Original Article

A High Dose of Short-term Intraperitoneal D-galactose-induced Aging Process in Male Rats

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Abstract

Aims: Intraperitoneal injection of D-galactose (D-gal i.p.) can accelerate aging has been used to develop models of aging previously, long-term use of D-gal has been used to induce aging in mice. Researchers are trying to determine whether short-term administration of high doses of D-gal i.p. in rats is able to induce significant signs similar to natural aging, namely increased oxidative stress and myostatin. **Methods:** Rats aged 6 months were induced D-gal i.p dose of 300 mg/mL/kg for 1 week. Biochemical analysis was performed for the estimation of systemic antioxidants and inflammation. **Results:** Short-term administration of D-gal i.p significantly increased systemic inflammation and myostatin level. In the treatment group, there is an increased superoxide dismutase activity although lower compared with NaCl 0.9% i.p. **Conclusion:** D-galactose intraperitoneally indeed accelerates aging in animal models by inducing oxidative stress and other aging-related biochemical changes, even with short-term high-dose administrations in one week.

Keywords: High-dose short-term D-galactose i.p, inflammation, stress oxidative, superoxide dismutase

Résumé

Objectifs: L'injection intrapéritonéale de D-galactose (D-gal i.p.) peut accélérer le vieillissement et a déjà été utilisée pour développer des modèles de vieillissement. L'utilisation à long terme de D-gal a été utilisée pour induire le vieillissement chez la souris. Les chercheurs tentent de déterminer si l'administration à court terme de doses élevées de D-gal i.p. chez le rat est capable d'induire des signes significatifs similaires au vieillissement naturel, à savoir une augmentation du stress oxydatif et de la myostatine. **Méthodes:** Des rats âgés de 6 mois ont reçu une dose i.p. de D-gal induite de 300 mg/mL/kg pendant 1 semaine. Une analyse biochimique a été réalisée pour l'estimation des antioxydants systémiques et de l'inflammation. **Résultats:** L'administration à court terme de D-gal i.p. a augmenté de manière significative l'inflammation systémique et le niveau de myostatine. Dans le groupe de traitement, il y a une activité accrue de la superoxyde dismutase, bien que inférieure à celle du NaCl 0,9 % i.p. **Résumé:** L'injection de D-gal présente des symptômes similaires au vieillissement naturel qui peuvent se développer chez la souris dès 1 semaine.

Mots-clés: D-galactose i.p. à court terme à haute dose, inflammation, stress oxydatif, superoxyde dismutase

Introduction

One type of amonosaccharide that can be found in fruits, vegetables, and dairy products is called D-galactose (D-gal). Due to its reducing sugar nature, D-gal can be metabolized to glucose at normal quantities but, at high doses, galactose oxidase can convert it to aldoses and hydroperoxides, which can lead to the formation of superoxide anion and oxygen derivatives as free radicals.[1]

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Chronic D-galactose induction is thought to be a model of accelerated aging since it can cause cognitive and motor ability deterioration, which is a sign of aging.[2] When large dosages of D-galactose are taken quickly after one another, the pathomechanism by which D-galactose causes aging is still unknown. D-galactosa is used at a dose of 60–150 mg/kg body weight, usually within 4-6 weeks of surgery.

Overconsumption of D-galactosa can produce reactive oxygen species (ROS) via oxidative metabolism of the sugar and glycation end products. It's interesting to note that rats given d-galactose injections on a regular basis for a duration of 6–10 weeks discovered that the systemic circulation of mice produced more free radicals. But since it takes a long time to conduct research, using modest amounts of D-galactose over an extended period of time can be challenging for researchers.[3]

D-galactose-induced muscle aging

Age-related diseases and the aging population are growing, yet simulating aging is expensive and technically challenging. In order to solve this problem, d-galactose has been demonstrated to augment the oxidative changes that occur in the normal aging process in d-galactose aging models of skeletal muscle and can be utilized to accelerate the aging process in diverse tissues in mice models. Measuring protein carbonyl groups, advanced oxidation protein products, lipid hydroperoxides, total thiols, and Cu, Zn-superoxide dismutase activity allowed researchers to evaluate the aging of Gastrocnemius muscle. Oxidative stress markers were shown to significantly increase in the gastrocnemius muscle model aged by D-galactose. Cellular senescence, an increase in dead cells in the cell cycle, is another aspect of the aging process.[4]

Some biomarker such as TNFα, IL-6, and NF-κB overactivation are examples of pro-inflammatory cytokines that are produced by senescent cells, also known as Senescence-Associated Secretory Phenotype.[5] Furthermore, the telomere/telomerase system is related because short telomeres, a sign of DNA damage, can promote cellular senescence. Myostatin protein is a marker of aging muscle that increases along with inflammation during the aging process.[6]

The aim of this study was to find out the pathways of muscle aging in rats as a result of administration of high-dose short-time D-galactose.

Methods

The research design of an experimental study, *in vivo*, was carried out in the integrated laboratory of FKIK Atma Jaya Catholic University, Jakarta, using 22 Sprague–Dawley rats, male, aged 6–12 weeks, 200–350 g. Eleven rats were induced intraperitoneally (G-ip) D-gal 300 mg/kg/day for 7 days. The remaining 11 rats were induced by NaCl 0.9% i.p (N-ip). In this study, measurements of BW, gastrocnemius circumference, C-reactive protein (CRP), SOD, and myostatin blood ELISA levels were compared between day 7 and day 0 to see the systemic effect of D-gal injection. Data were analyzed using IBM SPSS Statistics 20.

Results

Characteristics of research subjects

Twenty-eight mice in this study included six mice included in the exclusion criteria because 1 mouse died, and 5 mice had blood samples lysed, so that a total of 22 mice were used.

Twenty-two groups were divided into two groups, namely the galactose i.p and NaCl i.p treatment. Each group was measured for BW and gastrocnemius muscle circumference on day 0. From these data on the weight of the rats and foot circumference on day 0, found no significant difference in weight of all mice [Table 1].

Differences in rat weight and gastrocnemius circumference after D-galactose induction i.p. compared to NaCl 0.9% i.p

On the 7th day, the group that had received D-gal i.p induction at a dose of 300 mg/kg/time for 7 consecutive days experienced a significant decrease in BW compared to the group that received NaCl 0.9% i.p according to Table 2.

Analysis on the 7th day, there was no significant change in the diameter of the gastrocnemius muscle after getting

Table 1: Research results of the rat group (day 0)

Group	BW (g)	*P*	Gastrocnemius muscle circumference (cm)			
			Right	*P*	Left	*P*
D-galactose i.p	265±35.6	0.734	4.2±0.3	0.313	4.2±0.3	0.325
NaCl i.p	264.5±42.8		4.1±0.5		4.1±0.4	

Data mean±SD, independent *t*-test. BW=Body weight, SD=Standard deviation, i.p=Intraperitoneally

Table 2: The results of the difference in body weight on the 0th and 7th day after injection D-galactose intraperitoneally compared to NaCl 0.9% intraperitoneally

	BW (g) day–0	BW (g) day–7	ΔBW day 7–0	*P*
D-galactose i.p (*n*=11)	264.4±20.5	236.5±18.5	−27.3±−2	0.049*
NaCl 0.9% i.p (*n*=11)	264.1±31.2	267.4±45.2	3.3±13.5	0.975

*Significance $P < 0.05$. Data mean±SD, independent *t*-test. SD=Standard deviation, i.p=Intraperitoneally, BW=Body weight

Table 3: Differences in gastrocnemius muscle circumferences after getting D-galactose intraperitoneally treatment compared to NaCl 0.9% intraperitoneally

Gastrocnemius muscle circumferences	D-galactose i.p (*n*=11)	NaCl 0.9% i.p (*n*=11)	*P*
Day 0			
Right	4.0±0.3	4.1±0.5	0.313
Left	4.1±0.3	4.1±0.5	0.325
Day 7			
Right	4.2±0.3	4.2±0.7	0.303
Left	4.3±0.4	4.2±0.7	0.591

Data mean±SD, independent *t*-test. SD=Standard deviation, i.p=Intraperitoneally

D-gal i.p. treatment compared to NaCl 0.9% i.p according to Table 3.

Differences in C-reactive protein, superoxide dismutase, and myostatin levels in experimental animals after induction of galactose treatment after day 7

In the group of rats induced by D-gal at a dose of 300 mg/kg/ for 7 days intraperitoneally (i.p), an increase in inflammation was indicated by a significant increase in CRP in the treatment group, $P = 0.041$. In the oxidative stress analysis, it was found that the group that received D-gal i.p induced, as well as those that received NaCl 0.9% i.p control, had an increase in SOD, but the control group NaCl 0.9% i.p experienced a higher increase in SOD than the D-gal group significantly, $P = 0.044$. Analysis of changes in myostatin markers in the group that received D-gal i.p treatment experienced a significant increase in myostatin compared to 0.9% NaCl. i.p, $P = 0.049$ according to Table 4.

Discussion

Effect of D-galactose i.p induction compared to NaCl i.p on changes in body weight

The results of this study, the group of mice that received D-gal i.p induced at a dose of 300 mg/kg/day i.p for 7 days experienced a significant decrease in BW after the 7th day of injection ($P = 0.049$). This is in accordance with a study by Fatemi *et al*., which reported that mice induced by D-gal at a dose of 500 mg/kg/BB had a weight loss effect. Weight loss after administration of large doses of D-gal is caused by an increase in hydration (increased drinking intake due to increased thirst) which will lead to weight loss due to decreased food intake, and loss of fat, through increased lipolysis.[7,8]

According to Thornton, in rodents, high doses of D-gal can cause oxidative damage to various tissues and organs because systemic exposure to D-gal accelerates the biochemical and morphological processes of aging including the central nervous system. 73 Damage to the central nervous system will affect the central nervous system. Central or peripheral renin-angiotensin. This situation will lead to increased drinking response and weight loss. This is related between chronic hypohydration (extracellular dehydration) and increased levels of the hormone angiotensin II. Furthermore, angiotensin II will stimulate the release of antidiuretic hormone, aldosterone secretion, and thirst.[9]

Table 4: Differences in C-reactive protein, superoxide dismutase, and myostatin levels on days 0 and 7 in the D-galactose intraperitoneally group ($n=11$) compared to NaCl 0.9% intraperitoneally ($n=11$)

	Hari ke-0	Hari ke-7	ΔH7-H0	Nila i.p
CRP				
D-gal i.p	1.47±0.55	1.71±0.56	0.3±0.2	0.041*
NaCl 0.9% i.p	1.38±0.42	1.06±0.86	−0.3±0.8	
SOD				
D-galactose i.p	19.15±1.45	29.36±1.41	10.22±1.61	0.044*
NaCl 0.9% i.p	18.96±1.47	32.37±4.44	13.41±4.49	
Myostatin				
D-galactose i.p	909.2±87.3	998.3±58.7	88.8±18.7	0.049*
NaCl 0.9% i.p	902.0±96.6	870.6±28.6	−121.3±34.6	

*Significance $P < 0.05$. Data mean±SD, independent *t*-test. SD=Standard deviation, SOD=Superoxide dismutase, CRP=C-reactive protein, i.p=Intraperitoneally, D-Gal=D-galactose

Weight loss in mice induced by D-gal at a dose of 300 mg kg/day for 7 days is thought to be due to an increase in ROS which will trigger the activity of SOD, resulting in a decrease in the hormone ghrelin. The decrease in the hormone ghrelin will cause a decrease in appetite, so that food intake in rats will decrease.[9,10]

In contrast to the study by Zhao *et al*., mice given D-gal at a dose of 80 mg/kg/week for 8 weeks had a weight gain effect, similar to research by Chen *et al*., in mice given D-gal of 150 mg/kg/BW for 8 weeks also resulted in weight gain. This difference in results was due to the different doses of D-gal given (small doses over a period of 8 weeks).[11,12]

Research from Azman and Zakaria, the average value of the variable leg muscle mass in the rat group given D-gal 100 mg/kg/day for 8 weeks decreased to 0.50 g, whereas with a dose of 150/kg/day for 8, it tended to decrease again that is equal to 0.45 g.[13]

Effect of induction of D-galactose i.p compared to NaCl i.p on changes in C-reactive protein levels

Analysis of inflammatory biomarkers in experimental rat models after being given D-gal 300 mg/kg/day for 7 days showed an increase in inflammation which was marked by a significant increase in Δ CRP in the group that received D-gal induction (0.3 ± 0.2 pg/mL) compared to the control group induced by NaCl 0.9 i.p (−0.3 ± 0.8) significantly ($P = 0.041$). This happens because of the accumulation of glucose in the body, resulting in increased inflammation. Protein or fat that combines with sugar in the bloodstream will produce advanced glycation end products which can cause oxidative stress and inflammation.[14]

In Petrushev *et al*.'s study, rats were given a D-gal solution of 100 mg/kg orally for 42 days; there would be an increase in 8-iso-prostaglandin F (2α), IL-6, and TNF-α in plasma which is a sign of increased inflammatory markers. 77 inflammatory markers higher circulating enzymes such as IL-6, TNF α, and CRP are significantly positively correlated with skeletal muscle strength and decreased muscle mass in sarcopenia.[15]

In line with Azman and Zakaria's research, administration of 100 mg/kg/day D-gal and 150 mg/kg/day of D-gal was shown to both increase IL-6 levels, 150 mg/kg/day D-gal administration increased IL-6 levels are even higher.[13]

D-gal activates the extrinsic and intrinsic pathways of apoptosis. D-gal also triggers mitochondria to release cyt c, decreases expression levels of anti-apoptotic Bcl2, and

increases apoptotic Bax. The process of apoptosis will trigger neuroinflammation and neurodegeneration.[16] The dose of D-gal to start inducing apoptosis is 100 mg–500 mg/kg/day, with a duration of 6–9 weeks.[17]

Hence, hyperglycemia has a harmful effect on cells and organ systems because it can affect the immune system and act as an inflammatory mediator. Hyperglycemia causes the secretion of pro-inflammatory cytokines, thereby triggering systemic inflammation.[18]

Effect of induction of D-galactose i.p compared to NaCl i.p on changes in superoxide dismutase level

This study showed that in the control group that received NaCl 0.9% i.p, there was a significant increase in Δ SOD (13.41 ± 4.49 pg/mL) compared to the group that received D-gal (10.22 ± 1.61 pg/mL) with $P = 0.044$.

D-gal can cause mutations in mitochondrial DNA and decline enzymes for DNA repair and will result in mitochondrial disturbances that trigger aging. Exposure to D-gal also causes a decrease in antioxidant enzymes such as glutathione, catalase, and SOD which can increase oxidative stress. D-gal is oxidized by galactose oxidase to become hydrogen peroxide (H_2O_2) which causes a decrease in SOD, whereas H_2O_2 reacts with reduced iron and forms hydroxide ions (OH-)/free radicals. Oxidative stress will reduce adenosin trifosfat synthesis which can damage mitochondrial membranes, damage mitochondrial structures, and ultimately induce apoptosis.[19,15]

This study shows that NaCl 0.9% i.p can function as an antioxidant to reduce the effects of free radicals caused by exposure to D-gal i.p. Shwe T *et al*. reported that NaCl can catalyze 1,1-diphenyl-2- pycrilhydrazil. The NaCl substance also functions as an antioxidant as superoxide anion scavenging. Another effect of NaCl is the catalysis of fat oxidation (lipid oxidation).[20,21]

Effect of induction of D-galactose i.p compared to NaCl i.p on changes in myostatin level

This study showed that in the control group that received D-gal i.p, there was a significant increase in Δ myostatin (88.8 ± 18.7 pg/mL) compared to the group that received NaCl 0.9% (−121.3 ± 34.6 pg/mL) with $P = 0.049$.

Myostatin, a member of transforming growth factor-β, functions as an autocrine inhibitor of skeletal muscle growth, induces muscle fiber atrophy by activating Smad2 and Smad3, and suppresses protein synthesis by inhibiting protein kinase B (Akt).[22] Increased myostatin expression plays a central role in integrating/mediating anabolic and catabolic responses. Myostatin negatively regulates the activity of the Akt pathway, which promotes protein synthesis, and increases the activity of the ubiquitin–proteasome system to induce atrophy. In addition, myostatin functions as a modulator of major catabolic pathways, including the ubiquitin–proteasome and autophagy–lysosome systems. Thus, myostatin pathway inhibits muscle growth through (1) cross-regulation between myostatin, growth-promoting pathways, and proteolytic systems; (2) myostatin inhibition causes muscle hypertrophy; and (3) regulation of translation by myostatin [Figure 1].[23,24]

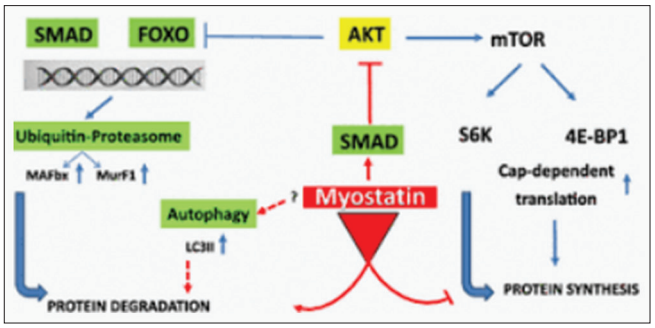

Figure 1: Myostatin function as a modulator of anabolic and katabolic pathway.[26] AKT = Protein kinase B, SMAD = Sma and Mad-related protein, FOXO: class O of forkhead box transcription factors, mTOR = mammalian Target of Rapamycin

The increase in myostatin protein due to the administration of D-gal will cause a risk of muscle atrophy,[25] even though in this study, there has not been a change in the diameter of the gastrocnemius muscle. The new D-gal takes effect in the 4th week.[26]

Conclusion

Giving D-gal high doses for a short time in rat animal models can induce muscle aging which is characterized by increased inflammation and myostatin. This is supported by weight loss which is a sign of the aging process.

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Conflicts of interest

There are no conflicts of interest.

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