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Therapeutic potential of flurbiprofen against obesity in mice

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ABSTRACT

Obesity is associated with several diseases including diabetes, nonalcoholic steatohepatitis (NASH), hypertension, cardiovascular disease, and cancer. Therefore, anti-obesity drugs have the potential to prevent these diseases. In the present study, we demonstrated that flurbiprofen, a nonsteroidal anti-inflammatory drug (NSAID), exhibited therapeutic potency against obesity. Mice were fed a high-fat diet (HFD) for 6 months, followed by a normal-chow diet (NCD). The flurbiprofen treatment simultaneously administered. Although body weight was significantly decreased in flurbiprofen-treated mice, growth was not affected. Flurbiprofen also reduced the HFD-induced accumulation of visceral fat. Leptin resistance, which is characterized by insensitivity to the anti-obesity hormone leptin, is known to be involved in the development of obesity. We found that one of the possible mechanisms underlying the anti-obesity effects of flurbiprofen may have been mediated through the attenuation of leptin resistance, because the high circulating levels of leptin in HFD-fed mice were decreased in flurbiprofen-treated mice. Therefore, flurbiprofen may exhibit therapeutic potential against obesity by reducing leptin resistance.

1. Introduction

Obesity is associated with several diseases including diabetes, nonalcoholic steatohepatitis (NASH), hypertension, cardiovascular disease, and cancer. Obesity has become a serious health concern worldwide. Therefore, effective medications are urgently needed.

Leptin is an anti-obesity hormone that was initially identified by Friedman's group in 1994 [1]. Leptin exerts its anti-obesity effects by activating the Ob-Rb leptin receptor, which is expressed in the hypothalamus [2–4]. However, leptin resistance, which is characterized by unresponsiveness to circulating leptin, may be one of the major causes of obesity [5,6]. Therefore, elucidating the mechanism by which leptin resistance develops is an important for the treatment of obesity. The endoplasmic reticulum (ER) is an organelle, that plays a role in protein folding. However, unfolded proteins accumulated when cells were exposed to stress conditions, and this resulted in ER stress [6,7]. We and another group recently demonstrated that ER stress may be involved in the development of leptin resistance [8,9]. Furthermore, we previously reported that flurbiprofen, a non-steroidal anti-inflammatory drug (NSAID), reduced ER stress, which consequently attenuated the leptin resistance associated with obesity [10]. We also showed that flurbiprofen attenuated obesity in mice [10]. However, these studies were performed by administering a simultaneous treatment with flurbiprofen when mice were fed a high-fat diet (HFD) [10]. Therefore, we do not currently know whether flurbiprofen has therapeutic potency. In the present study, we investigated whether flurbiprofen exhibited therapeutic potency against obesity by treating animals after the onset of obesity.

2. Materials and methods

2.1. Reagents

Flurbiprofen was obtained from Sigma (MO) or Cayman chemical (MI).

2.2. Animals

Adult male C57BL/6 Cr Slc mice were obtained from SLC (Hamamatsu, Japan). Mice were maintained in our animal facility at 22–24 °C under a constant day-night rhythm and given food and water, *ad libitum*. They were fed either a normal chow diet (NCD: D12450B Research diets, NJ) or high-fat diet (HFD: D12492; Research diets, NJ). The NCD and HFD contained 10 kcal % fat and 60 kcal % fat, respectively. Flurbiprofen was dissolved in sterilized water containing NaOH and mixed into the drinking water. The concentrations of flurbiprofen and other NSAIDs were estimated

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based on the average amount of water consumed to ensure the total intake of the drug was the desired dosage. All animal experiments were carried out in accordance with the NIH Guide line for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee at Hiroshima University.

2.3. Body weight

Body weight was measured once a week at 16:00–17:00.

2.4. Body length

Mice were fed a HFD for 6 months, followed by a NCD. Body length was measured in mice fed a NCD, and administered either flurbiprofen (3 mg/kg/day, 3 weeks) or vehicle. We measured body length from the nose to the beginning of the tail.

2.5. Leptin levels

Blood samples (including EDTA) were collected from animals by decapitation, and were then centrifuged (3000 rpm) at 4 °C for 15 min to obtain plasma samples. Plasma leptin levels were measured by ELISA according to the manufacturer's guidelines (R&D systems; MN).

2.6. Statistics

Results are expressed as the mean ± S.E. Statistical analyses were performed using the Student's *t*-test.

3. Results and discussion

To evaluate its therapeutic efficacy, we treated mice with flurbiprofen after feeding them a HFD. We fed mice a HFD for 6 months, followed by a NCD, and body weight was measured every week. We observed a gradual decrease in body weight in the NCD group (Fig. 1A). Body weight decreased after the diet was changed to a NCD after 3 weeks (Fig. 1A). We next examined whether flurbiprofen could further decrease body weight. We administered flurbiprofen (3 mg/kg/day) after the diet was changed to a NCD. Body weight was significantly lower in the flurbiprofen-treated group than in the NCD alone-fed group (Fig. 1A). This effect was not due to the inhibition of growth because no significant differences were observed in body length between the group (Fig. 1B). The total weight of visceral fat (adipose tissue) was measured in mice fed with the NCD, given either flurbiprofen or vehicle at the 3-week time point. Weight of visceral fat was significantly decreased in the flurbiprofen-treated group (Fig. 2). Therefore, the anti-obesity effects of flurbiprofen may be attributed to the inhibition of adipose tissue weight gain. Overall, these results suggest that flurbiprofen has anti-obesity effects.

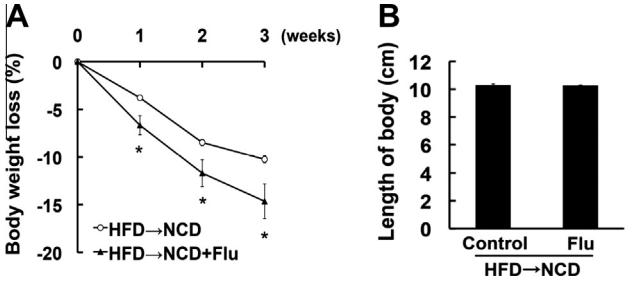

Fig. 1. Therapeutic potential of flurbiprofen against obesity. Mice were fed a high-fat diet (HFD) for 6 months, followed by a normal-chow diet (NCD). The flurbiprofen treatment (3 mg/kg/day) was simultaneously administered. (A) Body weight was significantly decreased in flurbiprofen-treated mice. $n = 14$ per group. $^{*}P < 0.05$ vs. HFD → NCD group. (B) The length of body was measured in NCD-fed mice, administered either flurbiprofen (3 mg/kg/day, 3 weeks) or vehicle. $n = 14$ per group.

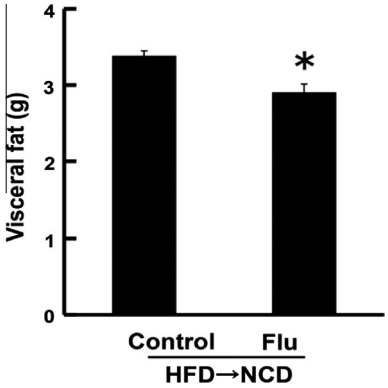

Fig. 2. Flurbiprofen reduced the HFD-induced accumulation of visceral fat. Mice were fed a HFD for 6 months, followed by a NCD. The total weight of visceral fat (adipose tissue) was measured in mice fed the NCD, and administered either flurbiprofen (3 mg/kg/day, 3 weeks) or vehicle. $n = 14$ per group. $^{*}P < 0.05$ vs. HFD → NCD.

Leptin also has anti-obesity effects. However, circulating leptin levels in obese patients are high, and leptin resistance is known to be one of the major causes of obesity [5,6]. Plasma leptin levels have already been correlated with body mass index (BMI) [11]. Therefore, high circulating leptin levels are a physiological indicator of leptin resistance. In the present study, we measured circulating levels of leptin in flurbiprofen-treated mice. Mice were fed a HFD for 6 months, followed by a NCD with or without flurbiprofen, and circulating leptin levels were then measured. We found that circulating leptin levels were significantly decreased by flurbiprofen (Fig. 3). Therefore, one of the anti-obesity effects of flurbiprofen may be mediated through the attenuation of leptin resistance. Hence, these results suggest that flurbiprofen may have a therapeutic effect against obesity by attenuating leptin resistance. Given that flurbiprofen exhibits chaperone activity, which has been shown to reduce the accumulation of unfolded protein [10], it has the potential to be applied as a fundamental treatment. Furthermore, flurbiprofen may also be beneficial for treating obesity-related diseases including diabetes, NASH, hypertension, cardiovascular disease, and cancer, and this will be an interesting topic for future studies.

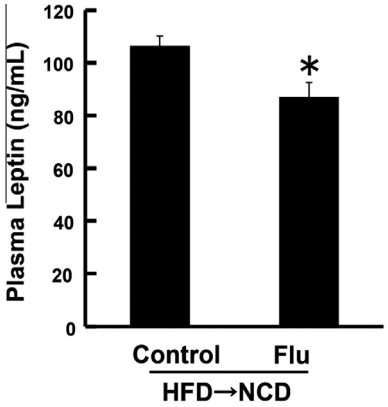

Fig. 3. Flurbiprofen reduced the HFD-induced elevation in circulating leptin levels. Mice were fed a HFD for 6 months, followed by a NCD. The flurbiprofen treatment (3 mg/kg/day) was simultaneously administered and plasma leptin levels were measured 3 weeks after the treatment. $n = 8$ per group. $^{*}P < 0.05$ vs. HFD → NCD group.

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References

[1] Y. Zhang, R. Proenca, M. Maffei, M. Baron, L. Leopold, J.M. Friedman, Positional cloning of the mouse obese gene and its human homologue, Nature 372 (1994) 425–432.
[2] C. Vaisse, J.L. Halaas, C.M. Horvath, J.E. Darnell Jr., M. Stoffel, J.M. Friedman, Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice, Nat. Genet. 14 (1996) 95–97.
[3] L.A. Campfield, F.J. Smith, Y. Guisez, R. Devos, P. Burn, Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks, Science 269 (1995) 546–549.
[4] T. Hosoi, T. Kawagishi, Y. Okuma, J. Tanaka, Y. Nomura, Brain stem is a direct target for leptin's action in the central nervous system, Endocrinology 143 (2002) 3498–3504.
[5] H. Münzberg, M.G. Myers Jr., Molecular and anatomical determinants of central leptin resistance, Nat. Neurosci. 8 (2005) 566–570.
[6] T. Hosoi, K. Ozawa, Endoplasmic reticulum stress in disease: mechanisms and therapeutic opportunities, Clin. Sci. (Lond) 118 (2010) 19–29.
[7] P. Walter, D. Ron, The unfolded protein response: from stress pathway to homeostatic regulation, Science 334 (2011) 1081–1086.
[8] T. Hosoi, M. Sasaki, T. Miyahara, C. Hashimoto, S. Matsuo, M. Yoshii, K. Ozawa, Endoplasmic reticulum stress induces leptin resistance, Mol. Pharmacol. 74 (2008) 1610–1619.
[9] L. Ozcan, A.S. Ergin, A. Lu, J. Chung, S. Sarkar, D. Nie, M.G. Myers Jr., U. Ozcan, Endoplasmic reticulum stress plays a central role in development of leptin resistance, Cell Metab. 9 (2009) 35–51.
[10] T. Hosoi, R. Yamaguchi, K. Noji, S. Matsuo, S. Baba, K. Toyoda, T. Suezawa, T. Kayano, S. Tanaka, K. Ozawa, Flurbiprofen ameliorated obesity by attenuating leptin resistance induced by endoplasmic reticulum stress, EMBO Mol. Med. 6 (2014) 335–346.
[11] M. Maffei, J. Halaas, E. Ravussin, R.E. Pratley, G.H. Lee, Y. Zhang, H. Fei, S. Kim, R. Lallone, S. Ranganathan, P.A. Kern, J.M. Friedman, Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects, Nat. Med. 1 (1995) 1155–1161.