Systemic inhibition of Janus kinase induces browning of white adipose tissue and ameliorates obesity-related metabolic disorders

Qurania, Kikid Rucira

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学位論文の内容要旨

Systemic inhibition of Janus kinase induces browning of white adipose tissue and ameliorates obesity-related metabolic disorders

Janus キナーゼ阻害は白色脂肪の褐色化を誘導し、肥満関連代謝異常を軽減する

神戸大学大学院医学研究科医科学専攻

循環器内科学

（指導教員：平田健一教授）

KIKID RUCIRA QURANIA

The epidemic of obesity is increasing worldwide. Obesity often causes systemic metabolic disorders that accelerate atherosclerosis, and thus its deleterious effects on health have been a serious issue. An imbalance between energy intake and expenditure could simply accounts for obesity; therefore, increasing energy expenditure is mostly effective to ameliorate obesity. Brown adipose tissue (BAT) is a thermogenic organ, and its high degree of specialization of lipolysis and fatty acid oxidation makes it a major contributor to the overall energy balance. BAT dissipates extra energy to generate heat through uncoupled respiration mediated by uncoupling protein 1 (UCP1). Oxygen consumption in BAT is enormously high, hence its thermogenic activity can counteract the obese phenotype. Also, a metabolically active BAT has been identified in adult human, and its activity showed negative correlations with body mass index and fat depot. Certain white adipose tissue (WAT) depots, called beige/brite adipocytes, are readily able to convert to a brown-like state upon prolonged cold exposure. This white-to-brown conversion is referred to as browning, and browning has been linked to obesity in mouse models. Browning of WAT is an attracting strategy to treat obesity by enhancing energy dissipation. Although several genetically modified mice showed metabolic alterations due to enhanced or reduced browning, little is known about chemical compounds that can induce browning *in vivo*. Recently, Janus kinase (JAK) inhibition has been revealed to induce browning in human adipocytes *in vitro*; however, effects of JAK inhibition on browning and metabolic homeostasis *in vivo* remained to be elucidated. A growing body of evidence suggests that the JAK/signal transducer and activator of transcription (STAT) signaling pathway is dysregulated in obesity and metabolic disorders. Furthermore, associations of genetic loci for JAK2 with obesity and metabolic syndrome have been reported. Mouse studies targeting the JAK/STAT signaling pathway showed various metabolic phenotypes depending on target genes and tissues/cells. Inhibition of this signaling in immune cells appears to have beneficial effects on metabolism, while disruption of this pathway in adipose tissue, liver, muscle, and pancreas mostly showed deleterious effects on metabolic health.

We first examined whether treatment with JAK inhibitor (JAKi) could induce browning in mouse 3T3-L1 adipocytes by using two JAKi, tofacitinib (JAK3 inhibitor) and ruxolitinib (JAK1 and JAK2 inhibitor). Treatment with both tofacitinib and ruxolitinib increased mRNA expressions of UCPs, indicating that JAK signaling inhibition could induce browning in mouse adipocytes as well. Expression of peroxisome proliferator-activated receptors (PPARs), which play crucial roles in lipid metabolism and adipocyte differentiation, showed a trend toward increase in adipocytes

treated with JAKi. Interestingly, the increase in UCPs expression was temporally, and their expression levels reduced to those in vehicle-treated control cells in 2 days after termination of JAKi-treatment. These data indicate that browning mediated by JAKi is reversible, and thus continuous administration of JAKi is preferable to investigate an effect of JAKi on browning *in vivo*.

We chose tofacitinib for further analysis because it has already been used in the clinical setting for the treatment of rheumatoid arthritis. Mice were fed with high-fat diet for 4 weeks, and then tofacitinib was administered via osmotic pump for ~10 weeks with continuous high-fat diet. Body weight in tofacitinib-treated mice did not differ from that in vehicle-treated mice. Also, body fat ratio assessed by CT analysis was similar between the groups. However, food intake in tofacitinib-treated mice showed a modest but significant increase comparing to that in vehicle-treated mice.

We then confirmed that tofacitinib-treatment sufficiently inhibited JAK/STAT signaling in both visceral and subcutaneous fat. Of note, expressions of UCP1 and PR/SET domain 16 (PRDM16), a transcriptional factor that regulates the thermogenic gene program in BAT, were increased in both visceral and subcutaneous fat of tofacitinib-treated mice compared with those in vehicle-treated mice. These data indicate that JAK inhibition could induce browning of WAT *in vivo*. On the other hand, UCP-1 and PPARs expression in BAT was not different between the groups, whereas expressions of UCP-3 and fatty acid oxidation genes showed a tendency toward increase in BAT of tofacitinib-treated mice. These data suggest that JAK inhibition has minimal effects on BAT functions. Nevertheless, core temperature was higher in tofacitinib-treated mice compared with that in vehicle-treated mice both under ambient and acute cold exposure condition, suggesting an enhanced thermogenic capacity due to browning of WAT in tofacitinib-treated mice. Increased UCP1 in WAT due to browning induces uncoupling of oxidative phosphorylation from ATP production, releasing energy as heat. Therefore, enhanced browning is expected to reduce obesity. Administration of tofacitinib induced browning of WAT, while it did not reduce body weight and adiposity. However, food intake was increased in tofacitinib-treated mice. Because JAK/STAT signaling is involved in leptin-mediated appetite loss, the increased food intake in tofacitinib-treated mice may partially due to impaired leptin function. These data collectively suggest that tofacitinib-treatment might have enhanced energy expenditure due to browning of WAT, which results in preservation of body weight and adiposity despite increased food intake.

Consistently, thermogenesis was enhanced in mice treated with tofacitinib. These findings strongly suggest that brown-like adipocytes in WAT induced by tofacitinib are metabolically active.

Notably, insulin sensitivity was significantly improved in tofacitinib-treated mice compared with that in vehicle-treated mice, despite similar body weight and adiposity between the groups. Somewhat unexpectedly, chronic inflammation in WAT was not ameliorated but rather deteriorated in visceral WAT by tofacitinib-treatment. JAK/STAT signaling is a vital pathway for inflammatory cytokines such as interleukins and interferons, and disruption of this pathway in immune cells reduced WAT inflammation. Therefore, we initially presumed that chronic inflammation in WAT might be ameliorated by tofacitinib-treatment. However, inflammatory gene expressions were not reduced, but rather showed an increase in visceral WAT of tofacitinib-treated mice. These data suggest a minimal contribution of WAT to the improved metabolic health in tofacitinib-treated mice.

Gluconeogenic genes expression in liver and glucose transporter genes expression in skeletal muscle was not different between tofacitinib- and vehicle-treated mice. On the other hand, serum levels of total-cholesterol, triglyceride, and free fatty acid were significantly reduced in tofacitinib-treated mice. Also, we found that hepatosteatosis was apparently ameliorated in tofacitinib-treated mice compared with vehicle-treated mice, without changes in lipogenic genes expression in liver. It has been reported that BAT is a master regulator of triglyceride-rich lipoproteins clearance and blood lipid abundance. Activation of BAT by cold exposure accelerated plasma clearance of triglycerides and improved glucose tolerance in obese mice. Therefore, it is intriguing that browning of WAT also enhanced blood lipid clearance, leading to improved serum lipid profiles that were observed in tofacitinib-treated mice. Also, improved serum lipid profiles may have contributed to the ameliorated hepatosteatosis at least partially, while tofacitinib might have direct effects on the lipid accumulation in the liver.

Together, our data revealed beneficial effects of JAK inhibition on metabolic health in association with the browning of WAT, improved serum lipid profiles, and ameliorated hepatosteatosis in diet-induced obese mice for the first time. Optimization for dose and duration of tofacitinib-administration as well as for compounds to inhibit JAK/STAT signaling may allow us to identify more beneficial effects of JAK inhibition on obesity and metabolic disorders. Although further analysis is required to elucidate the underlying mechanism, JAK inhibition is an attracting therapeutic approach for the treatment of obesity and its related metabolic disorders.

神戸大学大学院医学(系)研究科（博士課程）

論文審査の結果の要旨			
受付番号	甲 第2906号	氏　名	KIKID RUCIRA QURANIA
論文題目 Title of Dissertation	Janusキナーゼ阻害は白色脂肪の褐色化を誘導し肥満関連代謝異常を軽減する Systemic inhibition of Janus kinase induces browning of white adipose tissue and ameliorates obesity-related metabolic disorders		
審査委員 Examiner	主　査 Chief Examiner 小川 渉 副　査 Vice-examiner 古屋敷智之 副　査 Vice-examiner 中村俊一		

（要旨は１，０００字～２，０００字程度）

In this study, the candidate has investigated the effects of JAK inhibitors (JAKi) on browning of adipocytes. The candidate has shown that treatment with both tofacitinib and ruxolitinib increased mRNA expressions of UCPs, indicating that JAK signaling inhibition could induce browning in mouse adipocytes. Then, mice were fed with high-fat diet for 4 weeks, and then tofacitinib was administered via osmotic pump for ~10 weeks with continuous high-fat diet. Body weight in tofacitinib-treated mice did not differ from that in vehicle-treated mice. However, food intake in tofacitinib-treated mice showed a modest but significant increase comparing to that in vehicle-treated mice. The candidate also showed that expressions of UCP1 and PR/SET domain 16 (PRDM16) were increased in both visceral and subcutaneous fat of tofacitinib-treated mice compared with those in vehicle-treated mice. Core temperature was higher in tofacitinib-treated mice compared with that in vehicle-treated mice both under ambient and acute cold exposure condition, suggesting an enhanced thermogenic capacity due to browning of WAT in tofacitinib-treated mice. Administration of tofacitinib induced browning of WAT, while it did not reduce body weight and adiposity. However, food intake was increased in tofacitinib-treated mice. These data collectively suggest that tofacitinib-treatment might have enhanced energy expenditure due to browning of WAT, which results in preservation of body weight and adiposity despite increased food intake. Consistently, thermogenesis was enhanced in mice treated with tofacitinib. These findings strongly suggest that brown-like adipocytes in WAT induced by tofacitinib are metabolically active.

Notably, insulin sensitivity was significantly improved in tofacitinib-treated mice compared with that in vehicle-treated mice, despite similar body weight and adiposity between the groups. Somewhat unexpectedly, chronic inflammation in WAT was not ameliorated but rather deteriorated in visceral WAT by tofacitinib-treatment. JAK/STAT signaling is a vital pathway for inflammatory cytokines such as interleukins and interferons, and disruption of this pathway in immune cells reduced WAT inflammation. Therefore, the candidate initially presumed that chronic inflammation in WAT might be ameliorated by tofacitinib-treatment. However, inflammatory gene expressions were not reduced, but rather showed an increase in visceral WAT of tofacitinib-treated mice. These data suggest a minimal contribution of WAT to the improved metabolic health in tofacitinib-treated mice. Gluconeogenic genes expression in liver and glucose transporter genes expression in skeletal muscle was not different between tofacitinib- and vehicle-treated mice. On the other hand, serum levels of total-cholesterol, triglyceride, and free fatty acid were significantly reduced in tofacitinib-treated mice. The candidate also found that hepatosteatosis was apparently ameliorated in tofacitinib-treated mice compared with vehicle-treated mice, without changes in lipogenic genes expression in liver. It has been reported that BAT is a master regulator of triglyceride-rich lipoproteins clearance and blood lipid abundance. Activation of BAT by cold exposure accelerated plasma clearance of triglycerides and improved glucose tolerance in obese mice.

The candidate, having completed studies on the role of JAK in browning of adipocytes and having advanced the field of knowledge in the area of the molecular mechanism of browning of adipocytes, is hereby recognized as having qualified for the degree of Ph.D.(Medicine)