans le dosage de la carbamazépine Dimension Flex® carbamazepine. Immunoanal Biol Spec; 18: 354–59.
16. Dade Behring Inc. Syva Emit® 2000 Carbamazepine Assay. Package insert (revised October 2004).
17. Méndez-Alvarez E, Soto-Otero R, Sierra-Marcuño G (1986) The effect of storage conditions on the stability of carbamazepine and carbamazepine-10,11-epoxide in plasma. Clin Chim Acta; 154: 243–46.
18. Van Tyle JH, Winter ME (2004) Carbamazepine. In: Winter ME, editor. Basic Clinical Pharmacokinetics. Fourth Edition. Philadelphia: Lippincot Willians & Wilkins: 172–82.
19. Hoek FJ, Kemperman FAW, Krediet RT (2003) A comparison between cystatin C, plasma creatinine and the Cockcroft and Gault formula for the estimation of glomerular filtration rate. Nephrol Dial Transpl; 18: 2024–31.
20. Levey AS, Bosch JP, Lewis JB et al (1999) A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med; 130: 461–70.
21. Shah VP, Midha KK, Dighe S et al (1991) Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. Conference report. Eur J Drug Metab Pharmacokinet; 16: 249–55.
22. Shah VP, Midha KK, Findlay JWA et al (2000) Bioanalytical methods validation- A revisit with a decade of progress. Pharm Res; 17: 1551–57.

23. Abrahamson M, Olafsson I, Palsdottir A et al (1990) Structure and expression of the human cystatin C gene. Biochem J; 268: 287–94.
24. Pirttilä TJ, Manninen A, Jutila L et al (2005) Cystatin C expression is associated with granule cell dispersion in epilepsy. Ann Neurol; 58: 211–23.
25. Davies JA (1995) Mechanisms of action of antiepileptic drugs. Seizure; 4: 267–31.
26. Tutor-Crespo MJ, Hermida J, Tutor JC (2007) Phenytoin immunoassay measurements in serum samples from patients with renal insufficiency: comparison with high-performance liquid chromatography. J Clin Lab Anal; 21: 119–23.

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