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In Situ Metabolism of Levetiracetam in Blood of Patients with Epilepsy

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Summary: *Purpose:* Although levetiracetam undergoes minimum metabolism, B-esterases have been identified in whole blood that are capable of metabolising levetiracetam. The present study was designed to ascertain any variability in levetiracetam blood concentrations that could be attributed to in situ metabolism and which could impact on the utility of such concentration measurements in guiding therapeutic management.

Methods: Blood samples were collected from 40 patients that were prescribed levetiracetam. Sera (Groups 1 and 2) or whole blood (Groups 3 and 4) were compared. Paraoxan, an inhibitor of B-esterase activity, was added to samples assigned to Groups 2 and 4. Samples within each group were assigned to Time 0 (frozen within 30 min of sample collection), Time 2 days and Time 7 days (samples kept at ambient temperature for 2 and 7 days).

Results: For serum samples, mean levetiracetam concentrations at Time 2 days and Time 7 days were indistinguishable from Time 0, regardless of whether B-esterase activity was inhibited on not. In contrast, for whole blood, in the absence of B-esterase inhibition, mean levetiracetam concentrations declined over time (11% and 29%; 2 and 7 days) compared to baseline values. In the presence of B-esterase inhibitor, mean levetiracetam concentrations at 2 days were indistinguishable from baseline values, although at 7 days values declined by 4%.

Conclusions: If therapeutic monitoring of levetiracetam is to be undertaken, serum should be the matrix of choice and that whole blood should be separated as soon as possible after patient sampling so as to minimize in situ levetiracetam metabolism which could result in spuriously low concentrations and substantial intrapatient variability. **Key Words:** Levetiracetam—Metabolism—B-esterase—Therapeutic drug monitoring—New antiepileptic drug.

Levetiracetam ((S)-α-ethyl-2-oxo-1-pyrrolidine acetamide) is a new antiepileptic drug (AED) with significant efficacy in patients with intractable partial seizure but increasingly it is being identified to have efficacy in patients with other seizure types and indeed in other neurological conditions (Patsalos, 2004a). It appears to act via a specific binding site (Noyer et al., 1995) within the brain and produces a limited reduction in high-voltage-activated calcium currents and possibly modulates intracellular calcium transients (Pisani et al., 2004). Recently, molecular studies involving transgenic mice suggested that levetiracetam binds to a synaptic vesicle protein SV2A, which is involved in vesicle neurotransmitter exocytosis (Lynch et al., 2004).

A major pharmacokinetic feature of levetiracetam, that perhaps sets it apart from other AEDs, is its low propensity to interact with other drugs (Patsalos, 2005; Patsalos and Perucca, 2003). The primary reason for this can be attributable to its mechanism of elimination and the fact that it neither induces nor inhibits cytochrome P450 (CYP) isoenzymes, which are responsible for drug metabolism, primarily in the liver but also in other organs and tissues, of most drugs (Nicolas et al., 1999; Patsalos, 2000).

Levetiracetam is primarily eliminated by renal excretion with approximately 66% of an administered dose recovered as unchanged levetiracetam in urine (Patsalos, 2004b). Levetiracetam is minimally metabolized. The major pathway involves hydrolysis to produce one major metabolite ((2S)-2-(2-oxopyrrolidin-1-y) butanoic acid; ucbLO57; ~24% of dose) and two minor metabolites (~3% of dose) and all three are pharmacologically inactive (Strolin Benedetti et al., 2003, 2004). However, unlike other AEDs that are metabolized, the metabolism of levetiracetam does not involve the hepatic CYP system; instead it involves hydrolysis of levetiracetam by B-esterases primarily in the blood (Coupez et al., 2003; Strolin Benedetti et al., 2003, 2004) although some hydrolysis may also occur in the liver (Coupez et al., 2003).

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Furthermore, in vitro studies confirm that levetiracetam hydrolysis can be inhibited by paraoxon (a broad inhibitor of B-esterases) but not by other B-esterase type inhibitors that are specific towards cholinesterases and/or carboxylesterases such as physiostigmine and metoclopramide (Coupez et al., 2003).

As with all the newly licensed AEDs, there is a debate as to whether or not therapeutic drug monitoring of levetiracetam can be usefully employed to optimize the use of levetiracetam to treat patients with epilepsy. Nevertheless, numerous analytical techniques for the quantitation of levetiracetam in blood and other biological matrixes have been described (Vermaij and Edelbroek, 1994; Ratnaraj et al., 1996; Coupez et al., 2003; Issoherranen et al., 2003; Ivanova et al., 2003; Shihabi et al., 2003; Pucci et al., 2004) and also a putative target/therapeutic range for levetiracetam has been suggested (Johannessen et al., 2003). A major consideration in relation to routine monitoring is sample stability and with regard to levetiracetam, a particularly pertinent consideration is whether or not there is postsampling metabolism of levetiracetam in situ by blood B-esterases. Such metabolism would invariably impact on the concentration of levetiracetam measured and indeed its utility in guiding therapeutic management. Thus, the present study was designed to ascertain any variability in levetiracetam blood concentrations that could be attributed to in situ metabolism.

METHODS

Materials

Paraoxon (diethyl *p*-nitrophenyl phosphate) was purchased from Sigma Aldrich Co Ltd (Poole, UK). All other reagents and solvents used were purchased as the purest grade available.

Sample collection and patient demographics

Blood samples were collected from the first 40 consecutive patients (18 males/22 females, age range 19–73 years, weight range 33–120 kg), attending the outpatient clinics at the Chalfont Centre for Epilepsy that were prescribed levetiracetam and that were having their blood levetiracetam concentrations monitored as part of their routine clinical management. Six patients were on levetiracetam monotherapy, 17 patients were prescribed two AEDs, 13 patients were prescribed 3 AEDs, 3 patients were prescribed 4 AEDs, and 1 patient was prescribed 5 AEDs. The coadministered AEDs included carbamazepine (19 patients), valproate (9 patients), phenytoin (6 patients), lamotrigine (6 patients), topiramate (6 patients), clobazam (5 patients), oxcarbazepine (2 patients), and gabapentin (2 patients). Blood samples (10 ml) were collected by venepuncture from the antecubital vein during January–March 2004 and were randomly assigned to one of four groups (10 samples/group). Group 1—serum without esterase inhibitor (4 males/6 females, age range 33–47 years, weight range 46–120 kg); Group 2—serum with esterase inhibitor (5 males/5 females, age range 19–73 years, weight range 37–119 kg); Group 3—whole blood without esterase inhibitor (4 males/6 females, age range 20–66 years, weight range 50–81 kg); and Group 4—whole blood with esterase inhibitor (5 males/5 females, age range 24–72 years, weight range 48–120 kg).

Whole blood was either separated by centrifugation to obtain sera and then assigned to Groups 1 and 2 or directly assigned to Groups 3 and 4. Samples assigned to Time 0 were frozen within 30 min of sample collection (sera) or centrifuged first (whole blood) to obtain sera and subsequently frozen within 30 min of sample collection (–20°C). Samples assigned to Time 2-days and Time 7-days were kept at ambient temperature and processed, as described for the Time 0 samples, at 2 days and 7 days after sample collection, respectively. After processing, all samples were stored frozen at –70°C until batch analysis for levetiracetam content. Because previously it was observed that 100 μM paraoxan (esterase inhibitor) was associated with a 92% inhibition of levetiracetam hydrolysis to its primary metabolite ucb LO57 (Coupez et al., 2003), the present study employed 120 μM paraoxan so as to achieve inhibition of levetiracetam hydrolysis of >92%.

Levetiracetam analysis

Serum levetiracetam concentrations were determined by high performance liquid chromatography with ultraviolet detection by the method described by Ratnaraj et al. (1996). The limit of detection of the method is 1 μmol/L and the within-batch and between-batch precisions are 2–5% and 3–6%, respectively.

Statistical analysis

Levetiracetam concentrations were compared using the paired Student's *t*-test.

RESULTS

Table 1 shows the individual patient serum levetiracetam concentrations and the mean values in those samples to which paraoxon was not added (Group 1). It can be seen that compared to Time—0, mean levetiracetam concentrations were indistinguishable at 2 days and 7 days. Table 2 shows the individual patient serum levetiracetam concentrations and the mean values in those samples to which paraoxon (120 μM) was added (Group 2). The observed results are comparable to those observed for Group 1 in that levetiracetam concentrations were indistinguishable at 2 days and 7 days compared to Time 0.

Table 3 shows the individual patient serum levetiracetam concentrations and the mean values in those samples to which paraoxon was not added to whole blood (Group 3). It can be seen that in the absence of paraoxon, mean levetiracetam concentrations decline over time so that by 2 days there is a significant ($p < 0.0005$) 11% reduction

TABLE 1. *Levetiracetam concentrations in serum samples to which the B-hydrolase inhibitor paraoxon had not been added*

	Levetiracetam concentration (μmol/L)		
Patient number	Time 0	Time 2 days later	Time 7 days later
1	573	673	599
2	92	91	90
3	54	69	62
4	627	644	541
5	240	232	245
6	342	335	345
7	253	255	257
8	178	169	164
9	64	69	61
10	164	164	163
Mean	259	260[a]	253[b]

[a] $p < 0.316$, [b] $p < 0.270$ compared to Time 0.
Statistical evaluation of data was performed with the paired Student's *t*-test.

TABLE 3. *Levetiracetam concentrations in whole blood samples to which the B-hydrolase inhibitor paraoxon had not been added*

	Levetiracetam concentration (μmol/L)		
Patient number	Time 0	Time 2 days later	Time 7 days later
1	52	43	27
2	495	439	378
3	201	179	142
4	164	145	108
5	312	295	219
6	104	91	85
7	219	179	124
8	424	385	334
9	46	34	16
10	223	211	155
Mean	224	200[a]	159[b]

[a] $p < 0.0005$, [b] $p < 0.00008$ compared to Time 0.
Statistical evaluation of data was performed with the paired Student's *t*-test.

and by 7 days the reduction is 29% compared with mean baseline (Time 0) levetiracetam concentrations. In contrast, when paraoxon was added to whole blood (Table 4; Group 4), mean serum levetiracetam concentrations at 2 days were indistinguishable from baseline (Time 0) values, although at 7 days mean levetiracetam concentrations were associated with a small (4%) but statistically significant reduction ($p < 0.008$).

DISCUSSION

In the present study, we have compared levetiracetam concentrations in sera of patients prescribed levetiracetam for the management of their epilepsy so as to ascertain whether or not in situ metabolism (sera vs. whole blood) can affect concentrations over time. Sera were prepared either immediately from whole blood (Tables 1 and 2) or after standing at ambient temperature for 2 or 7 days (Tables 3 and 4). Furthermore, the B-hydrolase inhibitor paraoxon was added either directly to sera (Table 2) or to whole blood (Table 4), at a concentration chosen to inhibit the metabolism of levetiracetam by >92% (Coupez et al, 2003;), so as to identify the relative contribution, if any, of whole blood and serum to the in situ metabolism of levetiracetam.

Our data clearly show that for serum, levetiracetam concentrations do not change over time (up to 7 days) at ambient temperature, regardless of whether inhibition of B-hydrolase activity has occurred or not (Tables 1 and 2). Thus it can be concluded that serum is devoid of B-esterase activity and that as a matrix it is stable and can be used reliably for levetiracetam therapeutic monitoring; sera can be transported to the laboratory by post without undue concern of sample spoilage. In contrast for whole blood, in the absence of B-hydrolase inhibition, mean levetiracetam concentrations declined significantly over time so that compared to baseline (224 μmol/L) values, they

TABLE 2. *Levetiracetam concentrations in serum samples to which the B-hydrolase inhibitor paraoxon had been added*

	Levetiracetam concentration (μmol/L)		
Patient number	Time 0	Time 2 days later	Time 7 days later
1	199	193	184
2	96	86	82
3	26	24	25
4	87	85	86
5	89	78	87
6	103	99	101
7	202	220	199
8	188	190	199
9	244	238	234
10	236	233	215
Mean	148	145[a]	141[b]

[a] $p < 0.122$, [b] $p < 0.021$ compared to Time 0.
Statistical evaluation of data was performed with the paired Student's *t*-test.

TABLE 4. *Levetiracetam concentrations in whole blood samples to which the B-hydrolase inhibitor paraoxon had been added*

	Levetiracetam concentration (μmol/L)		
Patient number	Time 0	Time 2 days later	Time 7 days later
1	35	34	36
2	141	135	124
3	155	146	146
4	576	560	560
5	247	245	241
6	480	278	457
7	44	45	43
8	43	43	41
9	274	285	276
10	265	262	257
Mean	226	225[a]	218[b]

[a] $p < 0.126$, [b] $p < 0.008$ compared to Time 0.
Statistical evaluation of data was performed with the paired Student's *t*-test.

declined by 11% (200 μmol/L) and 29% (159 μmol/L) after 2 days and 7 days, at ambient temperature, respectively (Table 3). In the presence of B-hydrolase inhibition, whole blood mean levetiracetam concentrations at 2 days (225 μmol/L) were indistinguishable to values at baseline (226 μmol/L), although at 7 days values were associated with a small (4%) but significant reduction (218 μmol/L; Table 4). These whole blood data confirm previous data suggesting that metabolism of levetiracetam can occur in situ and that the primary route of such metabolism is hydrolysis (Coupez et al., 2003; Strolin Benedetti et al., 2003). Furthermore, that such hydrolysis can be associated with significant reduction in levetiracetam concentrations (11% at 2 days and 29% at 7 days), can be expected to be of clinical significance and therefore it would not be advisable to transport levetiracetam samples as whole blood when therapeutic drug monitoring is indicated.

In conclusion, if therapeutic monitoring of levetiracetam is to be undertaken, serum should be the matrix of choice and that whole blood should be separated as soon as possible after patient sampling so as to minimize in situ levetiracetam metabolism which could result in spuriously low concentrations and substantial intrapatient variability.

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