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Glutathione S-Transferase Pi is Involved in the Growth of Mice

Xiaoliang Dong[1], Bing Rui[1], Fangyuan Yin[1], Dan Chen[1], Ling Li[1], Zhimin Yin[2, *], and Lan Luo[1, *]

[1]State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing 210023
and
[2]Jiangsu Province Key Laboratory for Molecular and Medicine Biotechnology, College of Life Science, Nanjing Normal University, Nanjing 210046, People's Republic of China

Abstract

Glutathione S-transferase Pi (GSTpi) is the most important subtribe of GSTs protein superfamily, and plays an important role in the process of detoxification, antioxidant and anti-inflammation. Here we use a GSTpi inhibitor, 6-(7-nitro-2, 1, 3-benzoxadiazol-4-ylthio) hexanol (NBDHEX), to evaluate the effect of GSTpi on the growth of mice. Mice treated with NBDHEX have heavier weight and longer length. But there is no significant difference in ratios of the weights of brain, kidney, lung, spleen, liver and heart to the whole body weight and Lee's index between NBDHEX and control mice. These data suggested that GSTpi might inhibit mouse growth. Enzyme-linked immunosorbent assay (ELISA) showed that GSTpi inhibition induced a significant increase of growth hormone (GH) levels in blood and pituitary and insulin-like growth factor (IGF-1) levels in liver and blood in mice. Further observation demonstrated that GSTpi negatively regulated GH-Janus Kinase 2 (JAK2)-signal transducer and activator of transcription 5 (STAT5) axis through inhibiting STAT5 phosphorylation, and as a result of GSTpi decreased the expression of IGF-1.

Key Words: GH, GSTpi, IGF-1, mouse growth, NBDHEX

Introduction

Glutathione S-transferase pi (GSTpi) is a ubiquitously expressed protein that plays an important role in detoxification and xenobiotics metabolism through catalyzing the conjugation of many hydrophobic and electrophilic compounds with reduced glutathione. It has been shown that GSTpi acts as a ligand-binding protein and an endogenous switch for the control of the catalytic activity of stress-activated kinases, such as c-Jun NH2-terminal kinase (JNK) (1, 30, 34, 37). Both early passage and immortalized mouse embryonic fibroblast (MEF) cells from GSTpi$^{-/-}$ animals expressed significantly elevated activity of extracellular signal-regulated kinases ERK1/ERK2, which linked to cell proliferation pathways (28). GSTpi deficient mice show increased myeloproliferation rate due to deregulation of JNK and Janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway (12). Moreover,

Corresponding authors: [1] Lan Luo, Ph.D. State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing 210023, People's Republic of China. Fax: +86 25 89686657, E-mail: lanluo@nju.edu.cn; and [2] Zhimin Yin, Jiangsu Province Key Laboratory for Molecular and Medicine Biotechnology, College of Life Science, Nanjing Normal University, Nanjing 210046, People's Republic of China. Fax: +86 25 85891305, E-mail: yinzhimin@njnu.edu.cn
*Both authors contributed equally to this paper.
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GSTpi can prevent angiotensin II (Ang II)-triggered proliferative signaling in vascular smooth muscle cells (VSMCs) and enhance As_2O_3-induced cell growth inhibition (4, 22). These *in vitro* and *in vivo* data are consistent with the principle that GSTpi influences cell proliferation. In 2000, Wolf CR *et al*. found that GSTpi knockout mice are heavier in body weight than the wild type controls and the underling mechanism is still unknown (34). These researches remind us that GSTpi might be involved in the growth regulation.

Mouse growth is controlled by growth factors and hormones through their cellular signaling pathways (8). Among the classic growth factors, the insulin-like growth factors (IGF-1 and IGF-2) have been shown to be members of a major growth-promoting signaling system. IGF-2 is essential for normal embryonic growth, whereas IGF-1 is a ligand that has a continuous function throughout development (2, 6, 7, 21). Postnatally, a major growth regulator is growth hormone (GH) which has no apparent embryonic role despite the presence of its cognate receptor- GH receptor (GHR) in embryos. Thus, absence of GH action in mutant animals or experimental ablation of the pituitary does not impair prenatal growth (10, 11, 26).

NBDHEX, a non-GSH-peptidomimetic derivative of 7-nitro-2, 1, 3- benzoxadiazole (NBD), appears as a new suicide inhibitor of GSTpi. It binds at the hydrophobic portion (H-site) of the GSTpi active site and mainly interacts with the GSH-binding site (G-site) of the enzyme (19, 32, 38). Meanwhile, NBDHEX can also block the combination of GSTpi and JNK, which active the JNK/c-Jun apoptosis signaling pathway (27). To date, numerous investigations about NBDHEX have focused on the drug resistance of tumor therapy (23, 20), whereas less attention has been paid to the role of NBDHEX in mouse growth. This study was aimed to investigate the effect of GSTpi on mouse growth and the underlying mechanisms by using GSTpi inhibitor NBDHEX.

Materials and Methods

Reagents and Antibodies

4-Chloro-7-nitrobenzofurazan (NBD-CL) (#25455), 6-Mercapto-1-hexanol (#451088) and 3-bromopyruvate (#16490) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) was purchased from Amresco (Pkwy., Solon, OH, USA). GST activity assay kit (Colorimetric method, A004) and reactive oxygen species (ROS) assay kit (E004) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). JAK2 polyclonal antibody (BS5769), phosphor-JAK2 polyclonal antibody (BS4109), STAT5 polyclonal antibody (BS6031), phosphor-STAT5 polyclonal antibody (BS4185), β-actin antibody (AP0731) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) monoclonal antibody (MB001) were purchased from Bioworld Technology (St. Louis Park, MN, USA). GSTpi antibody was purchased from BD Biosciences (San Jose, CA, USA).

General Procedure for Preparation of NBDHEX

NBDHEX was prepared according to the previous report (27). 4-Chloro-7-nitrobenzofurazan (0.2 g) and 6-Mercapto-1-hexanol (0.27 ml) were dissolved in the mixture of ethanol and pH 7.0 phosphate buffer saline (PBS) in the proportion of 1:1, and stirred at 25°C. The reaction system was maintained with pH 7 by adding trimethylamine. 3-bromopyruvate was used to remove the rest of 6-Mercapto-1-hexanol. Reaction product was a dark yellow and water insoluble substance and was identified through thin-layer chromatography (TLC), high performance liquid chromatography (HPLC) and ^{1}hydrogen-nuclear magnetic resonance (^{1}H-NMR).

Preparation of GSTpi Protein and Detection of GSTpi Activity

The full-length cDNA encoding GSTpi was amplified by polymerase chain reaction (PCR) for ligation into pET-28a (Novagen) which contains a hexahistidine N-terminal tag. Fusion protein comprising a full-length GSTpi was engineered as described previously (36). His_6-GSTpi was purified by metal-affinity chromatography by using IDA-Ni^{2+} affinity column (Novagen). Unless otherwise indicated, all purification procedures were carried out either at 4°C or on ice. Glycerol was added with a final concentration of 20%. The purified proteins were stored at −80°C until use. GSTpi enzyme activity was detected by GST assay kit.

Cell Culture and Transfection

The normal human hepatocyte cell line L02 was purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). L02 cells were maintained in Dulbecco's modified Eagle's medium (Thermo Fisher Scientific Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum and antibiotics at 37°C in humidified air containing 5% CO_2. Transient transfection was performed by using the polyethylenimine reagent (Sigma-Aldrich) according to the manufacturer's instructions. The total amount of DNA was normal-

ized by the empty control plasmids.

Cell Viability Assay

Cells were seeded into 96-well plates and allowed to adhere for 24 h. After being treated with different doses of NBDHEX for 48 h, cells were subjected to viability detection by using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay kit (Sigma-Aldrich) according to the manufacturer's specifications. In brief, cells in each well were incubated with 10 μL MTT working solution at 37°C for 4 h. The absorbance of each well at 490 nm was measured using a Synergy2 Multi-Mode Microplate Reader (BIO-TEK, Winooski, VT, USA).

Animal Experiments

Both ICR mice and BALB/c mice were purchased from Vital River Laboratories (Beijing, China). Laboratory animal handling and experimental procedures were performed in accordance with the requirements of Provisions and General Recommendation of Chinese Experimental Animals Administration Legislation and were approved by Science and Technology Department of Jiangsu Province (Approval Number: SYXK 2002-0059). All animal experimental procedures were approved by the Animal Care Committee of Nanjing University (Nanjing, China). Mice were raised under conventional controlled conditions (22°C, 55% humidity and day-night rhythm) and had free access to a standard diet and tap water. All mice were allowed to acclimate to these conditions for at least 2 days before inclusion in experiments.

Enzyme-linked Immunosorbent Assay (ELISA) Assay

Mouse GH (F10601) and IGF-1 (F10670) ELISA detection kit were purchased from Shanghai Westang BIO-TECH CO., LTD. Labeled antibodies and the biotin were co-incubated with the test samples. The optical density (OD) values of the samples were detected using a Biotek microplate reader. Standard curves were plotted according to standard OD values, and test sample concentrations were calculated from the standard curve.

Western Blot Analysis

Tissues and cells were lysed by using lysis buffer and centrifuged at 13000 g, 4°C. Samples incubated with a sodium dodecyl sulfate (SDS) sample loading buffer were heated on the boiling water bath for 5 min, then subjected to 12% SDS-polyacrylamide gel (PAGE), and transferred onto polyvinylidene fluoride (PVDF) membranes. After blocked in 5% fat-free milk at room temperature (RT) for 1 h, membranes were incubated overnight at 4°C with primary antibodies, followed by horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at RT. The dilutions of anti-GSTpi, anti-β-actin, anti-p-JAK2, anti-JAK2, anti-p-STAT5, anti-STAT5 and anti-Flag antibodies for western blot are all 1:1000. The dilution of anti-GAPDH antibody for western blot is 1:2000. The dilution of anti-Flag antibody for Immunoprecipitation is 1:200. The dilutions for secondary antibodies are both 1:10000. Finally, membrane-bound antibodies were detected using a chemiluminescence reagent. The total protein content of loading was monitored by reprobing the same blots with loading control.

ROS Detection

ROS was detected by using the commercial assay kit. Briefly, tissue extracts were incubated with ROS specific dye, 2, 7-dichlorofuorescin diacetate (DCFH-DA), at 37°C for 30 min, and then were centrifuged, washed and suspended in PBS. ROS were detected by using a TECAN Infinite® 200 PRO Multimode microplate reader (Männedorf, Switzerland) at 525 nm.

Statistical Analysis

All statistical analysis was carried out by using GraphPad Prism software. Error bars for *in vitro* and *in vivo* analysis represent the standard deviation among intra-class data collected from more than 3 independent experiments. Statistical significance was determined using unpaired Student's two-tailed *t*-test for two data sets. Statistical significance was defined as *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Results

NBDHEX Is Synthesized and Identified

The general route to synthesize NBDHEX is outlined in Fig. 1A. After reaction, the synthesized compound was characterized by TLC. As shown in Fig. 1B, the left bands were reactants spots, and the right was new product spot. This result showed that all the substrates were consumed completely. The purity of the end product was measured by HPLC. As shown in Fig. 1C, the retention time of the major peak of the end product was 4.56 min. According to area normalization method, we calculated that the purity of end product was about 97.63%. Then the structure of end product was determined

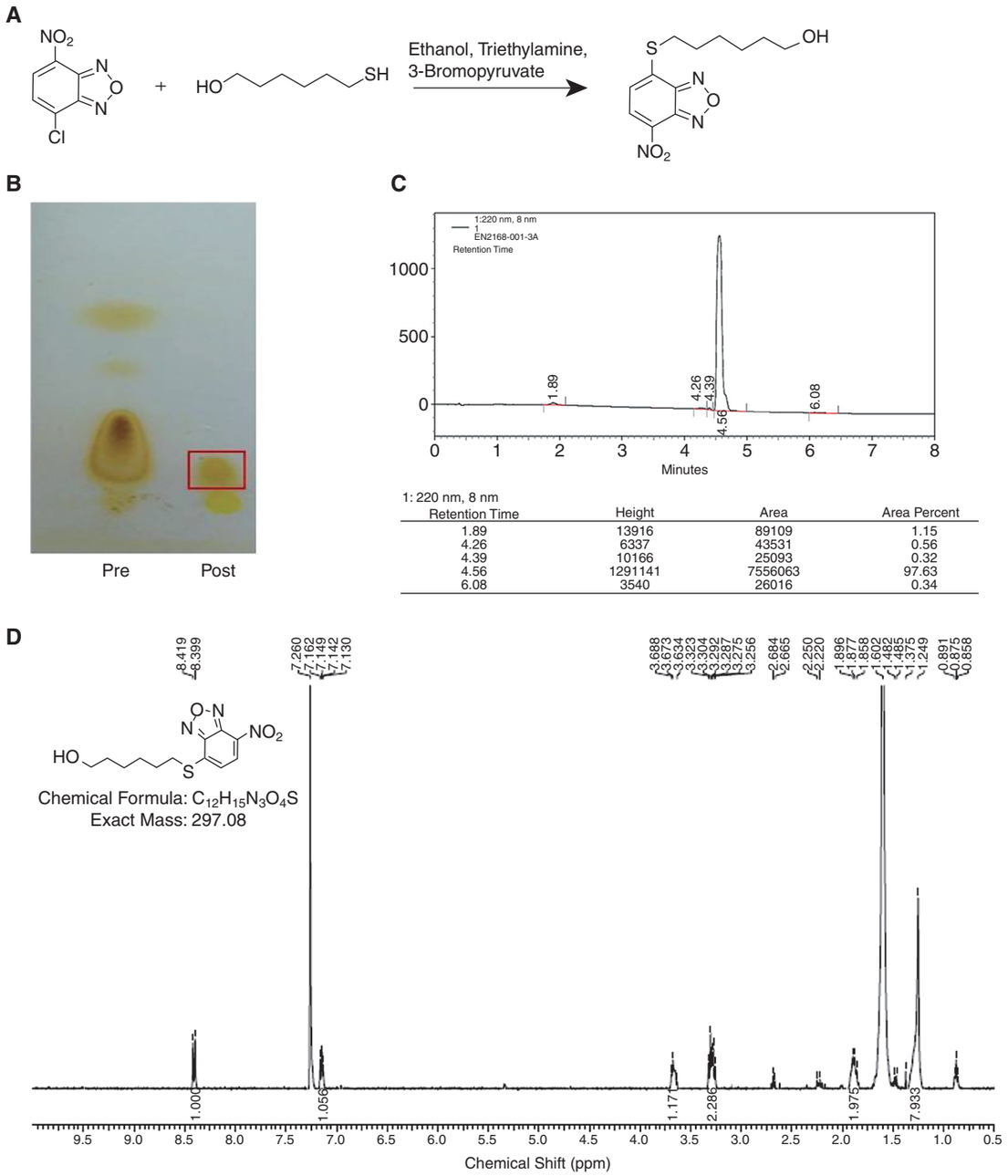

Fig. 1. NBDHEX is synthesized and identified. (A) General procedure of NBDHEX synthesis. Details were described in Materials and Methods. (B) TLC analysis. (C) HPLC analysis. (D) ^{1}H-NMR analysis.

by ^{1}H-NMR. As shown in Fig. 1D, in addition to a small amount of impurity peak, the chemical shifts of hydrogen shown in the spectra are in accord with that in NBDHEX, which was consistent with previous report (27). All the results suggested that the end product was in accordance with the GSTpi inhibitor NBDHEX.

NBDHEX Inhibits GSTpi Activity Efficiently In Vitro *and* In Vivo

Following, we determined the bioactivity of NBDHEX. After IDA-Ni^{2+} resin column purification, the recombinant GSTpi gave a single band on SDS-PAGE with an apparent relative molecular mass (*M*r)

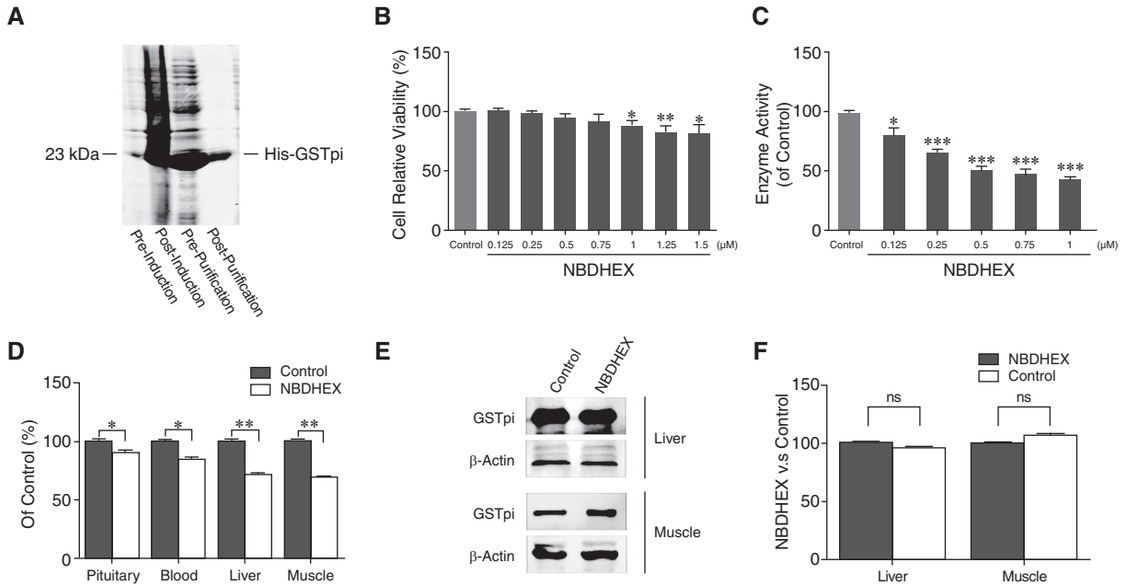

Fig. 2. NBDHEX inhibits GSTpi activity *in vitro* and *in vivo*. (A) Expression of GSTpi protein in *E. coli* before and after induction with IPTG was monitored by SDS-PAGE followed with Coomassie blue staining. (B) L02 cells were treated with different doses of NBDHEX (0, 0.125, 0.25, 0.5, 0.75, 1, 1.25 and 1.5 μM) for 36 h and the cell viability was detected by MTT. (C) 44 nM GSTpi protein was incubated with NBDHEX at concentrations of 0, 0.125, 0.5, 0.75 and 1 μM, and then GSTpi catalytic activity was measured by using a commercial assay kit. (D) Mice were injected intraperitoneally with NBDHEX, and then GSTpi catalytic activities in pituitary, blood, liver and muscle were detected. (E) Mice were injected intraperitoneally with NBDHEX, and the GSTpi protein levels in mouse liver and muscle were measured by Western blot. Values are mean ± standard deviation (SD); $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared with control; n = 6-8.

of 23 kDa (Fig. 2A). Following, MTT assay was used to evaluate the cytotoxic effect of NBDHEX on cell viability. As shown in Fig. 2B, low dose of NBDHEX (0.125, 0.25, 0.5 and 0.75 μM) did not affect human liver cell (L02) viability, while high dose of NBDHEX (1, 1.25 and 1.5 μM) slightly reduced cell viability, suggesting excessive inhibition of GSTpi activity might affect cell viability. We then incubated GSTpi (44 nM) (27) with different concentrations of NBDHEX (0, 0.25, 0.5, 0.75 and 1 μM), enzyme inhibitory experiment result showed that NBDHEX dose-dependently inhibited GSTpi enzyme activity (Fig. 2C). Furthermore, we detected the inhibition effect of NBDHEX on GSTpi activity *in vivo*. BALB/c mice were treated with 1 mg/kg NBDHEX by intraperitoneal injection, and GSTpi activities in pituitary, blood, liver and muscle were determined. As shown in Fig. 2D, GSTpi activities in all these tissues of NBDHEX treated mice were lower than control animals, which demonstrated that NBDHEX could inhibit GSTpi activity efficiently *in vivo*. Further western blotting showed that NBDHEX did not affect GSTpi protein level in liver and muscle tissues (Fig. 2E).

NBDHEX Facilitates Mouse Growth

We next intraperitoneally injected to mice with NBDHEX to evaluate if GSTpi was involved in animal growth. Five-week age BALB/c mice were treated with 0.5, 1 and 2 mg/kg/day NBDHEX for 5 weeks respectively. As shown in Fig. 3A, mice treated with 1 mg/kg NBDHEX gained body weight faster than control. However NBDHEX, at the dose of 0.5 and 2 mg/kg/day, just showed a weak effect on mouse body weight gain. After 10 week treatment, IVIS Lumina XR imaging indicated that 1 mg/kg NBDHEX treated mice had longer longitudinal bones compared with control mice (Fig. 3B). To verify whether the increase of mice weight was induced by one or several special organs, we detected weight of some organs in BALB/c mice. As shown in Fig. 3C, there was no significant differences between NBDHEX treated and control groups in the ratios of brain, kidney, lung, spleen, liver and heart weight to body weight. Lee's index which is used to evaluate the obese degree was higher in 1 mg/kg NBDHEX treated mice than the control group (Fig. 3D). Consequently, we treated two strains of mice, BALB/c and ICR, with 1 mg/kg/day NBDHEX

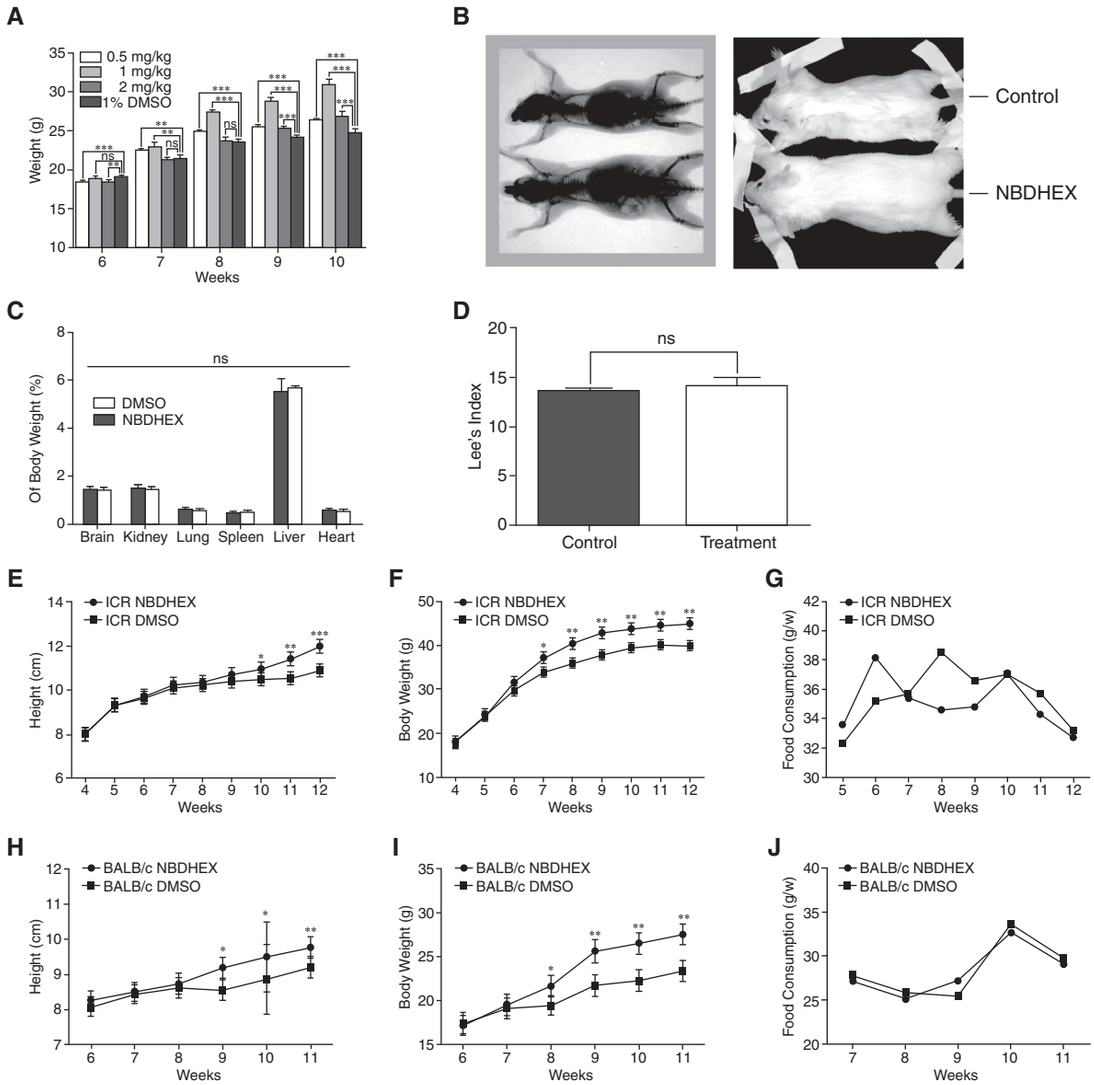

Fig. 3. NBDHEX facilitates mouse growth. (A) Five-week-old male ICR mice were intraperitoneally injected with NBDHEX at doses of 0.5, 1 and 2 mg/kg/day for 5 weeks and the body weight were monitored. (B-C) After 5 week treatment with 1 mg/kg/day NBDHEX, mice were observed under a small animal imager for measuring body longness (B), the relative organ weights of BALB/c mice were detected (C), and Lee's index of mice was determined (D). ICR (E-G) and BALB/c (H-J) mice were intraperitoneally injected with 1 mg/kg/day NBDHEX, the dynamic changes of weight, longness and food consumption of mice were detected. Values are mean ± SD; *$P < 0.05$, **$P < 0.01$ and ***$P < 0.01$ compared with control; n = 8.

and recorded the weight and length of mice for 12 weeks (Fig. 3, E-J). Both BALB/c and ICR mice from NBDHEX treated groups showed heavier in body weight than control mice. Meanwhile, the lengths of mice treated with NBDHEX increased faster significantly than control mice. But there was no significant difference in food consumption between animal groups, suggesting that NBDHEX induced the promotion of weight and length increase in mice was not related with food intake. These results indicated that GSTpi negatively regulated the growth of mice.

NBDHEX Enhances the Levels of Growth Factors in Mice

Since the mouse post-natal body growth was mainly regulated by the GH/IGF-1 axis, we further detected the levels of GH and IGF-1 in the mouse liver and blood through ELISA. As shown in Fig. 4A

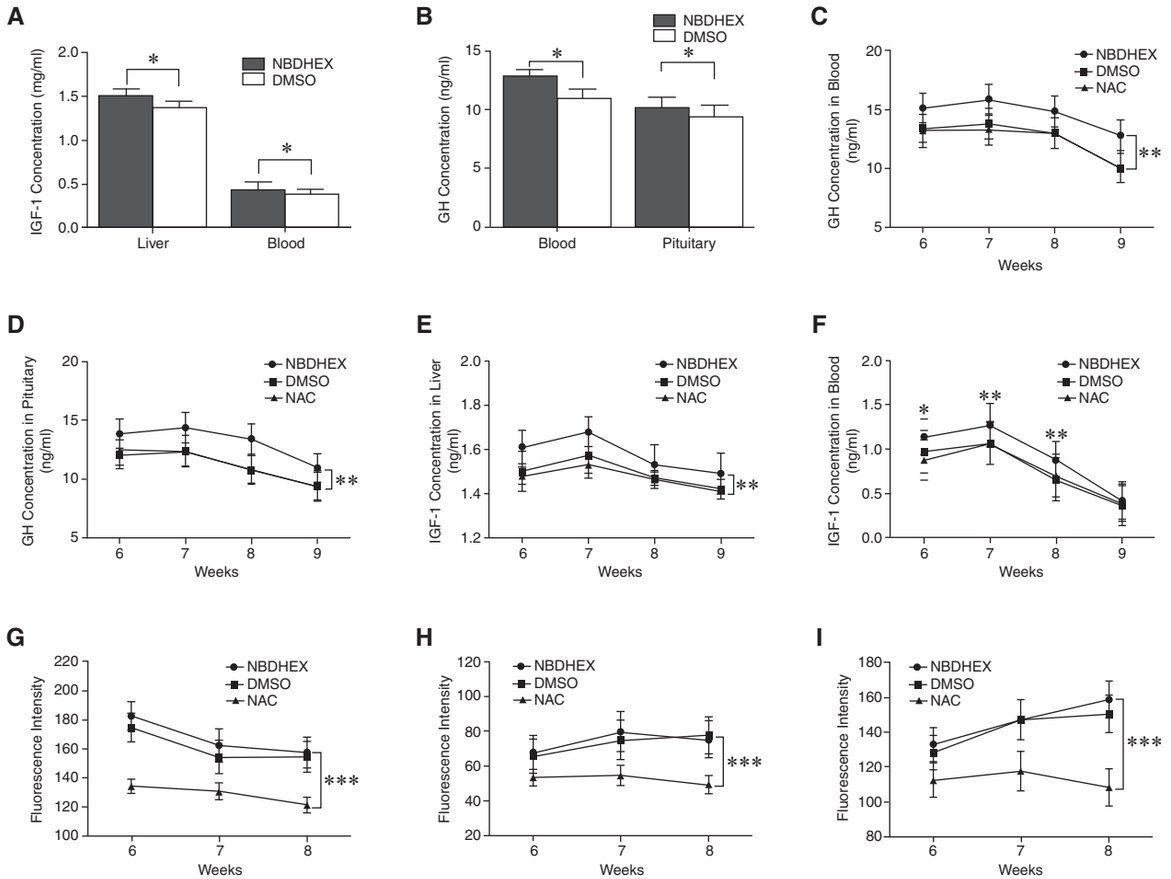

Fig. 4. NBDHEX enhances the levels of growth factors. (A and B) BALB/c mice were intraperitoneally injected with 1 mg/kg/day NBDHEX for 5 weeks, the levels of GH in blood and pituitary and IGF-1 in liver and blood were detected. (C-F) BALB/c mice were intraperitoneally injected with NBDHEX and NAC separately for 4 weeks, the levels of GH in blood and pituitary (C and D) and IGF-1 in liver and blood (E and F) were detected by using ELISA assay. (G-I) BALB/c mice were intraperitoneally injected with NBDHEX and NAC separately for 3 weeks, the levels of ROS in mice blood (G), muscle (H) and liver (I) were detected by using a commercial assay kit. (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with control; n = 8)

and B, both GH levels in mouse blood and pituitary and IGF-1 levels in mouse blood and liver were higher in the group treated with 1 mg/kg NBDHEX than those in control, which indicated GSTpi might have negative regulatory effects on the level of GH and IGF-1. ROS can act either upstream or downstream of several growth factors, and can affect the activity of various kinases in signaling pathways (17, 18). To verify whether GSTpi regulated growth factors or hormones through depending on ROS, we first detected the dynamic changes of ROS in the blood, skeletal muscle and liver of BALB/c mice. As shown in Fig. 4G-I, there was no significant difference in the ROS level between NBDHEX and DMSO-treated group, while N-acetyl cysteine (NAC), a synthetic precursor of GSH, apparently decreased ROS level. We then compared the dynamic changes of GH and IGF-1 levels in NBDHEX treated BALB/c mice with NAC treated one. Results from the ELISA detection showed that blood and pituitary levels of GH as well as liver and blood levels of IGF-1 in NBDHEX treated mice were higher than those in DMSO group. But there was no significant difference between NAC and DMSO group (Fig. 4, C-F). These results suggested that GSTpi restricted the levels of growth factors in an enzyme-dependent way but not the ROS related way.

GSTpi Inhibits the Secretion of IGF-1 through Reducing the Phosphorylation of STAT5

IGF-1 is mainly secreted from the liver under stimulation of GH. GH binds with GH receptor to lead to activation of JAK2/STAT5 cascade and the expression of IGF-1 promoted by activation of the transcription factor STAT5. To further investigate

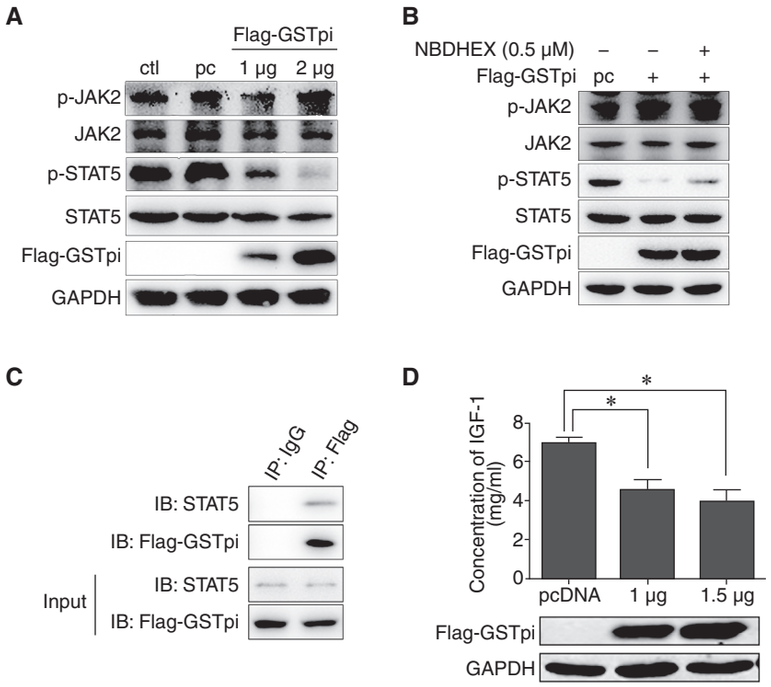

Fig. 5. GSTpi inhibits the phosphorylation of STAT5 and expression of IGF-1 through physically interacting. (A) Lysates from L02 cells transfected with GSTpi plasmid for 24 h were subjected to immunoblotting with anti-p-JAK2, anti-JAK2, anti-p-STAT5, anti-STAT5, and anti-Flag antibodies. GAPDH was used as a loading control. (B) L02 cells transfected with GSTpi plasmid for 24 h were treated with 0.5 μM NBDHEX, and the lysates of cells were detected by immunoblotting with anti-p-JAK2, anti-JAK2, anti-p-STAT5, anti-STAT5 and anti-Flag antibodies. GAPDH was used as a loading control. (C) Lysates from L02 cells transfected with GSTpi plasmid were subjected to immunoprecipitation with anti-Flag antibody, and were detected by immunoblotting with anti-STAT5 and anti-Flag antibodies. (D) The levels of IGF-1 secreted from L02 cells transfected with GSTpi plasmid were detected through ELISA assay (*$P < 0.05$ compared with control; n = 3).

the mechanism by which GSTpi negatively regulated the secretion of IGF-1, we investigated the intracellular signaling pathways which were involved in the IGF-1 secretion in human hepatocytes (L02). The Western blot detection revealed that overexpression of GSTpi significantly reduced the phosphorylation of STAT5 but not the protein level, suggesting that GSTpi inhibited IGF-1 activation. Meanwhile, GSTpi did not affect JAK2 both at phosphorylation and protein levels (Fig. 5A). When inhibiting GSTpi using NBDHEX, the inhibitory effect of GSTpi on STAT5 phosphorylation was reversed (Fig. 5B). Following we performed immunoprecipitation and immunoblotting experiments to detect the specific association of flag-GSTpi with STAT5. Lysates of L02 cells were immunoprecipitated using respective antibodies, and then the pellets were analyzed by immunoblotting with anti-flag monoclonal antibody. The results indicated that GSTpi combined with STAT3 in L02 cells (Fig. 5C). We then overexpressed flag-GSTpi in L02 and detected the concentration of IGF-1 in culture medium by using ELISA assay. The result showed that cells tranfected with GSTpi (1 μg and 1.5 μg) had lower IGF-1 secretion level than control cells (Fig. 5D), suggesting that overexpression of GSTpi significantly decreased IGF-1 secretion from L02 cells. These results suggested that GSTpi negatively regulated the level of IGF-1 through directly inhibiting the phosphorylation of STAT5.

Discussion

The growth (enlargement in size) of a mammalian organism is influenced predominantly by proliferative events outnumbering apoptosis and increase of total cell number. This process is controlled through the cellular signaling triggered by growth factors and hormones. In the present study, we utilized NBDHEX, a GSTpi inhibitor, to investigate the regulatory effect of GSTpi on mice growth and the underlying mechanism (24). Our results demonstrated that intraperitoneally injected NBDHEX to mice promoted their growth and enhanced the level of

GH and IGF-1. Previous studies show that GSTpi is involved directly in controlling cellular mitogenic pathways that influence proliferation (11, 28). According to its function on cell proliferation, we hypothesized that GSTpi might be involved in regulating animal growth. Interestingly, Turella P *et al*. found that the mice injected with NBDHEX intraperitoneally were heavier in body weight than that of control when they studied the toxicity of NBDHEX *in vivo* (32), but they did not perform the further investigation. Consistent with this report, in present study, we found that both ICR and BALB/c mice grew faster when they were treated with NBDHEX by intraperitoneal injection than the control. In addition, the body weight gain did not appear in the individual organ but the whole body.

GSTpi refers to a cytosolic phase II detoxifying enzyme, which catalyzes the nucleophilic attack of the sulfur atom of GSH on electrophilic groups of substrate molecules under some stress stimulations (31). It has been reported that NBDHEX at dose of 2 μmol/l did not affect GSH level in cultured tumor cells (32). These studies indicate that under physiological conditions, GSTpi might not increase GSH, the important antioxidant related with ROS generation, but NAC can significantly increase GSH level. Thus, in the present study, different from NAC, NBDHEX had no effect on the ROS level. Furthermore, JNK may be activated by a variety of stressors, the functional consequence of JNK activation appears to be cell-type and signal-specific (5, 16). As GSTpi could inhibit the activity of JNK (1), thus the GSTpi inhibitors, such as NBHDEX, might exert various effects in different cells and under different stimulations. On the other hand, it has been reported that inhibition of GSTpi or down-regulation of GSTpi result in cell proliferation (4, 12). Therefore, it is not surprised that NBDHEX could have antitumor activity and growth enhancing effect.

Previous reports demonstrated that, at least in rodents, the GH/IGF-1 axis provides the main conduit of postnatal body growth control (13, 33, 36). IGF-1 secretion in liver is mainly regulated by GH through activating JAK2/STAT5 signaling (25). The association of GH with GH results in the dimerization of GH receptor and activation of JAK2, which subsequently (among other effectors) phosphorylates STAT5b, a transcription factor activating the *Igf1* gene promoter acting either alone or synergistically with *HNF1a* (3, 14, 18). Our previous study showed that GSTpi physically associated with STAT3 to prevent Ang II-triggered binding of Src to STAT3 and thus suppressed Ang II-stimulated phosphorylation and nuclear translocation of STAT3 (4). STAT3 and STAT5 are two members of STAT protein family that mediate many aspects of cellular immunity, proliferation, apoptosis and differentiation. All STAT proteins share a common structural motif consisting of an N-terminal domain followed by a coiled-coil, DNA-binding linker, Src homology 2 (SH2), and C-terminal transactivation domain. These structural observations suggest a possibility that GSTpi may also interact with STAT5. In fact, our results demonstrated that GSTpi interacted with STAT5 and blocked the JAK2-STAT5 signaling.

In conclusion, our present study indicates that mouse growth was markedly promoted by GSTpi inhibitor NBDHEX through increasing GH and IGF-1. Further mechanism investigation demonstrated that GSTpi prevented JAK2-STAT5 signaling by inhibiting the phosphorylation of STAT5 and NBDHEX increased IGF-1 *via* reversing the effect of GSTpi function of on STAT5. These findings demonstrated, for the first time, a novel regulation of IGF-1 secretion by GSTpi which might be a potential target for growth related disease such as dwarfism.

Conflict of Interest

The authors declare that they do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Acknowledgments

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