ity of being free of disease than did patients with creatinine values within the reference interval. The probability against the occurrence of true RVD diagnosis in an atherosclerotic hypertensive patient with cystatin C <0.90 mg/L was 4.26:1, which appears a reasonable odds ratio for screening purpose. Conversely, the same probability in a patient with creatinine values in the reference interval was 1.22:1.

Compensatory hyperfunction of the unaffected kidney generally minimizes the consequences of unilateral ischemic damage, and this is indeed what currently happens in the so-called creatinine blinded area, in which creatinine concentrations exceed the upper limit of the reference interval only when GFR is reduced to 75 mL/min (5). In our study, such an increase in cystatin C reflected an earlier stage of renal damage because it increased progressively above the 90th percentile of the normotensive range as the lumen of renal artery was reduced by 65–70%, whereas a corresponding increase in creatinine was recognizable when at least 80% lumen stenosis was present. These data agree with the notion that serum cystatin C increases when GFR is ~88 mL/min (5). Additional evidence was also provided by our analysis of RVD patients treated with angioplasty and stenting of the stenosed vessels. Improved renal blood flow induced by successful angioplasty was followed by changes in cystatin C but not creatinine (Fig. 1), suggesting that relatively subtle GFR variations were adequately reflected by cystatin C but not by creatinine concentrations. This finding highlights the possibility that improvement of renal function may be underestimated or not recognized by creatinine measurements in some ischemic patients treated with angioplasty. It is possible that this reduced sensitivity is partially attributable to the aspecific chromogenic interferences inherent in the Jaffe method and that our conclusions cannot be applied to the enzymatic method, which does not imply such interferences. Although the Jaffe method is less expensive and more widely used, a comparison between enzymatically measured creatinine and cystatin could be an interesting issue for future research in the field. Taking into account that the value of the present results must be restricted to the specific clinical setting of atherosclerotic hypertensive patients, cystatin C assay may be of clinical utility in a rule-out diagnostic strategy for RVD.

This study was supported by grants from the Ministry of the University and Scientific and Technological Research, the Veneto Region Department of Health, and the Fondazione Cariverona-Progetto Sanità 1996–97.

References

Determination of Gabapentin in Plasma by Liquid Chromatography with Fluorescence Detection after Solid-Phase Extraction with a C18 Column, Donna Gauthie and Ram Gupta (Department of Laboratory Medicine, St. Joseph’s Hospital, 50 Charlton Ave. East, Hamilton, Ontario, L8N 4A6 Canada; *author for correspondence: fax 905-521-6185, e-mail dgauthie@stjosham.on.ca)

Gabapentin (Neurontin®), one of the relatively new anti-epileptic drugs, has a novel mechanism of action that is not fully understood. Gabapentin has been shown to increase γ-amino butyric acid concentrations in the brain of epilepsy patients (1, 2). There is an approximate linear relationship between dose and plasma concentrations. A therapeutic range of 2–20 mg/L has been commonly accepted (2), although a higher minimum concentration of 6 mg/L has also been suggested (1).

Various analytical methods have been used for the determination of gabapentin in plasma, with liquid chromatography being the most commonly used approach in clinical laboratories. Gabapentin does not have a natural chromophore; therefore, it must be derivatized to be detected spectrophotometrically or fluorometrically unless detected by mass spectrometry (3). Gabapentin has been derivatized with 2,4,6-trinitrobenzenesulfonic acid for photometric detection (4, 5). Several approaches have been described for the preparation of fluorescent derivatives. Garcia et al. (6) derivatized gabapentin with fluorescamine for determination by capillary electrophoresis. However, the preparation of o-phthalaldehyde (OPA) derivatives of gabapentin has been the most popular and practical approach for liquid chromatographic determinations (7–12).

In all of these procedures, plasma proteins are precipitated by different reagents and the resulting supernatant
is treated with the derivatizing reagent. As a result, all endogenous amino compounds produce fluorescent derivatives, which produce very large solvent peaks, decreasing the sensitivity and increasing the length of the chromatographic run times. These procedures have another limitation in that the OPA derivatives must be injected almost immediately after preparation because of their instability.

We evaluated an alternative liquid chromatographic procedure to reduce extraneous peaks and improve the stability of OPA derivatives for the determination of gabapentin in plasma.

A 200-μL aliquot of a plasma sample was mixed with 200 μL of a 5 mg/L solution of the internal standard [1-(aminomethyl)cycloheptane acetic acid] in a 15 g/L sodium borate (borax; Sigma-Aldrich) solution and applied to a 1-mL Bond Elut C18 extraction column (cat. no. 12102001; Varian) that had been activated by washing with one column volume of methanol and one column volume of water. High-purity Omnisolv brand solvents (methanol and acetonitrile) were obtained from Merck. Deionized water was distilled in a glass still. The sample was pushed through the column at a very slow rate (~90 s) by use of a 1-mL plastic syringe (cat. no. 309626; Becton-Dickinson) and an adapter (cat. no. 12131001; Varian) to fit the C18 extraction column. These authors passed the sample through a C18 extraction column. On the other hand, Lensmeyer et al. (4) isolated gabapentin in 92–98% yields with a Bond Elut C18 extraction column. These authors passed the sample through a C18 column at a very slow rate. We also showed fluorescence response without any delay. The linearity of the method was determined by serial dilution (eight concentrations) of a 40 mg/L gabapentin calibrator in drug-free plasma: $y = 0.1956x - 0.003$ ($r = 0.998$). The lower limit of quantification was 0.3 mg/L, which is well below the therapeutic range (2–20 mg/L).

Quality-control sera (Bio-Rad TDM levels 1 and 3) containing acetaminophen, amikacin, amitriptyline, caffeine, carbamazepine, chloramphenicol, desipramine, digoxin, disopyramide, ethosuximide, gentamicin, imipramine, lidocaine, lithium, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, phenobarbital, phenytin, primodone, procainamide, propranolol, quinidine, salicylate, theophylline, tobramycin, valproic acid, and vancomycin did not show any interfering peaks when processed. Hemolyzed or moderately lipemic samples did not affect the recovery of the drug or internal standard.

The chromatogram of drug-free plasma (Fig. 1A) showed only a few early-eluting peaks. There were no peaks eluting after the internal standard. Gabapentin is a polar amino acid and cannot be isolated by liquid–liquid extraction. For gas chromatographic procedures, gabapentin has been isolated with cation-exchange (13) or mixed-mode solid-phase extraction (14, 15). Cation-exchange extraction of gabapentin does not offer any advantage because all the endogenous amino compounds form cations and are coextracted. Furthermore, cation-exchange columns must be eluted with ammoniac methanol to recover gabapentin, and the ammonia must be completely removed by evaporation before derivatization with OPA. Kushnir et al. (15) reported that gabapentin is not significantly retained by commonly used 1-mL C18 or CN extraction columns. The peaks were detected by use of an excitation wavelength of 350 nm and an emission wavelength of 450 nm. The drug and the internal standard showed fluorescence response without any delay.

The within-run CVs were 3.0% (n = 10) for both the low (mean = 0.5 mg/L) and the high (mean = 20.7 mg/L) gabapentin concentrations. Between-run CVs (n = 10) were 6.7% (mean = 0.6 mg/L) for the low concentration and 3.7% (mean = 20.6 mg/L) for the high concentration.
found that both gabapentin and the internal standard are recovered from plasma in a 90–98% yield after extraction with 1-mL C18 extraction columns. The recovery of gabapentin is drastically reduced when the sample passes through the C18 sorbent at a relatively fast rate. However, endogenous amino compounds present in plasma are more polar than gabapentin and are not retained by C18 sorbent even when the sample is passed at a slow rate.

Different authors have used different compositions of OPA derivatizing agent, a mixture of a methanolic solution of OPA, a thiol, and borate buffer, but the fluorescent derivatives are produced in similar yields. The presence of water in the reagent makes the OPA derivative unstable, and the stability can be improved by decreasing the water content (16). In our described procedure, the derivatizing reagent does not contain borate buffer. The methanolic eluate obtained from the C18 extraction column contains traces of borate buffer, which is adequate to initiate the reaction. The resulting OPA derivatives are stable for at least 4 h at room temperature and for at least 24 h when stored at 4–8 °C.

Gabapentin OPA derivatives have been detected at excitation wavelengths of 230–250 or 330–350 nm with a common emission wavelength of 430–450 nm. Excitation at 230–250 nm provides ~2.5 times the sensitivity of that observed at 330–350 nm with the same attenuation, slit width, and emission wavelength. However, the baseline is unstable at excitation wavelengths of 230–250 nm, and the detector must be set at a higher attenuation to decrease the noise. The procedure presented here is extremely sensitive even at an excitation wavelength of 350 nm.

α-Aminocyclohexanepropionic acid (cat. no. 43,805-7; Aldrich) behaved similar to gabapentin and its analog for solid-phase extraction. It produced a fluorescent OPA derivative. Other OPA derivatizing agents, a mixture of a methanolic solution of OPA, a thiol, and borate buffer, were also observed at 330–350 nm with a common emission wavelength of 430–450 nm. Excitation at 230–250 nm gives ~2.5 times the sensitivity of that observed at 330–350 nm with the same attenuation, slit width, and emission wavelength. However, the baseline is unstable at excitation wavelengths of 230–250 nm, and the detector must be set at a higher attenuation to decrease the noise. The procedure presented here is extremely sensitive even at an excitation wavelength of 350 nm.

We thank Dr. A. Fraser of Victoria General Hospital, Halifax, and Pfizer Canada for supplying us with samples of gabapentin and 1-(aminomethyl)cyclohexane-1-acetic acid.

References