Preclinical evaluation of pharmacokinetic–pharmacodynamic rationale for oral CR metformin formulation

David Stepensky³, Michael Friedman³, Wassim Srour³, Itamar Raz¹, Amnon Hoffman¹,*

¹Department of Pharmaceutics, School of Pharmacy, The Hebrew University of Jerusalem, P.O. Box 12065, Jerusalem 91120, Israel
²Diabetes Unit, Hadassah University Hospital, Jerusalem, Israel

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Abstract

We examined the pharmacokinetic (PK) and pharmacodynamic (PD) rationales to develop controlled release (CR) formulations of metformin. Unrestrained diabetic rats received the drug as intravenous bolus (i.v.), oral solution (p.o.), intra-duodenal bolus, 4-h infusion, or intra-colonic bolus. In addition, we developed two CR-gastroretentive dosage forms (CR-GRDF) that released the drug over 3 or 6 h (in vitro), and retained in the rats’ stomach for 8–10 h. Metformin exhibited flip-flop PK. The colonic absorption was low but sustained and was associated with highly variable glucose-lowering effects, thus providing a PK rationale to develop CR-GRDF. In addition, the glucose-lowering effect was greater following p.o. vs. i.v. administration, despite equivalent AUC, indicating a first pass PD effect, thus, adding a PD rationale to develop metformin CR-GRDF. When administered to the diabetic rats, CR-GRDFs produced bioavailability and extent of glucose-lowering effects that were similar to those of the duodenal infusion and p.o. metformin administration. These findings are attributed to the adsorption of metformin to the intestine that yields slow and prolonged absorption even following p.o. administration of drug solution. The data indicates that unless the CR formulation could significantly extend the absorption period, it is not likely to improve glucose-lowering efficacy. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Metformin; Pharmacokinetics; Pharmacodynamics; Controlled release; Gastroretentive

1. Introduction

Metformin is a biguanide glucose-lowering agent that has been widely used for management of NIDDM [1,2]. Unlike other biguanide drugs, metformin does not induce lactic acidosis. However, current metformin therapy is suboptimal as it is associated with a high incidence of gastrointestinal side effects, seen in about 30% of patients [3], and the drug is commonly administered at high doses (as oral tablets) 2–3 times per day, to achieve effective glucose-lowering treatment. Since the pharmacological mechanism of action of metformin differs from that of the other antidiabetic drugs, it has a very important role in NIDDM treatment. There are
continuing efforts to improve its pharmaceutical formulation in order to optimize therapy. These efforts have been focused on the development of oral sustained release (SR) preparations [4–7], as well as more sophisticated controlled release (CR) gastroretentive dosage forms [8]. Currently, there is an increased interest in developing new metformin formulations because the metformin US patent expires this year.

Metformin has properties of a strong base \( (pK_a 11.5) \) and is protonated at physiologic pH. The ionized metformin has a tendency to adsorb to the negatively charged intestinal epithelium, thus affecting the drug absorption pattern. The high polarity of this drug also dictates its fast renal elimination with no significant metabolism. In addition to these unique pharmacokinetic (PK) properties, the pharmacodynamics (PD) of metformin is rather complex and does not follow a direct relationship between plasma drug concentration and magnitude of effect [9,10]. For instance, we have found that augmented effects were produced when metformin was administered via the GI tract in comparison to i.v. bolus despite similar systemic exposure to the drug (AUC) [11]. These PK and PD complexities may directly affect the efficacy of a CR formulation. In addition, the rate and site of drug release from the pharmaceutical dosage form may also influence the magnitude and duration of the pharmacological response [12,13]. Therefore, an essential step prior to the development of advanced CR preparations should be to establish a biological rationale that takes into account both the PK and PD properties of the drug [14]. The present investigation was aimed to provide this information, and to assess whether optimization of metformin therapy may be accomplished by using oral CR drug delivery systems.

A conventional oral SR formulation releases most of the drug content at the colon, which requires that the drug will be absorbed from the colon. As there are indications that metformin has poor colonic absorption in healthy human subjects [15,16], it is important to assess whether the absorbed amount is sufficient to produce effective glucose-lowering activity in a diabetic state. In the case of insufficient colonic absorption, clinical advantage may be accomplished by a CR-gastroretentive dosage form that is retained in the stomach and produces a constant input of the drug to the sites of absorption at the upper part of the gastrointestinal (GI) tract. In this work, we developed CR-gastroretentive dosage forms of metformin and assessed their PK and PD in comparison to other modes of drug administration. To accomplish this, we employed a commonly used preclinical NIDDM model of streptozotocin-induced diabetes in rats. The CR-gastroretentive dosage forms were based on polymeric slow release matrix tablets of the metformin that are retained for several hours in rat stomach.

2. Materials and methods

2.1. Materials

Metformin hydrochloride was kindly provided by Teva Pharmaceutical Industries, Ltd., Netanya, Israel. Phenformin hydrochloride was purchased from Sigma Israel Chemicals Ltd., Rehovot, Israel. All other reagents used in this study were of analytical or HPLC grade.

2.2. Animals

Male Sabra rats (weighing 200–250 g, Animal Breeding Unit, The Hebrew University of Jerusalem, Israel) were used in this study. The animals were housed under standard conditions with a 12-h light/dark cycle with free access to water and food (regular rat chow), with exception of food deprivation during the period of blood sampling throughout the PK/PD experiments.

An experimentally induced model of type 2 diabetes (NIDDM) was produced by intraperitoneal injection of streptozotocin (50 mg/kg, dissolved in 0.01 M sodium citrate buffer, pH 4.0, freshly prepared to avoid decomposition). The degree of diabetes was assessed 5 days later by measurements of blood glucose levels (from the tail artery) using a Glucometer Elite™ blood glucose meter (Bayer, Bruxelles). Rats with blood glucose below 140 mg/dl following overnight fast and above 300 mg/dl at fed conditions were selected for the experiment. The baseline time course of blood glucose concentrations was checked on several occasions to ensure that the
rats’ metabolic status remained stable throughout the whole experimental period.

2.3. Surgery

To enable drug administration directly to the duodenum or to the colon, cannulas (PE-50 Intramedic Polyethylene Tubing, Becton Dickinson, MD) were implanted in the above-mentioned parts of the rat gastrointestinal tract; an additional indwelling cannula was placed in the jugular vein. The surgery was performed under ketamine/xylazine anesthesia (9% ketamine and 1% xylazine solution, i.p., 1.0 ml/kg weight) at least 5 days prior to initiation of the PK/PD experiments. The cannulas were exteriorized at the dorsal part of the neck, which made it possible to carry out the investigation in non-anesthetized and unrestrained rats.

2.4. CR tablets

Metformin tablets (CR tablets I) were prepared by direct compression using a 5 mm die. Each tablet weighed 76 mg and contained 25% w/w of Methocel K100M. An additional formulation (CR tablets II) was produced by ethylcellulose coating of CR tablets I. In vitro dissolution profiles of both types of tablets were determined using a Type II Tablet Dissolution Tester (dissolution media: 900 ml of DDW, 37°C, 100 rev./min) [5].

To ascertain the gastrentretivity of the CR matrix tablets, two metformin tablets (either CR tablets I or II) containing small metal pellets (as radiocontrast markers) [17] were inserted noninvasively with an intragastric tube into the rat’s stomach and their position was determined radiographically over 10 h (Philips Oralix X-Ray, Eindhoven, The Netherlands).

2.5. Experimental protocols

Metformin was administered to the diabetic rats (n=5–6) via the following modes:

1. intravenous (i.v.) bolus 200 mg/kg;
2. an oral bolus dose (p.o.) 450 mg/kg;
3. intraduodenal bolus 450 mg/kg;
4. a constant rate intraduodenal infusion (4 h, a total dose of 450 mg/kg);
5. intra-colonic bolus 450 mg/kg;
6. intragastric administration of CR tablets I (two tablets, a total dose of 450 mg/kg);
7. intragastric administration of CR tablets II (two tablets, a total dose of 450 mg/kg);
8. vehicle bolus administration (saline and double distilled water via intravenous and intraduodenal routes, respectively).

The doses of metformin were selected based on preliminary experiments to produce measurable glucose-lowering effects and similar systemic exposure (AUC) following i.v. and gastrointestinal modes of drug administration. For i.v. administration, metformin was dissolved in saline, and for p.o., intraduodenal and intra-colonic administration, metformin was dissolved in double distilled water.

For each mode of metformin administration, blood samples (120 μl) were collected from the tail artery at the following time points: 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 10 h. Blood glucose levels were immediately measured by Glucometer Elite™. Plasma samples were obtained from the rest of the blood (centrifugation at 3500 rev./min for 10 min) and stored at −20°C pending analysis.

2.6. Analytical procedures

Plasma metformin concentrations were determined by a HPLC method applying a Kontron chromatograph (pump 420, auto-injector 465, detector 432, Data Acquisition System 450, Kontron, Zurich, Switzerland) and LiChrospher 100 RP-18 column (Merck, Darmstadt, Germany). The mobile phase consisted of 0.01 M disodium hydrogenphosphate solution (pH=6.5), methanol, and acetonitrile (10:1.5:3, v/v). Detection was UV at 234 nm and phenformin was used as the internal standard. The quantitation limit was 100 ng/ml. Intra-assay and inter-assay coefficients of variation were 5 and 9%, respectively.

For each of the different modes of administration, individual rat’s glucose-lowering effect was calculated as percent of reduction in blood glucose values relative to the baseline of blood glucose at each time point:
% effect (at time $t$)

\[
\text{blood glucose following metformin administration} \times 100%.
\]

2.7. Data analysis

Analysis of pharmacokinetic and pharmacodynamic data was performed using the WinNonlin program (Version 1.1, Pharsight Corporation, Mountain View, CA) by means of the noncompartmental analysis method.

The Kruskal–Wallis ANOVA with subsequent Tukey’s multiple comparisons test were applied for analysis of area under the concentration vs. time curve (AUC), the area under the effect vs. time curve (AUEC), and nadir effect values following different modes of metformin administration. A $P$ value of less than 0.05 was termed significant.

3. Results

3.1. I.v. vs. p.o. administration

The pharmacokinetic and pharmacodynamic data following intravenous and p.o. bolus modes of metformin administration are presented in Figs. 1 and 2. The rate of elimination of metformin as determined by the log terminal slopes following the two modes of administration are distinctly different (0.31±0.06 h$^{-1}$ and 0.57±0.08 h$^{-1}$ for p.o. and i.v. bolus, respectively, $P<0.001$). This difference indicates that the pharmacokinetics of metformin following oral administration is characterized by flip-flop pharmacokinetics, which means that the rate of absorption is much slower than the renal elimination, and it governs the concentration–time profile of the drug.

The calculated values of AUC and AUEC following the studied modes of metformin administration are presented in Table 1. Similar magnitude of systemic exposure (as indicated by the AUC values) was produced following p.o. and i.v. bolus administration. However, the nadir effects and overall magnitude of glucose-lowering effect (AUEC) were significantly lower following i.v. administration in comparison to p.o. administration. As shown in Table 1, drug effect following i.v. bolus administration was also significantly lower in comparison to intraduodenal bolus and infusion. The PK and PD results following p.o. and intraduodenal bolus administrations of metformin were just the same, as expected. The relationship between the magnitude of glucose-lowering effect and systemic drug concentration was characterized by a profound counterclockwise hysteresis (graph not shown).

3.2. Intra-colonic administration

The concentration vs. time profile following intra-
Table 1
The observed area under concentration and effect curves and nadir effects for different modes of metformin administration (mean±S.D.)

<table>
<thead>
<tr>
<th>Mode of administration</th>
<th>AUC mg min/ml</th>
<th>AUEC mg min/dl (×10⁻⁷)</th>
<th>Nadir effect % of baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.v. bolus 200 mg/kg</td>
<td>6.19±1.19</td>
<td>22.7±9.0</td>
<td>21.8±5.2</td>
</tr>
<tr>
<td>P.o. bolus 450 mg/kg</td>
<td>5.65±0.65</td>
<td>53.2±18.4</td>
<td>44.1±6.8±d</td>
</tr>
<tr>
<td>Intraduodenal bolus 450 mg/kg</td>
<td>5.72±0.72</td>
<td>56.8±20.6</td>
<td>45.4±7.6±d</td>
</tr>
<tr>
<td>Constant rate intraduodenal infusion 450 mg/kg</td>
<td>5.90±1.23</td>
<td>55.3±22.4</td>
<td>42.3±8.8±d</td>
</tr>
<tr>
<td>CR tablets I 450 mg/kg</td>
<td>7.07±1.04</td>
<td>68.2±17.0</td>
<td>57.6±4.9±d</td>
</tr>
<tr>
<td>CR tablets II 450 mg/kg</td>
<td>5.81±1.35</td>
<td>48.9±19.6</td>
<td>36.3±7.1±d</td>
</tr>
<tr>
<td>Intra-colonic bolus 450 mg/kg</td>
<td>1.07±0.33</td>
<td>31.7±17.3</td>
<td>24.2±13.7</td>
</tr>
</tbody>
</table>

* Significantly different from other modes of administration at P<0.001.
* Significantly different from i.v. bolus at P<0.01.
* Significantly different from i.v. bolus at P<0.05.
* Significantly different from intra-colonic bolus at P<0.01.
* Significantly different from intra-colonic bolus at P<0.05.
* Significantly different from CR tablets I at P<0.01.

Colonic metformin administration in comparison to intraduodenal bolus administration is presented in Fig. 3. It can be seen that the overall extent of absorption (AUC) was at least fivefold lower compared to intraduodenal bolus, and also lower than that of the other modes of administration (see Table 1). However, the modest absorption of metformin from the colon of the diabetic rats was steady, and consistent between the tested animals. On the other hand, the corresponding glucose-lowering effect, shown in Fig. 4, was highly variable. This notable effect (approximately twofold lower compared to the intraduodenal bolus), represents the mean data of six rats, where a significant glucose-lowering effect was found in only 33% of the animals, and a negligible effect was observed in other rats. Due to the high variability of intra-colonic effects, a significant difference in AUC values was found only in comparison to CR tablets I. On the other hand, the mean nadir effect of intra-colonic bolus was significantly lower than the nadir effect of most other modes of administration (see Table 1).

### 3.3. CR tablets

An in vitro dissolution test showed that metformin was gradually released from the matrix tablets. All the drug content of the CR formulations was released within 3 h for formulation CR I, and within 6 h for formulation CR II. Following intragastric administration to the rats, both CR tablets I and II were
retained in the stomach for at least 8 h, as confirmed radiographically, thus proving to be gastroretentive in this model.

The metformin serum concentration vs. time curves following intraduodenal infusion, p.o. administration of metformin solution and administration of the gastroretentive CR tablets are presented in Fig. 5, and corresponding AUC values are summarized in Table 1. The overall amount of metformin that reached the systemic blood circulation (as indicated by AUC values) following all the abovementioned modes of metformin administration, was similar. The PK profile attained following administration of the CR I formulation was similar to that found after p.o. administration of metformin solution. On the other hand, the gastroretentive CR II formulation yielded a metformin concentration–time profile that was similar to a 4-h continuous infusion of the drug to the duodenum, i.e. it was characterized by a slow initial rate of elevation of metformin plasma concentrations and delayed peak levels in comparison to p.o. administration.

The time course of glucose-lowering effect following CR-gastroretentive tablets, p.o. and intraduodenal modes of metformin administration is shown in Fig. 6. The nadir effects were reached 4 h after the p.o. drug administration, similarly to those following administration of CR tablets I. Following intraduodenal infusion of metformin and administration of CR tablets II the glucose-lowering effect increased gradually over 6 h and then decreased. Significant differences of the nadir effect were observed between CR tablets I and II ($P<0.01$). However, the overall extent of glucose-lowering effect was similar when the same dose of metformin was given as a CR tablet, p.o. or intraduodenal bolus, or as a continuous infusion of the drug solution to the duodenum (see Table 1).

4. Discussion

Earlier studies examined the PK profile of oral sustained release dosage forms of metformin [4,5]. There is a pharmaceutical challenge in developing such dosage forms due to the high dose of metformin used clinically. Since regular metformin tablets contain 500–1000 mg of the drug, the dimensions of the tablet are close to the upper limit of the size that is plausible for patient intake. Due to the high water solubility of the drug, a considerable amount of polymeric excipients would be required to produce a slow release formulation of the drug, which makes the development of such a formulation much more complex. However, prior to the actual development stage, the PK and PD of the drug should be carefully examined, to identify the proper sites of absorption and action, and thereby the preferred input function.
4.1. Correlation between systemic drug concentration and magnitude of effect

As in many other cases, the development of CR dosage forms of metformin has been somewhat empirical and has been based on an intuitive rationale assuming a direct relationship between the drug plasma concentration and the magnitude of response elicited. The results of this investigation highlight the lack of a direct correlation between the magnitude of glucose-lowering effect (AUEC) and systemic drug exposure (AUC). For instance, while the elimination of the drug from the central circulation following i.v. bolus administration was completed within 1.5 h, the glucose lowering effect lasted much longer (Figs. 1 and 2). In addition, similar AUC values attained following administration of the drug to a peripheral vein and to the duodenum were not associated with a similar magnitude of response (AUEC values).

The data suggest that there is a first pass pharmacodynamic effect that produces an augmented effect when the drug reaches the systemic circulation following intestinal absorption, in comparison to administration of the drug directly to the systemic circulation. This finding indicates that the presystemic sites of metformin glucose-lowering action that are located in the GI tract and the liver are of major importance for the overall effect. Recently, we have shown a similar phenomena of first pass pharmacodynamic effect for niacin and bezafibrate where the magnitude of lipid-lowering action was found to be mode-of-administration-dependent [18,19]. The findings of improved glucose-lowering activity of metformin following sustained exposure of presystemic sites to the drug in the present study indicate that continuous intestinal absorption of metformin could be a preferred mode of administration, thus supporting the rationale for development of an oral CR formulation of the drug.

4.2. Colonic metformin administration

In general, a non-disintegrated solid SR dosage form given orally will arrive in the colon within 5 to 6 h, where the drug content will continue to be released. Therefore, it is essential to ascertain that colonic absorption of the drug is significant enough to produce the required pharmacological effect. Previous studies have found very low absorption of metformin from the colon [15,16,20]. However, these studies were performed in healthy volunteers and did not assess the kinetics and extent of the glucose-lowering effect. As discussed above, in the case of metformin, low drug absorption is not necessarily an indication for low extent of glucose-lowering effect because even limited exposure of presystemic sites of action may be sufficient to elicit meaningful drug response.

Despite the fact that pharmacokinetic data in our study shows sustained (but poor) colonic absorption in the diabetic rats, the corresponding PD data was too inconsistent. It was negligible in most cases, while pronounced in the other cases. The same degree of PD variability was found in our preliminary studies, indicating that this finding is typical, and represents the high degree of PD variability associated with colonic absorption of metformin. The PK and PD data suggest that optimization of oral dosage forms of metformin should not rely on colonic metformin absorption. Rather, the emphasis should be placed on adequate release of the drug in the upper parts of the GI tract.

4.3. Gastroretentive dosage forms

For drugs with a narrow absorption window, gastroretentive controlled release dosage forms may produce higher bioavailability and superior effects compared to immediate release and CR oral dosage forms. This is because gradual release of the drug from the CR dosage form in the stomach results in a continuous and prolonged input of the drug to the main absorption sites located in the small intestine. In addition, the indications of first pass PD effect of metformin (discussed above) also provide a PD rationale for sustained administration of the drug to the small intestine.

In our study, we developed two formulations of metformin with different release rates in vitro. The basic composition of the two CR formulations was the same with the slower release rate of CR II achieved by a thin ethylcellulose film coating. The dimensions of these non-degradable matrix tablets were relatively large in comparison to the rat’s stomach and ensured the gastroretentivity. This novel
preclinical noninvasive gastroretentive model enabled a better insight into the PK and PD impact of the CR-gastroretentive mode of administration. The simultaneous PK and PD data provided a comprehensive assessment of the actual advantages of this pharmaceutical approach to optimizing the pharmacological response.

We compared the concentration–effect–time data obtained by the CR-gastroretentive formulations to the two extreme cases: a bolus p.o. administration of the drug in aqueous solution (the simplest oral dosage form), and a zero order input function obtained by a continuous infusion of the drug directly to the duodenum. The two CR-gastroretentive dosage forms of metformin indeed exhibited different concentration–time profiles that directly correlated with their in vitro release profiles. The CR II formulation closely resembled the PK profile of duodenal infusion, thus providing assurance that the gastroretentive dosage form developed in this work and assessed in this preclinical model was appropriate.

The unique finding of this work is the fact that there were no statistically significant differences between the amount of drug absorbed when the drug was given by a rapid bolus mode of administration or by slow infusion to the duodenum. These results are due to the high affinity of metformin to the negatively charged intestinal wall. For instance, Wilcock and Bailey have shown that following oral administration of metformin to streptozotocin diabetic mice, high concentrations of the drug are retained for several hours in the upper parts of the GI tract [21]. This adsorption phenomenon derives from the basic properties of the biguanide molecule (positive charge) and produces a depot-like situation. As a result, metformin is released from its GI adsorption sites in a sustained manner thereby yielding an absorption rate profile that mimics the SR formulation [21,22]. This property of metformin, that is clearly seen from the flip-flop PK of p.o. administration of aqueous solution of the drug (Fig. 1), suggests that pharmaceutical manipulations that modify the release rate do not seem to improve the extent of metformin absorption.

The extent of glucose-lowering effect was similar for the two CR formulations, oral solution, and intraduodenal administrations. On the other hand, different rates of metformin release from the formulations led to differences in the nadir effect. Although this finding may suggest that modifications in the CR formulation produce different drug effects, it should be noted that the faster release CR formulation yielded effects that did not differ from simple oral solution, and the slower formulation, CR II, produced a lower nadir effect than the oral solution and the CR I tablets. Thus, practically, the changes in the CR formulation did not improve the glucose lowering effect of metformin.

The PK and PD profiles obtained following the p.o. and intraduodenal infusion construct guidelines for further development of improved oral dosage forms of metformin. They indicate that unless the CR formulation can significantly extend the absorption period, it is not likely to improve glucose-lowering efficacy. As shown in this work, continuous infusion of the drug for 4 h to the absorption sites at the upper GI tract was not sufficient.

Despite the limited advantages in bioavailability and extent of glucose-lowering effect that are expected from CR-gastroretentive dosage forms of metformin, they may be clinically beneficial, due to reduced incidence of gastrointestinal adverse effects, that could possibly result from the gradual input of the drug to the upper GI tract [5]. However, this issue has to be clinically demonstrated.

5. Conclusions

The rat model was found to be an effective strategy for the evaluation of the PK and PD of CR-gastroretentive dosage forms, thereby providing a means to assess the PK and PD rationale to develop these formulations in order to optimize drug treatment. Although initially there seemed to be both a PK and a PD rationale to develop such formulations, the actual findings indicate that the differences in input rate of the drug to the upper GI region did not significantly affect the extent of metformin action. This interesting finding evolves most probably from the affinity of the positively charged drug to the GI wall, thus yielding a slow rate of drug absorption, even following p.o. administration of drug solution. The work stresses the importance of
establishing a PK and PD rationale prior to the development of CR formulations.

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References