NOTE

Sustained Release of Metformin via Red Blood Cell Accumulated Sulfenamide Prodrug

LAURI PEURA, KRISTIINA M. HUTTUNEN

School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Kuopio FI-70211, Finland

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ABSTRACT: Metformin is a first-line antidiabetic drug to treat type 2 diabetes. It is rapidly eliminated from plasma but also accumulated into red blood cells (RBCs) from which it is slowly released back into plasma. The aim of the study was to evaluate whether the amount of metformin in the RBCs could be increased by a sulfenamide prodrug approach, which could provide longer duration of metformin in systemic circulation. Pharmacokinetic properties of metformin and its cyclohexyl sulfenamide prodrug were evaluated in plasma and in whole blood after intravenous and oral administration in rats. Once the sulfenamide prodrug reached the bloodstream, it was rapidly and efficiently accumulated into the RBCs, where it was converted to metformin by free thiols. The RBC–whole blood ratio of metformin was increased approximately from 42% to 96% when metformin was administered intravenously as its sulfenamide prodrug, and the proportion of metformin in the RBCs was found to be concentration and time independent. Because metformin was slowly liberated into plasma, the prodrug showed a sustained-release pharmacokinetic profile and longer plasma half-life for metformin after oral administration. Therefore, this sulfenamide prodrug has great potential to improve metformin therapy as the daily doses could be reduced. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:2207–2210, 2014

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INTRODUCTION

Metformin is an oral antihyperglycemic agent that suppresses hepatic glucose production and intestinal glucose absorption, as well as enhances peripheral glucose uptake and increases insulin sensitivity in patients with noninsulin-dependent diabetes mellitus (type 2).[1–3] This bisguanidine-structured small molecule is a strong base and therefore protonated in physiological fluids. Because metformin is slowly and incompletely absorbed from the upper gastrointestinal tract, its oral bioavailability in human is only 40%–60%.[4] Therefore, the therapeutic dose range of metformin is high (0.5–3.0 g/day), which in turn causes uncomfortable gastrointestinal adverse effects.[5] Once in the bloodstream, metformin is rapidly eliminated from plasma, without metabolism, having an elimination half-life of only few hours. Although plasma protein binding of metformin is negligible, it is known to distribute into the red blood cells (RBCs, erythrocytes).[6,7] RBCs partitioning favors basic and small drugs, such as metformin, as they can enter the cells through aqueous channels.[8] Furthermore, the cytosolic pH of the RBCs is 7.1–7.3 and thus lower than the pH in plasma (~pH 7.4), which can cause an ion-trapping effect of basic drugs into the RBCs.

Accumulation into the RBCs can be used to prolong the lifetime of drugs in the bloodstream. It has been reported that binding of polymeric nanoparticles to the surface of the RBCs have increased their circulation half-life.[9] However, accumulation inside the RBCs can also be utilized to produce sustained-release drug delivery. Prodrug technology can be one attractive approach to increase the RBCs accumulation of a drug. Generally, there are not many sustained-release prodrugs reported in the literature; only few examples of amide or carbamate prodrugs that are hydrolyzed to their parent drug in plasma more slowly than conventional ester prodrugs.[10–12]

The aim of the present study is to show that a cyclohexyl sulfenamide prodrug (Scheme 1) can prolong the circulation lifetime of metformin. Sustained release of metformin is achieved by fast and efficient accumulation of more permeable sulfenamide prodrug into the RBCs, where the prodrug is rapidly converted to metformin by free thiols, such as reduced glutathione (GSH), and slowly released back into plasma. The intestinal absorption and oral bioavailability of metformin have previously shown to be improved by this more permeable prodrug most probably via increased passive diffusion across the cell membranes.[13,14] In the present study, pharmacokinetic properties of metformin and its cyclohexyl sulfenamide prodrug are evaluated in plasma and in whole blood after intravenous and oral administration in rats to determine their RBC partitioning and the mechanism of the sustained release of metformin from the prodrug. The sustained-release pharmacokinetic profile together with the improved oral absorption the prodrug may have the potential to reduce daily dosing of metformin therapy, not only the size of the tablets but also the number of tablets required to be taken to retain the glycemic control. Together with ameliorated gastrointestinal side effects, this would be expected to lead to better patient acceptance.

RESULTS

In present study, 50 μmol of metformin or its sulfenamide prodrug was administered to rats both intravenously ($n = 3$) and orally ($n = 3$). The collected blood samples were divided in two parallel proportions and each proportion was prepared as

Abbreviation used: RBC, red blood cell.

Correspondence to: Kristiina M. Huttunen (Telephone: +358-40-3553-684; E-mail: Kristiina.Huttunen@uef.fi)

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Scheme 1. Proposed bioconversion of the cyclohexyl sulfenamide prodrug by free thiols to metformin.

described in Supporting Information; one half representing separated plasma and cell precipitate and the other half the whole blood sample. The mean fraction of metformin partitioned into the RBCs was calculated from the RBC–whole blood ratio and represented as percentage (%) partitioned into the RBCs. After the intravenous administration of metformin, its accumulation was approximately 41.80 ± 9.89%. The fraction of released metformin in the RBCs was increased to 96.24 ± 1.12% after the intravenous administration of the sulfenamide prodrug of metformin. The fraction partitioned into the RBCs was concentration independent in both cases. However, the variation was greater with metformin than with the prodrug. In both cases, the partitioning was fast; approximately 42% (after metformin administration) and 96% (after prodrug administration) of metformin were found to be accumulated into the RBCs at the first time points (1.5 min). After oral administration of metformin, accumulation into the RBCs was almost same as after intravenous administration. In the case of the sulfenamide prodrug, the fraction of metformin found in the RBCs was reduced to approximately 65%–75% because of the bioconversion of the sulfenamide prodrug to metformin during its absorption and possible distribution to other cells.

The mean plasma and whole blood concentration–time curves of metformin after administration metformin or the prodrug intravenously are illustrated in Figure 1a. More close inspection of the first 10 min (Fig. 1b) reveals that metformin is eliminated more rapidly from the systemic circulation, whereas the prodrug is able to keep metformin levels higher for longer period of time. However, the maximum plasma concentration (C_{max}) was over seven times greater and the approximate area under the plasma concentration–time curve ($AUC_{0-\infty}$) nearly 1.5 times greater after intravenous administration of metformin relative to the sulfenamide prodrug (Table 1). This clearly shows that the prodrug was more efficiently distributed from plasma to the deep compartment. Furthermore, the volume of distribution (V_d) values, which were 3.40 ± 0.11 L for metformin and 9.06 ± 0.76 L for the sulfenamide prodrug in plasma and 3.00 ± 0.16 and 4.97 ± 0.15 L, respectively, in whole blood, represent that the prodrug was distributed more efficiently to the RBCs than metformin after intravenous administration. After RBC accumulation, the prodrug was rapidly converted to metformin, which in turn was slowly released back in plasma. As released metformin stayed for longer period of time in the systemic circulation, the exposure to metformin by the sulfenamide prodrug was much greater and therefore the whole blood $AUC_{0-\infty}$ of released metformin after intravenous administration of the sulfenamide prodrug was over 10 times greater relative to metformin itself (Table 1; Fig. 1). Therefore, the sulfenamide prodrug of metformin achieved an extended-release pharmacokinetic profile.

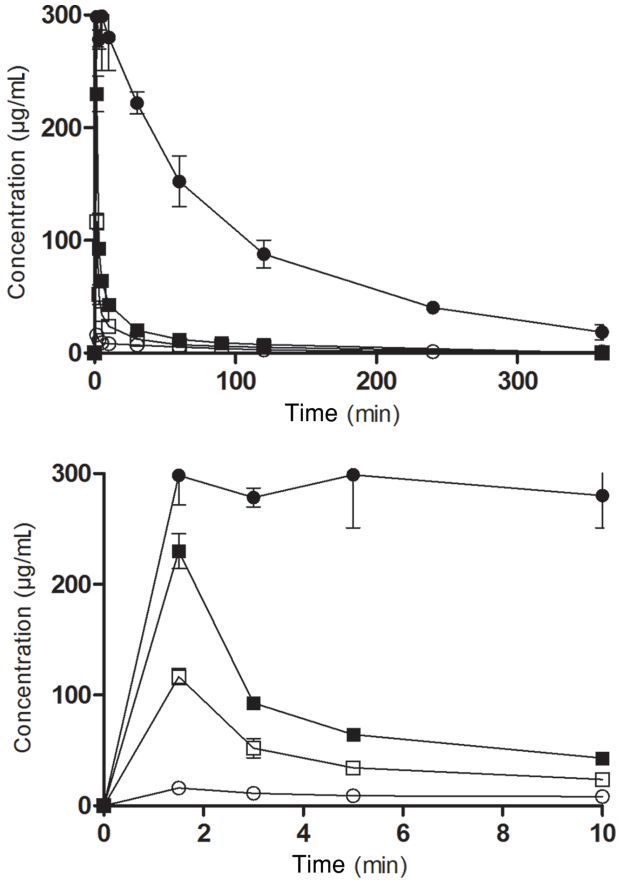

Figure 1. Mean plasma (□, open squares) and whole blood (■, filled squares) concentration–time curves of metformin after intravenous administration of metformin at the dose of 33.13 mg/kg (50 µmol) to rats ($n = 3$) and mean plasma (○, open circles) and whole blood (●, filled circles) concentration–time curves of metformin after intravenous administration of the sulfenamide prodrug of metformin at the dose of 48.67 mg/kg (50 µmol) to rats ($n = 3$) from 0 to 360 min (a) and from 0 to 10 min (b).

The mean plasma and whole blood concentration–time curves of metformin after oral administration metformin or the prodrug are illustrated in Figure 2. After oral administration, the situation was little more complex as metformin was only partly absorbed, and the proportion that was absorbed was quite rapidly eliminated from plasma. At the same time, the sulfenamide prodrug was absorbed more efficiently, but a portion was apparently converted to metformin during the absorption. However, as seen in Figure 2, the total amount of metformin in plasma was much higher after oral administration

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