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Comparison of potential risks of lactic acidosis induction by biguanides in rats

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ABSTRACT

Lactic acidosis has been considered to be a side effect of some biguanides, after phenformin was withdrawn from the market because of its association with lactic acidosis. The potential of lactic acidosis induced by biguanides at human therapeutic exposure levels, however, has not been examined. Then, we compared the risk of lactic acid at doses providing exposure levels comparable to human therapeutic doses. Metformin and phenformin were orally administered to rats for up to 28 days, and plasma drug concentrations and blood lactic acid levels were examined. Metformin did not elevate lactic acid levels at the dose corresponding to higher systemic drug exposure than human therapeutic level, even for repeated doses. In contrast, phenformin elevated lactic acid levels at the dose corresponding to lower exposure than human therapeutic level, and sustained high levels were observed up to 24 h post-dose; furthermore, these changes were enhanced by repeated doses. Direct comparison at each rat equivalent dose clearly indicated that lactic acid levels of phenformin were higher than those of metformin. These non-clinical findings suggest that metformin dose not increase lactic acid levels like phenformin does, and therefore may not increase the risk for lactic acidosis at human therapeutic exposure level.

1. Introduction

Metformin, a biguanide oral antihyperglycemic agent, is widely used for type 2 diabetes mellitus. The principal mode of action of biguanides has been thought to be inhibition of hepatic gluconeogenesis, which could decrease the usage of lactic acid (Bailey, 1992, 1993; Wollen and Bailey, 1988). Therefore, metformin, as well as other biguanide agents, may intensify the accumulation of lactic acid under certain circumstances (Wiholm and Myrhed, 1993). Lactic acidosis, one of the most severe adverse effects of biguanide treatments, is a life-threatening condition characterized by low arterial pH (<7.35) and elevated arterial lactate levels (5.0 mEq/L (45 mg/dL) in humans) (Mizock and Falk, 1992; Stacpoole, 1993). One of the biguanides, phenformin was withdrawn from the market as an "imminent hazard" by the FDA in 1977 (http://ftp.resource.org/courts.gov/c/F2/587/587.F2d.1128.76-1308.html), because it caused hundreds cases of lactic acidosis, which has a 50% fatality rate. The estimated rate of phenformin-associated lactic acidosis ranged from 40 to 64 cases per 1,00,000 person-years (Aguilar et al., 1992; Bailey, 1992). In contrast, the association of metformin with lactic acidosis shows a lower incidence of approximately 0–9 cases per 1,00,000 person-years, a 10- to 20-fold lower incidence than for phenformin (Bailey and Turner, 1996; Berger, 1985; Defronzo et al., 1995; Misbin et al., 1998; Stang et al., 1999), and paper and review indicate that metformin is not associated with an increased risk of lactic acidosis (Brown et al., 1998; Salpeter et al., 2010). Several animal experiments were conducted to compare the potential of lactic acidosis induced by biguanides previously (Jalling and Olsen, 1984; Owen et al., 2000; Schlienger et al., 1979; Wang et al., 2003); however, the potential of lactic acidosis in human exposure of therapeutic doses has not been compared among these drugs, because these studies did not fully reflect conditions of clinical use.

In the present study, we evaluated the effects of metformin or phenformin on blood lactate levels in rats following a repeated oral administration of these drugs, and determined concentrations of the drugs in plasma. Furthermore, we determined rat doses that provided comparable exposure to human therapeutic dose exposure levels of each drug (rat equivalent dose), and then compared the lactic acid levels induced by those equivalent doses.

2. Materials and methods

2.1. Chemicals

Metformin hydrochloride (101.9%) was obtained from the manufacturing division of Dainippon Sumitomo Pharma. Co. Ltd.

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(Osaka, Japan). Phenformin hydrochloride (100.5%) was purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA).

2.2. Animals

Male and female F344/Jcl rats (SPF) were purchased from CLEA Japan, Inc. (Ishibe Breeding Facility, Shiga, Japan). Animals were housed individually in stainless steel wire mesh cages, maintained in an air-conditioned animal room (temperature: 21.0–25.0 °C, relative humidity: 40.0–70.0%) with a 12-h light–dark cycle (fluorescent lighting from 7:00 to 19:00) and 16 clean, fresh air changes per hour. They received pelleted basal diet (CRF-1; Oriental Yeast Co., Ltd., Chiba, Japan) and tap water *ad libitum*. After quarantine/accumulation, administration was started at 7 weeks of age (males: 118–145 g, females: 97–123 g body weight).

2.3. Animal study design

Animals were randomly assigned to 14 groups as shown Table 1: 5 or 8 animals/sex/group for single-dose, and 10 or 13 animals/sex/group for repeated-dose administration were allocated. Three animals in each group were used for determination of plasma drug concentration, and the others used for determination of blood lactic acid level. The dosage levels of metformin hydrochloride were 50, 100, 200 mg/kg/day, and those of phenformin hydrochloride were 100, 200, 400 mg/kg/day. These dosages were selected as dosage levels expected to be comparable to the human exposure levels of therapeutic doses of each drug.

Both drugs were suspended in 0.5% methylcellulose aqueous solution. Dosing formulation volumes were calculated based on latest body weights of animals (10 mL/kg). The dosing formulations were drawn into polypropylene syringes while being stirred using a magnetic stirrer, and were promptly administered orally via intubation. Treatment period of repeated dosing group was 28 days.

Observation for clinical signs and mortality was conducted daily, and body weight was measured twice a week throughout the treatment period. On Days 1 and 28, blood samples were obtained at 1, 2, 6 and 24 h post-dosing (4 time points) via jugular vein under ether anesthesia.

This study was conducted in compliance with the "Law for Partial Amendment of the Law Concerning the Protection and Control of Animals (Law No. 68, Jun. 22, 2005, Japan)" and in-house guidance, fully accredited by AAALAC International.

Table 1
Study design.

Drug	Dosage (mg/kg)	Number of animals							
		Day 1				Day 28			
		LA		PK		LA		PK	
		M	F	M	F	M	F	M	F
Control	0	5	5	–	–	10	10	–	–
Metformin hydrochloride	50	5	5	3	3	10	10	3	3
	100	5	5	3	3	10	10	3	3
	200	5	5	3	3	10	10	3	3
Phenformin hydrochloride	100	5	5	3	3	10	10	3	3
	200	5	5	3	3	10	10	3	3
	400	5	5	3	3	10	10	3	3

Control: 0.5% methylcellulose aqueous solution.
LA: group for determination of lactic acid levels, PK: group for determination of plasma drug concentrations, M: male, F: female.
Rats were repeatedly treated with drugs once a day for 28 days, and blood samples were obtained at 1, 2, 6, and 24 h post-dosing on Days 1 and 28 via jugular vein.

2.4. Drug exposure levels

Blood samples were treated by heparin and promptly chilled in ice water, and then centrifuged at 1600*g* for 10 min at 4 °C, and supernatant (plasma) was transferred into micro-tubes.

Metformin or phenformin concentrations in plasma were analyzed using liquid chromatography (1100 Series; Agilent Technologies Inc., CA, USA)–tandem mass spectrometry (TSQ; Thermo Fisher Scientific Inc., MA, USA) following deproteinization of plasma with methanol. Pharmacokinetic parameters were calculated from the concentrations of metformin or phenformin in plasma: maximum observed plasma concentration (C_{max}), time to reach the maximum observed plasma concentration (T_{max}), and the area under the plasma concentration–time curve (AUC_{0-t}, trapezoidal rule).

2.5. Blood lactic acid levels

Blood samples were placed in polypropylene sample tubes containing 0.8 mol/L perchloric acid of the same volume as the blood, mixed well, and allowed to stand for approximately 1 h at room temperature. These samples were centrifuged (1600*g*, 10 min, 4 °C), and the deproteinized supernatant fractions were used as samples for analysis of lactic acid. Lactic acid was measured by means of an enzymatic method using a Hitachi 7180 automatic analyzer (Hitachi, Ltd., Japan).

2.6. Statistical analysis

Mean and standard deviation of lactic acid levels in each group were calculated. Statistical significance was evaluated as follows: homogeneity of variance of groups was analyzed using Bartlett's test (5% level of significance), and then in case of homogenous variance of each group, Dunnett's was performed for statistical difference. In case of heterogamous variance of each group, Steel's test was performed. Differences were evaluated at a two-tailed 1% or 5% level of significance and presented as $P < 0.01$ and 0.05.

3. Results

3.1. Drug exposure levels

Tables 2 and 3 show pharmacokinetic parameters calculated from metformin and phenformin concentrations in plasma. C_{max} and AUC_{0-t} values tended to increase depending on dose levels for metformin or phenformin. There were no changes in C_{max} or AUC_{0-t} values of metformin between Day 1 and 28, and thus repeated doses of metformin did not change systemic exposure. On the contrary, C_{max} and AUC_{0-t} values of phenformin of Day 28 were higher (C_{max}: 1.5- to 7.5-fold, AUC_{0-t}: 1.2- to 3.2-fold) than those of Day 1, and an accumulation of phenformin was observed after repeated doses.

AUC at human therapeutic dose is 24,400 and 549–779 ng h/mL for metformin (2550 mg/patient/day) (Sambol et al., 1996) and phenformin (50 mg/patient/day) (Oates et al., 1982, 1983), respectively. On the basis of systemic exposure in rats and humans, human therapeutic dosages of metformin and phenformin were comparable to rat dosages as follows: for metformin, 100 mg/kg/day in both sexes for single- or repeated-dose; for phenformin, 200–400 mg/kg/day in both sexes for single-dose, and 200 mg/kg/day in males, 100–200 mg/kg/day in females for repeated-dose.

3.2. Blood Lactic acid levels

Blood lactic acid levels of metformin-treated animals were almost comparable with control levels on Day 1 and 28 in all dose