METFORMIN RETENTION INDEPENDENT OF RENAL FAILURE IN INTESTINAL OCCLUSION

J.D. LALAU (1), J.M. RACE (1), F. ANDREELLI (2), C. LACROIX (3), J.P. CANARELLI (4)

SUMMARY - Metformin is eliminated by the kidneys, and metformin accumulation has always been noticed in oligo-anuric patients. We have reported an exception to the rule with the case of a metformin-treated patient having metformin accumulation contrasting with a mild increase in serum creatinine in the context of a volvulus of the sigmoid colon. This case prompted us to examine the association between intestinal occlusion and plasma metformin concentrations. For this purpose, we developed an experimental animal model of mechanical obstruction of the intestine. Rats were pre-treated during 3 weeks via drinking solution at a dose of ≈ 100 mg/kg/day of metformin. They underwent at day 0 either sham-operation (n = 7) or operation (n = 8) to place a plastic tube around the ileum near the ileocaecal valve. Metformin administration was pursued on days 1, 2, and 3 giving a single dose of 100 mg/kg by intragastric gavage. Four days after the surgery, i.e. 24 h after the last metformin administration, the surviving intestinal obstructed rats (n = 8) developed overt intestinal dilation but no biochemical abnormality compared to sham-operated animals (n = 7; arterial lactate concentrations respectively 4.87 ± 0.63 mmol/l and 3.97 ± 0.30 mmol/l, NS, and serum creatinine concentrations 69.0 ± 1.7 µmol/l and 68.7 ± 1.9 µmol/l, NS). By contrast, there was a striking difference with regard to metformin concentrations, decreasing from 2.95 ± 0.94 mg/l at day 0 to 0.12 ± 0.03 mg/l at day 4 (p < 0.001) in the sham-operated group but remaining unchanged (1.65 ± 0.76 mg/l and 1.61 ± 0.51 mg/l) in the operation group. In conclusion, this is the first experiment showing that intestinal occlusion may be responsible for metformin retention in the absence of renal failure. Whether this observation may be relevant to other drugs remains to be established.

Key-words: metformin, lactic acidosis, drug accumulation, intestinal occlusion, renal failure.

RESUME - Retention de metformine independante de la fonction re nale lors d'une occlusion intestinale. La metformine est eliminee par les reins, et l'accumulation de metformine a toujours ete rapportee chez des patients oligo-anuriques, sauf dans un cas. Il s'agissait d'un patient traité par metformine et qui a developpe dans le contexte d'une occlusion intestinale liee a un volvulus du sigmoide une accumulation de metformine contrastant avec une faible el evation de la creatininemie. Cette observation nous a conduit a rechercher l'impact de l'occlusion intestinale sur les concentrations plasmatiques de metformine. Nous avons developpe pour cela un model animal experimental d'obstruction intestinale mecanique. Des rats pre traites pendant 3 semaines par ≈ 100 mg/kg/jour de metformine apporte par l'eau de boisson ont ete operes a J0 soit pour pseudo-occlusion, soit pour occlusion vraie a l'aide d'un fragment de tube plastique place en manchon autour de l'illeon a la jonction ileo-caecale. L'administration de metformine a ete poursuivie les jours 1, 2, et 3 par gavage gastrique a la dose de 100 mg/kg. Le quatrieme jour apres la chirurgie, soit 24 h apres la derniere administration de metformine, les animaux survivants du groupe "occlusion" (n = 8) ont developpe une obstruction intestinale patente mais pas d'anomalie biochimique comparativement aux animaux de l'autre groupe (n = 7) : lactatemie arterielle respectivevement 4.87 ± 0.63 mmol/l et 3.97 ± 0.30 mmol/l, NS, et creatininemie 69.0 ± 1.7 µmol/l et 68.7 ± 1.9 µmol/l, NS. Il y avait en revanche une difference frappante de metforminemie, decroissante dans le groupe "pseudo-occlusion" de 2.95 ± 0.94 mg/l a J0 a 0.12 ± 0.03 mg/l a J4 (p < 0.001) et demeurue inchanges dans l'autre groupe (1.65 ± 0.76 mg/l et 1.61 ± 0.51 mg/l). En conclusion, il s'agit de la premiere demonstration experimentale d'une retenion de metformine sans insuffisance re nale, liee a une occlusion intestinale. Il reste a etablir dans quelle mesure cette observation peut etre etendue a d'autres medications que la metformine.

Mots-cles : metformine, acidose lactique, accumulation medicamenteuse, occlusion intestinale, insuffisance re nale.

Metformin is a drug widely used as an effective antihyperglycemic agent for the treatment of type 2 diabetes mellitus. Although rarely, metformin therapy has been associated with lactic acidosis [1, 2]. Accumulation of the drug has been implicated in the development of lactic acidosis. Since metformin is eliminated as the parent compound exclusively by the kidneys [3], accumulation of the drug can only be caused by renal failure or overdosage.

The observation that plasma metformin concentrations were very high in a man presenting with a volvulus of the sigmoid colon secondary to a megacolon but no overt renal failure was therefore unexpected [4]. With the exception of electrolytes disturbances due to duodenal obstruction or vomiting, biochemical abnormalities are indeed uncommon in mechanical obstruction of the intestine. It is in particular not known that this abdominal processus may lead to the retention of a drug. Therefore, in an attempt to clarify the relationship between metformin accumulation and intestinal occlusion per se, i.e. regardless of renal function, we have constructed an animal model mimicking the case report to examine the effect of intestinal occlusion on metformin kinetics.

**MATERIAL AND METHODS**

**Case report**

The case of a metformin-treated patient with intestinal occlusion, lactic acidosis, and major metformin accumulation contrasting with mild increase in serum creatinine has been reported in details elsewhere [4]. Briefly, this was a 65-year-old man with type 2 diabetes treated with metformin 850 b.i.d. and admitted with distended abdomen, abdominal pain and vomiting. Biochemistry showed the patient to have: arterial pH 7.13, arterial lactate 24.5 mmol/l, serum creatinine 129 µmol/l, and plasma metformin concentration 61 mg/l (normal 0.6 ± 0.5 mg/l). An exploratory laparotomy revealed a volvulus of the sigmoid linked to a megacolon.

**Animal study**

**Animals**

The experiment was performed on Wistar adult female rats weighing 250 g purchased from the Centre d’Elevage d’Ardenay (France). They were fed on a standard laboratory chow until the end of the experiment.

**Metformin pre-treatment**

Animals were treated with metformin via drinking solution during 3 weeks before surgery (day 0) and the day after. Knowing a daily drink intake of ≈ 50 ml per animal, metformin was added in the drinking solution at the concentration of 5g/l in order to deliver ≈ 100 mg/kg/day.

**Surgery**

Rats were divided into two groups: the sham-operated group (n = 8), and the mechanical obstruction group (n = 13). The surgical intervention was performed on day 0 at 8 AM. Animals underwent midline laparotomy under ether-anaesthesia. The sham-operated animals then had gentle manipulation of the small bowel before closing. The intestinal occlusion animals had a ring of sterile plastic cannula (nasobronchial cannula, Pharma-Plast ref. 12, Lynge, Danemark) placed and tied around the ileum near the ileocaecal valve in order to create a simple model of progressive non ischemic obstruction. The diameter of the cannula was chosen so as to encircle the intestine without narrowing it (Fig. 1).

**Metformin therapy after surgery**

Because of the risk of reduced water intake due to the occlusion, metformin was given on day 1, 2, and 3 by direct intragastric gavage as a single dose of 100 mg/kg.

**FIG. 1.** Animal model of progressive non ischemic obstruction: the intestinal obstructed animals had a ring of plastic cannula placed around the ileum near the ileocaecal valve. The diameter of the cannula was chosen so as to encircle the intestine without narrowing it (bottom). See Methods.
End of the experiment

On day 4 after surgery and twenty-four hours after the last metformin administration, surviving animals (7 in the sham-operated group and 8 in the occlusion group) were sacrificed by ether overdose at 8 AM. The abdomen was incised to look for intestinal dilation.

Blood samples

Blood was drawn for measurement of metformin on two occasions:
- on day 0 during the surgical procedure by incision of the tail. This was to ascertain the metformin intake from drinking solution and to allow ultimately the comparison in each experimental group between pre- and postsurgical metformin concentrations;
- on day 4 on sacrifice immediately after watch from the aorta before the bifurcation point of the arteries using fine heparinized needles. From these samples of day 4, the following biochemical parameters were also determined: glucose, creatinine, lactate, and pH.

Analytical techniques

Glucose and creatinine were determined using an autoanalyser (Technicon DAX 24, Bayer Diagnostics). Arterial blood pH was measured using an electrode (ABL System, Radiometer, Copenhagen, Denmark). Lactate was measured immediately after blood collection by an enzymatic method without deproteinization using a spectrophotometer (Boehringer Manheim, Meylan, France). L(+-)-lactate dehydrogenase was used; it is specific for L(+-)-lactate (relative rate = 100) and does not cross-react with D(-)-lactate. Plasma metformin levels were measured in duplicate in the same laboratory with high-performance liquid chromatography in accordance with the method of Lacroix et al. [5].

Statistical analysis

Results are presented as means ± SE. Comparisons were made using the Mann–Whitney’s non parametric test.

RESULTS

Metformin concentrations before occlusion (day 0)

The mean value was 2.95 ± 0.94 mg/l in the sham-operated group and 1.65 ± 0.76 mg/l in the intestinal occlusion group. The difference was not significant, due to the large variation within each group.

Evolution after surgery

During the period from surgery to sacrifice, animals of the occlusion group were not less reactive than those of the sham-operated group. However, 5 animals died in the first group (2 at day 1, 1 at day 2, and 2 at day 3), while 1 animal died in the other group (at day 2).

Data on sacrifice (day 4 after surgery)

Sham-operated animals were noted to have normal bowels, i.e. with a diameter = 0.3 cm. By contrast, all the intestinal obstruction animals had marked dilation of the small bowel proximal to the obstruction, with a diameter > 1 cm. There was no significant difference in biochemical parameters between the intestinal occlusion group and the sham-operated group: blood glucose 9.91 ± 0.58 mmol/l and 9.07 ± 0.31 mmol/l, respectively, serum creatinine 69.0 ± 1.7 µmol/l and 68.7 ± 1.9 µmol/l, blood lactate 4.87 ± 0.63 mmol/l and 3.97 ± 0.30 mmol/l, and arterial pH 7.69 ± 0.03 and 7.59 ± 0.03. By contrast, compared to day 0 values, plasma metformin concentrations varied differently in the two experimental groups, dropping to a low value in the sham-operated group (0.12 ± 0.03 mg/l, p < 0.001 compared to day 0) while not significantly changing at 1.61 ± 0.51 mg/l in the occlusion group (Fig. 2). In the latter, all individual metformin values were above those of the sham-operated group.

FIG. 2. Plasma metformin concentrations in the two experimental animal groups. Blood samples were obtained before (day 0) and four days (day 4) after either sham-operation or occlusion (24 hours after the last metformin administration by intragastric gavage).
DISCUSSION

This is the first time that results of this nature, obtained in rats with intestinal obstruction, have been reported. Present findings are in perfect accordance with those of a metformin-treated patient with lactic acidosis and intestinal occlusion previously reported [4], both clinical and experimental situations showing retention of metformin associated with intestinal occlusion in the absence of marked increase in serum creatinine.

In the present experiment, the rats were given metformin in drinking water to deliver a dose of about 100 mg/kg/day. This dose corresponds, on a weight-related basis, to twice the maximal clinical daily dose in man. However, since rats are less sensitive to the metabolic effects of metformin than man [6] and since metformin clearance is 4 to 5 times that of creatinine [3, 7, 8], the present selection of the metformin dose was considered appropriate for experimental purposes.

The route of administration of metformin explains the great variability of plasma metformin levels obtained just before the occlusion within each experimental group. However no significant difference was evidenced between the two groups, allowing the final comparison of metformin concentrations between these groups, that ultimately evidenced a 25-fold decrease in the sham-operated group but no change in the occlusion group.

Whether the striking difference in the evolution of metformin concentrations between both experimental groups is due to abnormal decrease of these concentrations in sham-operated animals or to abnormal lack of decrease in operated animals must be determined. In this regard, data on pharmacokinetics of metformin are available, in particular in rodents, to favour the second hypothesis of a metformin retention due to occlusion. Indeed, metformin is normally rapidly eliminated. Studies of accumulation of metformin by plasma and tissues of the normal and diabetic mouse have shown that plasma metformin concentrations after oral administration of 50 mg/kg/day had declined from 61.5 ± 8.0 µmol/l at 0.5 h to 2.4 + 0.9 µmol/l at 24 h, and that the greatest accumulation of metformin was observed in the gastrointestinal tract where the concentrations of metformin declined by 24 h to < 2% of peak values [9]. After oral administration of radio-labelled metformin in mouse, autoradiographic images of whole-body sections have shown that metformin was not stored in the organism, and that no radioactivity was detected at 18 h. Such findings in mouse are consistent with studies in man showing that metformin is rapidly eliminated unchanged as the parent compound [3, 7, 8]. Excretion data revealed that > 95% of the absorbed metformin dose is excreted in the urine in 24 h [7, 8]. The very high clearance of metformin indicates that it enters the renal tubules by glomerular filtration and is actively secreted by cells lining the proximal region.

What could account for the difference in metformin concentrations is not easy to explain. Knowing that metformin concentrations in the intestinal wall are normally 10-100 times greater than plasma concentrations, whether the drug is administered enterally or parenterally [9, 10], and that metformin has a slow disappearance from the intestine compared to other tissues [10], a first possibility is that the intestinal wall traps metformin. This would reinforce the concept that the intestine might represent the deep compartment of metformin distribution first proposed by Noel [11]. However, this does not explain why metformin once retained is not eliminated from plasma, since the drug is normally rapidly eliminated, as already stated.

Thus, metformin retention implies finally defective elimination, which may be of glomerular or tubular origin. In intestinal occlusion, the deterioration of general condition and/or transudation of fluids into the peritoneal cavity may cause hypovolemia and glomerular hypofiltration. However, creatinine concentrations were strictly identical in both experimental groups. Moreover, even if creatinine clearance does not parallel glomerular filtration rate in the special situation of renal failure [12], the creatinine clearance may not exceed the inulin clearance by more than 50 per cent while metformin clearance is 4 to 5 times that of creatinine [3, 7, 8]. Thus, the hypothesis of metformin retention implying the glomerulus may reasonably be excluded. An alternative possibility is that of an interaction between organic cation transport systems of the renal proximal tubule which normally eliminate biguanides [13] and an unknown substance possibly related to the intestinal occlusion. For example, the organic cation cimetidine has been reported to inhibit metformin elimination [14].

It should be noted that there was no relevant difference in lactate levels between both experimental groups. This is because there was metformin retention following intestinal but no true metformin accumulation. Indeed, plasma metformin concentrations were high after occlusion relatively to the low values in the other group but not in absolute terms, being lower than the maximal plasma concentrations of normal mice given acutely the same dose of metformin orally [9]. Finally, metformin-induced lactic acidosis did not occur in obstructed animals, as it was the case in the clinical report but with major metformin accumulation. In this regard, it remains to know whether the development of true metformin accumulation in our experimental model could occur with more time than that elapsed between surgery and sacrifice.

CONCLUSION

In the light of these and data presented previously, intestinal occlusion may represent a new mechanism
for metformin accumulation. Whether this may concern other drugs than metformin remains to be determined.

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REFERENCES

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