Kinetics of plasma and erythrocyte metformin after acute administration in healthy subjects

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SUMMARY
Objectives: Although the existence of a deep compartment for metformin has long been hypothesized, there is still little direct information concerning metformin distribution in individual tissues in man. The only available study involves chronic metformin therapy. In that study, the measurement of metformin in erythrocytes provided a reliable indicator of metformin distribution and of potential accumulation. To determine the kinetics of metformin in plasma and in erythrocytes after acute oral administration, we performed the present study in healthy subjects after a single oral dose of metformin and compared the pharmacokinetics parameters in erythrocytes to those in plasma.

Methods: Six nondiabetic participants took the study dose of 850 mg metformin at 8:00 AM after a non-standardized breakfast (i.e., as recommended in clinical practice). Blood samples were collected for metformin measurement in plasma and in erythrocytes at 0, 1, 2, 3, 4, 6, 9, 24, 33, 48, 57, and 72 h.

Results: Maximum metformin concentration was attained at 3.0 ± 0.3 h in plasma and 4.7 ± 0.5 h in erythrocytes. This difference was not significant. Metformin concentrations peaked at a maximum almost 6 times higher in plasma than in erythrocytes (1.7 ± 0.1 and 0.3 ± 0.0 mg/l, respectively). However, because the elimination half-life of metformin was much longer in erythrocytes (23.4 ± 1.9 h vs. 2.7 ± 1.2 h), there was no difference in area under the curve between plasma and erythrocytes. The distribution volume (plasma) was calculated to be 146 ± 11 l. Plasma and erythrocytes concentration-time curves showed that metformin was not detectable in plasma 24 hours after the oral administration, while it remained detectable in erythrocytes up to 48 hours. Metformin concentrations crossed approximately 13 hours after having reached their maximum values in plasma, approximately 16 h after metformin intake.

Conclusion: Having demonstrated the rapid elimination of metformin from plasma and its slow disappearance from erythrocytes, the presents results should contribute to adjustment of metformin dosage to renal function, assessment of drug compliance, and retrospective analysis (when blood samples are drawn with delay) of the link between metformin and development of lactic acidosis. Most importantly, the present findings should help to ascertain the optimal dosages of metformin, particularly in elderly patients.

Key-words: Type 2 diabetes • Metformin • Pharmacokinetics • Erythrocytes.

RESUME
Objectif : Bien qu’un compartiment profond ait été suspecté de longue date pour la metformine, une seule travail a étudié directement la distribution tissulaire et en dosant la concentration de metformine dans les érythrocytes de façon transversale chez des sujets diabétiques en traitement chronique. Nous avons voulu compléter ce travail par une détermination des paramètres pharmacocinétiques après administration d’une dose unique de metformine chez des sujets non diabétiques.

Méthodes : Six sujets non diabétiques ont pris une dose unique de 850 mg de metformine par voie orale à 08 h après un petit-déjeuner non standardisé (i.e. tel qu’il est recommandé en pratique clinique). Des prélèvements sanguins ont été réalisés aux temps 0, 1, 2, 3, 4, 6, 9, 24, 33, 48, 57, et 72 h pour déterminer la concentration de metformine dans le plasma et les érythrocytes.

Résultats : La concentration maximale de metformine a été atteinte à 3.0 ± 0.3 h dans le plasma et à 4.7 ± 0.5 h dans les érythrocytes (différence non significative). Les valeurs pic ont été près de 6 fois plus élevées dans le plasma : 1.7 ± 0.1 et 0.3 ± 0.0 mg/l. Cependant, dans la mesure où la demi-vie d’élimination était bien plus longue dans les érythrocytes (23.4 ± 1.9 h vs. 2.7 ± 1.2 h), les aires sous la courbe ne se sont pas avérées différentes entre le plasma et les érythrocytes. Le volume distribution (plasma) a été mesuré à 146 ± 11 l. La metformine n’était plus détectable dans le plasma 24 heures après l’administration orale, tandis qu’elle l’était encore dans les érythrocytes à 48 heures avec un croisement des courbes de concentrations à environ 13 heures après le maximum dans le plasma, soit environ 16 h après la prise de metformine.

Conclusion: Ayant ainsi démontré directement que la metformine disparaît rapidement dans le plasma mais lentement dans les érythrocytes, le dosage érythrocytaire de la metformine peut aider à ajuster la posologie de metformine à la fonction rénale, en particulier chez le sujet âgé, à évaluer la compliance au traitement, et enfin à analyser rétrospectivement (quand le sang est prélevé avec délai) le lien entre la metformine et la genèse d’une acidose lactique.

Mots-clés : Diabète de type 2 • Metformine • Pharmacocinétique • Erythrocytes.

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Metformin is an agent widely used in the treatment of type 2 diabetes for its antihyperglycemic properties. Target tissues for these properties include insulin-sensitive tissues such as liver and peripheral tissues, and non-insulin-sensitive tissues such as the small intestine and erythrocytes [1].

Metformin is a stable compound. It is not metabolized, undergoing urinary excretion unchanged at a rate of approximately 90% in 12 hours [2]. Previous studies have shown a plasma elimination half-life ranging from 2.0 to 6.0 hours after oral administration of varying doses (0.5 to 1.5 g/d) [3-10]. Urine studies have indicated a further terminal elimination phase with a half-life of between 8 and 20 hours, involving only a small fraction (<5%) of the administered dose [11]. Such studies are compatible with the existence of a deep compartment, although little transfer into cells is expected in view of the physicochemical properties of metformin. A strongly basic compound, metformin is almost completely ionized at the pH of plasma. Despite poor lipid solubility due to its markedly polar nature, a certain proportion of the drug enters the cells. Studies in healthy and diabetic mice have shown that metformin may accumulate in many tissues, i.e. at concentrations greater than those of plasma [12]. Subcellular studies of metformin in rat liver have shown that, in cells, it is primarily located in the cytosol [13].

While kinetic studies have convincingly suggested a deep compartment, definite evidence was obtained in a study of metformin elimination by dialysis therapy [14]. That removal of metformin by dialysis continued despite an equilibrium between blood and dialysate concentrations clearly indicated storage of metformin in a deep compartment at a concentration superior to that of the blood compartment. However, there is still no direct information regarding metformin distribution in individual tissues in man after acute administration. The measurement of metformin in erythrocytes, serving as a marker for a deep compartment, has been shown to reliably assess metformin distribution and potential accumulation in chronic metformin therapy [15]. Applying the same method, the authors examined the kinetics parameters of metformin in erythrocytes compared to those in plasma after acute oral administration.

Subjects and methods

Subjects

Six healthy subjects (four men and two women) were enrolled in the study after informed consent. Their average age was 32.2 ± 3.0 years (range, 24.5-46.0). The mean body mass index was 20.6 ± 0.85 kg/m² (range, 17.4-23.8). All were free of glycosuria and had normal fasting blood glucose levels. None of the subjects took medication. The study was approved by the ethics committee of Picardie (Amiens, France).

Methods

Participants took the study dose of metformin at 8:00 AM after a non-standardized breakfast, as recommended in clinical practice. Blood samples were collected for metformin measurement both in plasma and in erythrocytes at 0, 1, 2, 3, 4, 6, 9, 24, 33, 48, 57, and 72 hours after a single oral dose of 850 mg of metformin chlorhydrate (Merck-Lipha, Lyon, France), which corresponds to 663 mg of metformin in the form of a base. Serum creatinine was measured at time 0.

Data analysis

The maximum attained plasma and erythrocyte concentrations (Cmax) and the time of its occurrence (tmax) were obtained from the measured data points without interpolation. The unextrapolated area under the concentration-time curve during the dosing interval (AUC) was calculated by the linear trapezoidal method and corrected for infinity using the rate constant of the last exponential phase (k).

The k value was calculated from the final 3 detectable concentrations of the log concentration-time curve using linear regression. The elimination half-life (t1/2) was calculated as 0.693/k. Clearance/bioavailability (Cl/F) was estimated by dose/AUC in which the dose is expressed in units of basic metformin and bioavailability is 50% as commonly reported for metformin chlorhydrate [2]. Volume of distribution/bioavailability (Vd/F) was estimated by dividing Cl/F by k.

Analytical Methods

Metformin concentrations were measured in duplicate in the same laboratory with high-performance liquid chromatography according to the technique described by Lacroix [16]. For measurement in erythrocytes, venous blood was centrifuged as rapidly as possible after collection to minimize plasma-cell exchanges. The pellet was washed three times with 0.9% sodium chloride, then deproteinized using 10% trichloroacetic acid. After centrifugation, 20 µl or 50 µl of the supernatant was injected into the exchange column. Results are expressed as basic metformin. Detection limit was 0.02 mg/l for plasma and 0.03 mg/l for erythrocytes. For interday precision, the coefficients of variation ranged from 4.9% to 11.2% for plasma and erythrocytes. For intraday precision, the coefficients of variation ranged from 0.8% to 12.2% for plasma and erythrocytes.

Statistical analysis

Data are presented as mean ± SEM. Differences between plasma and erythrocyte concentrations were analyzed for
statistical significance using the nonparametric Wilcoxon matched-pairs signed-ranks test. Statistical significance was defined as $p \leq 0.05$.

**Results**

The subjects studied had normal serum creatinine (85.8 ± 6.2 μmol/l; range, 64-105 μmol/l). The pharmacokinetic parameters for metformin in plasma and erythrocytes are presented as mean values in Table I. The apparent difference in times of maximal concentration between plasma and erythrocyte concentrations of metformin did not reach statistical significance ($p = 0.068$). Metformin concentrations peaked at a maximum concentration almost 6 times higher in plasma than in erythrocytes (respectively, 1.7 mg/l and 0.3 mg/l; range, 1.3-2.0 mg/l, and 0.2-0.5 mg/l). However, because of the far longer elimination half-life of metformin in erythrocytes (23.4 h vs. 2.7 h, range 18.5 h-31.5 h, and 2.2 h-3.6 h, respectively), there was ultimately no difference in area under the curve between plasma and erythrocytes. The distribution volume for metformin was calculated to be 146 ± 11 l.

Plasma and erythrocyte concentration-time curves are shown in figure 1, with inter-individual variations. This figure shows that 24 hours after oral administration, metformin was not detectable in plasma, while it was still detectable in erythrocytes up to 48 hours. Metformin concentrations crossed approximately 13 hours after having reached their maximum values in plasma, roughly 16 h after metformin intake.

**Discussion**

Although plasma pharmacokinetic parameters for the antidiabetic drug metformin have long been known [6, 7, 17], to the best of our knowledge, no deep compartment kinetic studies of cellular metformin concentrations have previously been reported. The kinetic parameters for plasma in this study are consistent with prior reports [3-10], in which plasma concentration was shown to attain a maximum of 1 to 2 mg/l (approximately $10^{-5}$ M) after an oral dose of 500 to 1,000 mg of metformin. When the present results are combined with those of the reports in which metformin was taken orally at the same dose of 850 mg [3, 4, 10], the ranges of mean maximal concentration, time of maximal concentration, and elimination half-life were narrow, from 1.5 to 1.8 mg/l, from 2.5 to 3.3 h, and from 2.4 to 4.6 h, respectively.

From a technical point of view, we may wonder about submission of erythrocytes to deproteinization. Indeed, because metformin bears a single net positive charge at physiological pH, and because ionic interactions are likely to contribute to its membrane binding properties, such a procedure is expected to remove at least a part of membrane-bound

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### Table I

<table>
<thead>
<tr>
<th>Kinetics parameters for metformin in plasma and in erythrocytes in six healthy subjects after a single oral dose of 0.85 g of metformin (mean ± SEM).</th>
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<tr>
<td><strong>Plasma</strong></td>
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<td>Time of maximal concentration (h)</td>
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<td>Maximal concentration (mg/l)</td>
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<td>Elimination half-life (h)</td>
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<td>Area under the curve (mg.h/l)</td>
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<td>Distribution volume (l)</td>
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**Figure 1**

Individual plasma and erythrocyte metformin concentration-time curves in six healthy subjects after a single oral dose of 0.85 g of metformin. P: plasma concentration, E: erythrocyte concentration. A different number is attributed to each subject (P1 and E1, for example, indicate plasma and erythrocyte concentrations in the same individual).
metformin. However, whether the values determined correspond to the part or the total erythrocyte metformin should not modify the trend of the concentration-time curve.

In the present study, metformin was administered as recommended in clinical practice, i.e. at the end of a meal (breakfast in this case), while in previous kinetic studies metformin was given after overnight fasting. Moreover, the breakfast of our participants was not standardized. However, as already stated, our results for plasma do not differ from those of previous studies. That was to be expected, because food has little influence on the distribution of metformin. In one study, in which metformin was taken after an overnight fast or after one of four different types of breakfast (low protein, low fat, low carbohydrate, or standard), the maximal metformin concentrations and values of area under the curve were roughly equivalent [18]. Concerning the variations in methodology among the available studies, it is also noteworthy that no significant difference in metformin kinetics has been reported between patients with type 2 diabetes and healthy subjects [10].

Compartmental analysis suggests that metformin pharmacokinetics are best described by a two-compartment open model, with rapid elimination from a central compartment and slower elimination from a deep compartment [11]. Urine studies have indicated a terminal elimination phase with a half-life of between 8 and 20 hours, between 12 and 14 hours in the majority of subjects. The present results provide a more detailed picture of metformin kinetics, showing an elimination half-life from erythrocytes of 23.4 ± 1.9 hours, nearly 10-fold higher than the elimination half-life from plasma.

What can we infer from data obtained in erythrocytes, in addition to the findings already available for plasma? The new information should contribute to adjusting metformin dosage to renal function, especially given that metformin is eliminated by the kidneys, in apparently unchanged form. Because of the rapid elimination of metformin from plasma and its slow disappearance from erythrocytes, it should be easier to perform the adjustment of dosage on the basis of the more stable, erythrocyte levels. In the same spirit, the determination of metformin concentration in erythrocytes may help improve assessment of patient compliance to metformin therapy. Formerly, if metformin concentration was determined several hours after metformin intake, no conclusion was possible regarding compliance to treatment. Lastly, measurement of metformin concentrations in erythrocytes now provides a reliable indicator of accumulation of the drug. This may be of particular help in retrospective attempts to establish a link between metformin use and development of lactic acidosis [19]. Under emergent conditions, blood sampling at admission for metformin determination is not necessarily the primary preoccupation. Moreover, in patients with lactic acidosis accompanied by shock syndrome, renal failure may be either primary, subsequently leading to metformin accumulation, and/or secondary to renal hypoperfusion. This distinction is important when considering prognosis during the course of various situations of metformin accumulation, because there is no mortality related to metformin alone, while mortality is still high in metformin-unrelated lactic acidosis [19, 20].

It is true that the assay of metformin is not readily available for most clinicians. However, the idea is not to generalize the assay in clinical practice but, instead, on the grounds of studies such as the present one, to generalize the ability to determine indirectly these concentrations based upon improved knowledge of each determinant, i.e. pharmacokinetics of metformin renal function and its course, metformin dosage, and lag-time between blood sampling and the last administration of metformin. Let us take, for example, the case of elderly subjects: the measurement of metformin concentrations performed in one study now permits adjustment of metformin dosage to creatinine clearance [21].

It may be concluded that metformin erythrocyte concentration measurements primarily contribute to adjusting metformin dosage to renal function. Optimizing dosage would help providing appropriate counseling in patients at risk of decline in renal function, particularly in elderly patients in whom the continuation of metformin therapy should not be discussed in terms of “the usual dose or zero”. The reduction in metformin dosage, if possible or necessary, should improve compliance of the patient and reduce the incidence of side effects.

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References