Research Paper

Rapamycin treatment benefits glucose metabolism in mouse models of type 2 diabetes

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ABSTRACT

Numerous studies suggest that rapamycin treatment promotes insulin resistance, implying that rapamycin could have negative effects on patients with, or at risk for, type 2 diabetes (T2D). New evidence, however, indicates that rapamycin treatment produces some *benefits* to energy metabolism, even in the context of T2D. Here, we survey 5 mouse models of T2D (KK, KK-Ay, NONcNZO10, BKS-*db/db*, TALLYHO) to quantify effects of rapamycin on well-recognized markers of glucose homeostasis within a wide range of T2D environments. Interestingly, dietary rapamycin treatment did not exacerbate impaired glucose or insulin tolerance, or elevate circulating lipids as T2D progressed. In fact, rapamycin *increased* insulin sensitivity and reduced weight gain in 3 models, and decreased hyperinsulinemia in 2 models. A key covariate of this genetically-based, differential response was pancreatic insulin content (PIC): Models with low PIC exhibited more beneficial effects than models with high PIC. However, a minimal PIC threshold may exist, below which hypoinsulinemic hyperglycemia develops, as it did in TALLYHO. Our results, along with other studies, indicate that beneficial or detrimental metabolic effects of rapamycin treatment, in a diabetic or pre-diabetic context, are driven by the interaction of rapamycin with the individual model's pancreatic physiology.

INTRODUCTION

Rapamycin increases lifespan in mice and several other organisms [1-8], presumably via inhibition of mTORC (mechanistic Target Of Rapamycin Complex). mTORC activation is associated with the response to nutrients, and it is involved in the regulation of insulin and glucose homeostasis [9-13]. Both glucose and insulin can hyperactivate mTOR, creating a negative feedback loop via S6 kinase to degrade insulin receptor substrate 1/2, impairing insulin signaling, and leading to insulin resistance [14]. Rapamycin can reduce glucose-stimulated insulin secretion and pancreatic islet cell proliferation in mice and in cell lines [reviewed in 9, 10]. Studies of humans taking rapamycin after tissue transplant or as an anti-cancer agent have shown that glucose metabolism is unaffected in most patients; however, a minority (~3–22%) can develop hyperglycemia, with rates depending on dose of rapamycin, patient population, and individual study [9]. Because rapamycin may alter glucose homeostasis, researchers have evaluated effects of rapamycin on glucose clearance, insulin sensitivity, and adiposity in mice [10-22]. Studies principally used C57BL6 or heterogeneous stocks, but a few have tested models of type 2 diabetes (T2D), such as KK/Hl [15], NONcNZO10 [21], and BKS-*db/db* [17, 18]. In normoglycemic strains, rapamycin treatment delays glucose clearance but reduces weight gain and adiposity (particularly when mice are fed a high fat diet). However, rapamycin inconsistently affects insulin sensitivity and serum insulin values. Effects of rapamycin on glucose clearance in already glucose intolerant strains have not been reported, but increased insulin sensitivity has been shown in the insulin resistant BKS-*db/db* mouse [17]. Rapamycin treatment also reduces weight gain in strains that are models of T2D [17, 18, 21]. Furthermore, effects can change over time. In a non-diabetic heterogeneous mouse model, negative effects on glucose clearance and insulin sensitivity faded with treatment duration [13]. It is difficult to compare these studies because of the differences among the methods of rapamycin treatment

(intraperitoneal injections or encapsulated in diet), fat content of diets, lengths of treatment, and phenotypic evaluations. Therefore, the present study analyzes the effects of rapamycin across a broad range of diabesity models using a consistent protocol to delineate both common and idiosyncratic responses to the compound. The 5 models selected represent distinct type 2 diabetic etiologies (Table 1), with differing severities of obesity, hyperglycemia, and hyperinsulinemia [23-28].

RESULTS

We analyzed effects of rapamycin treatment on 10 T2D phenotypes in 5 models of diabetic mice that represent distinct etiologies. Encapsulated rapamycin was administered through the diet (rapa-treatment), which replicates the alimentary mode of administration used for humans. We evaluated responses, as T2D initially progressed, through 2–6 weeks of rapa-treatment that

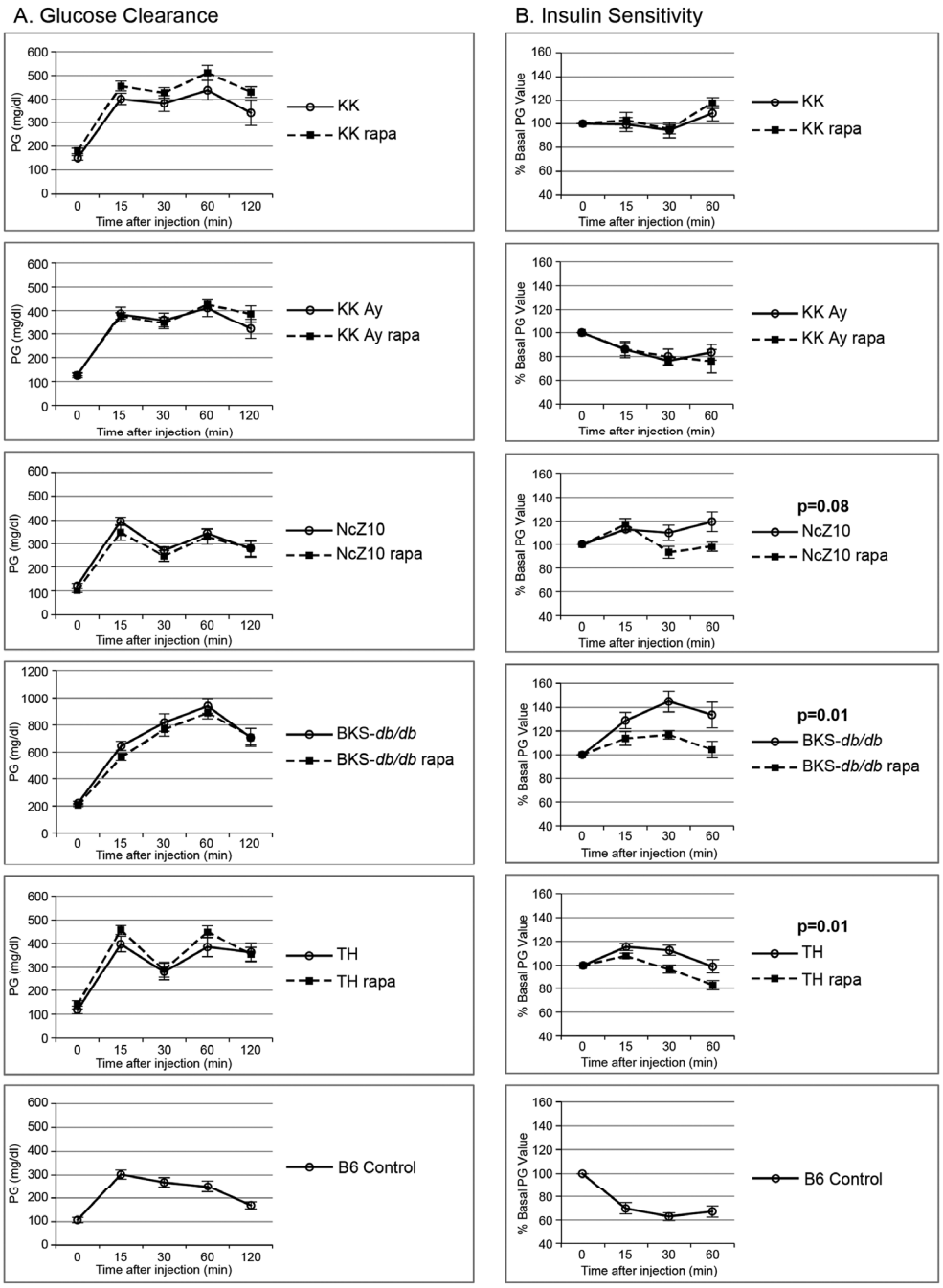

Figure 1. Effect of rapamycin on glucose clearance and insulin sensitivity in 5 diabesity models. (**A**) Rapamycin does not exacerbate glucose intolerance in 5 glucose intolerant strains. (**B**) Rapamycin improves insulin sensitivity in the insulin-resistant NcZ10, BKS-*db/db*, and TH strains. Two or three B6 controls were tested with each strain (cumulative data shown, n = 13–16) to serve as positive controls for the glucose and insulin injections, as quality controls, and for reference values. P values are given for repeated measures MANOVA (n = 5–6 per strain/treatment group except for NcZ10, n = 11).

began at 8–11 weeks of age. Rapa-treatment did not exacerbate the expression of 5 T2D phenotypes (insulin resistance, glucose intolerance, circulating lipids [triglycerides, cholesterol, non-esterified fatty acids]) in any of these diabesity models (Figure 1 and Table 2).

Rapa-treatment elevated hyperglycemia in only one of the 5 models (TH, Table 2). In fact, rapa-treatment produced some benefits. Weight gain was diminished and insulin sensitivity was improved in 3 models (BKS-db/db, NcZ10, TH), and hyperinsulinemia was reduced

Table 1. Characteristics of the 5 T2D mouse models used in the study.

Diabetes strain	Pancreatic insulin content	Hyper-phagia	Obesity	Hyper-insulinemia	Hyper-glycemia	Glucose intolerance	Insulin resistance
KK	High	Yes	Moderate	Severe, by 8 wk	Mild, by 10 wk	Yes	Yes
KK-Ay	High	Yes	Moderate	Very Severe, by 8 wk	Severe, by 16 wk	Yes	Yes
NcZ10	Intermediate	No	Moderate	Mild, by 12–20 wk	Moderate, by 12–20 wk	Yes	Yes
BKS-*db/db*	Low	Yes	Morbid	Severe, by 4–8 wk	Severe, by 4–8 wk	Yes	Yes
TH	Low	No	Moderate	Moderate, by 10–14 wk	Moderate, by 10–14 wk	Yes	Yes

Data references [23-28].

Table 2. Rapamycin treatment effects on markers of metabolism in 5 T2D strains.

Strain	Group (n)	Glucose clearance (GTT) 3 wk*	Insulin sensitivity (ITT) 2 wk*	Glucose (mg/dl)			Insulin (ng/ml)		TG (mg/dl) fed 6 wk	Cholesterol (mg/dl) fed 6 wk	NEFA (mEq/L) fed 6 wk	Pan-creatic insulin content (ng/mg) 6 wk
				Overnight fasting 3 wk	Fed 2 wk (Glu-2)	Fed 6 wk (Glu-6)	Overnight fasting 3 wk	Fed 6 wk (Ins-6)				
KK	Untreated (6)	Glucose intolerant	Insulin resistant	151 ± 9	190 ± 25	210 ± 14	1.11 ± 0.16	13.9 ± 2.8	388 ± 43	180 ± 2	4.66 ± .24	175 ± 50
	Rapa (6)	No change	No change	176 ± 11	197 ± 23	250 ± 25	3.67 ± 1.56	23.9 ± 9.4	385 ± 96	171 ± 9	4.01 ± .45	167 ± 34
KK-Ay	Untreated (6)	Glucose intolerant	Insulin responsive**	126 ± 9	350 ± 47	519 ± 64	1.27 ± 0.12	168.7 ± 34.6	938 ± 129	162 ± 8	5.92 ± .33	161 ± 43
	Rapa (6)	No change	No change	129 ± 5	373 ± 24	418 ± 64	0.83 ± 0.10 **p = .02**	54.6 ± 9.3 **p = .01**	691 ± 140	185 ± 8 **p = .06**	5.44 ± .33	73 ± 8 **p = .06**
NcZ10	Untreated (11)	Glucose intolerant	Insulin resistant	120 ± 11	177 ± 14	218 ± 21	0.45 ± 0.10	1.80 ± .51	286 ± 18	129 ± 5	3.66 ± .11	63 ± 5
	Rapa (11)	No change	Increased sensitivity	102 ± 9	173 ± 10	258 ± 20	0.26 ± 0.03 **p = .10**	1.23 ± .18	249 ± 15	127 ± 5	3.34 ± .10 **p = .06**	43 ± 4 **p = .004**
BKS-*db/db*	Untreated (5–6)	Glucose Intolerant	Insulin resistant	220 ± 11	321 ± 20	638 ± 47	1.81 ± 0.38	7.86 ± 1.15	117 ± 11	207 ± 9	1.92 ± .15	25 ± 4
	Rapa (6)	No change	Increased sensitivity	207 ± 11	375 ± 17 **p = .07**	707 ± 29	1.86 ± 0.23	5.96 ± 1.03	98 ± 7	201 ± 7	1.55 ± .10 **p = .06**	16 ± 2 **p = .08**
TH	Untreated (6)	Glucose intolerant	Insulin resistant	118 ± 15	222 ± 33	365 ± 60	1.79 ± 0.25	4.17 ± 0.84	259 ± 33	226 ± 8	2.97 ± .14	26 ± 6
	Rapa (6)	No change	Increased sensitivity	140 ± 16	278 ± 18	502 ± 16 **p = .05**	1.20 ± 0.25	1.21 ± 0.34 **p = .009**	272 ± 22	247 ± 12	3.31 ± .10 **p = .08**	11 ± 2 **p = .03**

p-values from 1-way ANOVA

*weeks of treatment. See Figure 1 for graphs.

**See Discussion for commentary regarding this result.