Univerzita Komenského
Lekárska fakulta v Bratislave

ZBORNÍK VEDECKÝCH PRÁC

VI. vedecká konferencia doktorandov LF UK

50. fakultná konferencia študentskej vedeckej a odbornej činnosti

Univerzita Komenského
Lekárska fakulta v Bratislave

14. apríla 2011

ZBORNÍK VEDECKÝCH PRÁC

VI. vedecká konferencia doktorandov LF UK

50. fakultná konferencia študentskej vedeckej a odbornej činnosti

Recenzenti: prof. MUDr. Viera Štvrtinová, CSc., doc. MUDr. Daniela Ostatníková, PhD., MUDr. Pavol Janega, PhD.

Editor: Branislav Zagrapan

Za obsahovú a jazykovú správnosť príspevkov zodpovedajú autori.

Výroba CD Tribun EU s.r.o., Gorkého 41, 602 00 Brno

ISBN 978-80-223-3088-6

2011

THE EFFECT OF CHRONIC LIQUID NUTRITION INTAKE ON DEVELOPMENT OF MAXILLA, MANDIBLE AND TEETH IN RATS

Ioannis Konstantopoulos[1], Nikolaos Konstantopoulos [2]

[1]graduated on Molecular Biology and Genetics on Aristotle's University of Thessaloniki-Greece, Dentistry Year 4
[2]graduated on Biology Faculty on Aristotle's University of Thessaloniki-Greece, Dentistry Year 3

Supervisor: doc. MUDr. Boris Mravec, PhD.

Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava

Introduction

Development of maxilla and mandible, as well as teeth, is under control of genetic and environmental factors. Therefore, we investigated the size of maxilla, mandible, and teeth in Wistar rats feed by standard pellet chow or by liquid nutrition.

Materials and Methods

Three experimental groups were used:
- animals feed from weaning till the age of 150 days by standard pelleted chow (n=12; control)
- animals feed from weaning till the age of 150 days by liquid nutrition (n=12; liquid nutrition juvenile)
- animals feed from weaning till the age of 90 days by standard pelleted chow and than feed by liquid nutrition till the age of 150 days (n=12; liquid nutrition adult)

After the termination of experiment, animals were sacrificed. Sculls were cleaned from muscles and other soft tissues.

Using special manual caliper specific distances of the teeth and bones of the heads were measurement. Following distances were measured (1):
- L4: posterior edge of the condyle junction of the mesial surface of the first molar with alveolar bone
- L5 posterior edge of the condyle - most anterior extension of the maxillary bone between the incisors
- L6: posterior edge of the condyle - posterior rim of the mental foramen
- L7: most superior surface of the condyle - line tangential to the inferior border of the mandible
- L12: anterior teeth length of maxilla: the distance between the incisive edges of anterior tooth and the margin of gingiva.
- L13: anterior teeth length of mandible: the distance between the incisive edges of anterior tooth and the margin of gingiva.

***Tab. 1*:** Size of selected parts of mandible and maxilla. Each value is displayed as mean ± SEM. Statistical significance compared to control group: *-p<0.05; *** - p<0.005. Statistical significance between liquid nutrition groups: + + + - p<0.005

	L4 (mm)	**L5** (mm)	**L6** (mm)	**L7** (mm)	**L12** (mm)	**L13** (mm)
Control	1,915±0,0151	2,507±0,0166	2,077±0,0218	1,121±0,0495	0,819±0,0100	1,251±0,0484
Liquid nutrition juvenile	1,987±0,0169 ***	2,577±0,0233 *	2,142±0,0157 *	1,106±0,0106	0,805±0,0129	1,183±0,0172
Liquid nutrition adult	1,885±0,0085 + + +	2,472±0,0143 + + +	2,039±0,0142 + + +	1,189±0,0082 + + +	0,928±0,0905	1,258±0,0428

Results

We have found significant differences in size of mandible, maxilla, and teeth between experimental groups (Tab. 1).

Discussion

The teeth, maxilla and mandible of the rats are growing in relation to animals nutritional habits (2-3). Standard feeding of rats, with solid nutrition, makes the teeth grinding and trimming, provoking a faster rebirth of teeth (3), but a smaller final length. Therefore, distances L12 and L13, representatives for length of upper and lower incisors respectively, belong to normal rates. In addition, the development of the length of the mandible, being represented by distances L4, L5 and L6, belong to normal rate of values, having the necessary space for attachment of masseter muscles (4, 5). The same is also valid for the height of mandible, in the area of ramus, being represented by distance L7.

Feeding the rats with liquids (liquid nutrition) reduces demand on teeth, maxilla and mandible for mastication (6). Therefore, at an early stage of their development, when they are still juveniles, teeth are growing normally (as being represented by L12 and L13). However, the mandible is growing significantly faster than the normal, according to length (as being represented by L4, L5 and L6), because it has no forces coming against to its development (the function of mastication), and as a second reason, because the teeth which are larger than the normal, for the specific developmental stage of rats, need better support. The height of mandible, represented by L7, is not involving in any role for its development at this stage, so it is growing up under normal rate.

At the last stage of their life, the adult rats yet, being under liquid nutrition for the rest of experiment, have larger teeth, especially the upper incisors. The anatomy of their teeth gives the formation the upper incisors to be external of mouth, when lower incisors, inside the mouth, when the rat is having the mouth closed. Therefore, the lower incisors have a minimum retraction on their development, being caused by the touch of occlusion on the body of upper incisors. Of course, these values are much less, than the forces needed for mastication of solid food. Anyway, it causes a minimum retraction on final length of tooth, which is again bigger than the normal. Therefore, both upper and lower incisors are growing freely reaching a final length, bigger than those of control rats. In addition, the length of mandible has been significantly reduced (7), as the incisors don't need any special support, and masseter muscles are smaller than those needed in solid nutrition (5).

In conclusion, we showed that reduced masticatory activity of rats fed from weaning by liquid nutrition significantly alters development of maxilla and mandible, and teeth of these animals. Our experimental data therefore clearly showed that development of maxilla and mandible, as well as teeth, is significantly influenced by the physical composition of food.

Supported by the grant VEGA (1/0260/10).

References

1. Kara C, Orbak R, Dagsuyu IM, Orbak Z, Bilici N, Gumustekin K. In vivo assessment of zinc deficiency on craniofacial growth in a rat model. Eur J Dent 2009; 3: 10-5.
2. Herzberg F, Schour I. The pattern of appositional growth in the incisor of the rat. Anat Rec 1941; 80: 497-506.
3. Law KT, Lee CK, King NM, Rabie AB. The relationship between eruption and length of mandibular incisors in young rats. Med Sci Monit 2003; 9: 47-53.
4. Watts DG, Williams CH. The effect of the physical consistency of food on the growth of the mandible and maxilla of the rat. Amer J Orthodont 1951; 37:895-928.
5. Yonemitsu I, Muramoto T, Soma K. The influence of masseter activity on rat mandibular growth. Arch Oral Biol 2007; 52: 487-93.
6. Yamamoto S. The effects of food consistency on maxillary growth in rats. Eur J Orthod 1996; 18: 601-15.
7. Beecher RM, Corruccini RS. Effects of dietary consistency on craniofacial and occlusal development in the rat. The Angle Orthodontist 1981; 51: 61-9

ATORVASTATIN AND HIGH-FAT DIET INDUCED TISSUE CHANGES IN EXPERIMENTAL ANIMALS

Branislav Zagrapan

(General Medicine, Year 3)

Co-authors: RNDr. Oľga Uličná, CSc.[2], Ing. Oľga Vančová[2], PharmDr. Jarmila Kucharská, CSc.[2]

Supervisor: MUDr. Pavol Janega, PhD.[1]

[1] *Institute of Pathological Anatomy FM CU*
[2] *Pharmaco-biochemical laboratory at the III. Internal Medicine Clinic of Comenius University, Faculty of Medicine*

Introduction

Atorvastatin is one of the lipid-lowering agents called statins. They act 1) directly via inhibition of HMG-CoA reductase and 2) indirectly by increasing the number of liver LDL receptors, to decrease plasma LDL-C concentration. Indications include primary prevention of hypercholesterolaemia-related disorders such as atherosclerosis and secondary prevention of myocardial infarction (MI) and cerebrovascular accidents (CVA) in symptomatic atherosclerosis (1).

Statins are safe (2) and widely prescribed drugs (3). Nevertheless, patients may experience adverse effects such as myalgia, gastrointestinal disturbance, insomnia and rash (1); in extreme cases, rhabdomyolysis may rarely (5/100 000 patient/year) occur (2). More frequent (4.2/1000 patients) is the elevation of serum transaminases (4), fatal liver failure is extremely rare (3). Even though the side effects are rare, they may be crucial in patients with other co-morbidities under the high-dose statin treatment.

The aim of our study was to evaluate the effect of atorvastatin therapy in the high-fat diet animal experimental model.

Method

Male 12-week old Wistar rats were randomly divided into 6 groups (10 animals in each). 3 groups received the standard chow: control group (K) and group with 10mg/kg/day (KA10) and 80 mg/kg/day (KA80) atorvastatin administration. 3 groups received the high-fat diet: without (HFD) and with 10 mg/kg/day (HFDA10) and 80 mg/kg/day (HFDA80) atorvastatin treatment. Atorvastatin was given for 28 days dissolved in drinking water by oral gavage. Animals on HFD were given chow containing 4% cholesterol and 10% pork lard for 28 days prior to and during the atorvastatin treatment.

At the end of the experiment, the markers of liver injury - AST and ALT - were measured in the plasma and the cholesterol and triglyceride content was evaluated in liver tissue homogenates.

The liver and heart tissues were fixed in 10% formalin, routinely processed in paraffin and stained with hematoxylin and eosin (HE). To examine myocardium collagen content, modified picrosirius red staining technique was used (5). The slides were evaluated in a light microscope and measured using the ImageJ software (NIST, USA). The percentage of optically empty space (OES), reflecting the steatosis and dystrophic changes in liver was measured in HE slices.

Statistical analysis

Values were expressed as means ± standard error of mean. Variance across groups was analyzed by ANOVA or Kruskal-Wallis ANOVA. Fisher Least Significant Differences test was employed for comparisons between groups. Analyses were done using DATAPLOT software (NIST, USA).

Results

HFD led to considerable periportal microvesicular steatosis, which significantly increased OES, when compared to controls (10.07±0.15 vs 9.19±0.23, ***Tab. 1***). The cholesterol and triglyceride content were highly increased in HFD groups and only high dose of atorvastatin (HFDA80) provided significant reversal (*Tab. 1*).

This correlates with the decrease of liver steatosis in HFD animals treated with 80mg/kg/day of atorvastatin, characterized by decrease of OES, which is not significant when compared to controls (***Tab. 1***).

The mild hydropic and vacuolar degeneration of hepatocytes was found in all animal groups. It was more prominent in KA10, where it increased the OES (9.31±0.18 vs. 9.19±0.23, $p<0.01$). No changes were found in KA80 group (***Tab. 1***).

aminotransferase as another marker of liver damage was not found to be significantly increased in any group.

Picrosirius red staining of heart tissue showed significant increase of collagen content in HFD animals with atorvastatin treatment (*Tab. 3*). Heart samples in HFDA10 appeared to have on average 75±8.57% more collagen over control (K, $p<0.01$); the increase in HFDA80 was 91 ±7.86% (vs. K, $p<0.01$). Nonetheless, the collagen content was lower in KA10 when compared to both K and KA80 (0.27±0.027 vs. 0.44±0.031 (K) and 0.45±0.049 (KA80), $p<0.01$).

Tab. 1: Liver histology. Mean % OES per field ± SEM [number of animals per group]; $^{**}p<0.01$ vs. K, $^{\dagger\dagger}p<0.01$ vs. HFD, $^{\ddagger}p<0.05$ vs. both KA80 and HFD10.

group	% OES per viewing field
K	9.19 ± 0.23 [10]††
KA10	9.31 ± 0.18 [10]††
KA80	9.20 ± 0.17 [8]††
HFD	10.07 ± 0.15 [10]**
HFDA10	10.47 ± 0.21 [9]**
HFDA80	9.87 ± 0.20 [9]‡

Tab. 2: Liver tissue fats mean content ± SEM [number of animals per group] ** $p<0.01$ versus K. †† $p<0.01$ versus HFD.

group	cholesterol [mmol/kg tissue]	triglycerides [mmol/kg tissue]
K	5.55 ± 0.26 [10]††	8.77 ± 0.38 [10]††
KA10	5.58 ± 0.28 [10]††	8.33 ± 0.52 [9]††
KA80	6.46 ± 0.18 [8]$^{**\,\dagger\dagger}$	7.79 ± 0.77 [8]††
HFD	107.66 ± 9.15 [10]**	41.32 ± 4.66 [10]**
HFDA10	103.09 ± 16.06 [9]**	43.92 ± 2.71 [9]$^{**\,\dagger\dagger}$
HFDA80	43.61 ± 6.87 [10]$^{**\,\dagger\dagger}$	22.07 ± 2.61 [9]$^{**\,\dagger\dagger}$

Serum alanine aminotransferase (ALT) was significantly increased in KA10 (by 28.92 ± 12.00%) and in all HFD groups – (HFD 26.09 ± 6.56%, HFDA10 by 26.09 ± 8.85%; and HFDA80 by 58.04 ± 9.15%) when compared to controls (K). Nonetheless, serum aspartate

Discussion

Periportal steatosis was observed in liver from all HFD groups. 80mg atorvastatin in HFD was a slight improvement over the animals without or with low-dose atorvastatin treatment. Liver

tissue cholesterol and triglyceride content corresponds with the histological changes. Although both parameters are essentially halved in high-dose atorvastatin treated HFD animals, cholesterol was still increased 7.8-times and triglycerides 2.5-times compared to controls. This and similar published results (7) suggest that statin treatment alone without dietary changes might not do enough to prevent organ injury resulting from diet-induced dyslipidaemia.

The mild hepatocyte degeneration was found also in control animals with the atorvastatin administration, accompanied by increased serum ALT. It is possible, that this finding can be explained by known hepatotoxic effects of atorvastatin, described in other studies (4, 6).

Interesting is the increased collagen content in hearts of atorvastatin treated HFD animals. Although, these data are limited by our sample size, similar association between statins and collagen content was discussed in previously published experiments (8, 9), which underlines its importance. We hypothesize that, similar to other statins, atorvastatin inhibits the expression of matrix metalloproteinases (MMP) modifying the Rac1 and RhoA signaling. The suggested mechanism is that of inhibition of membrane targeting of the small GTPases through decreased prenylation both HMG-CoA reductase-dependently and independently (8, 9). Perhaps exogenous cholesterol might potentiate the HMG-CoA reductase-dependent mechanism by its own inhibitory effect on this enzyme, thus lowering MMP expression and increasing fibrosis in some tissues. These mechanisms are incompletely understood and beg further study.

Podporené Európskym fondom regionálneho rozvoja v rámci Operačného programu Výskum a vývoj pre projekt "Vybudovanie Centra excelentnosti pre náhle cievne mozgové príhody na Lekárskej fakulte UK v Bratislave (ITMS 26240120015)," spolufinancované zo zdrojov Európskeho fondu regionálneho rozvoja.

References

1. Rang, PH et al. Rang and Dale's Pharmacology, 6th Ed. Edinburgh, Churchill-Livingstone, 2007, 321 – 329.
2. Law M, Rudnicka AR. Statin safety: a systematic review. Am J Cardiol. 2006; 97(8A): 52C-60C
3. Perger L, Kohler M, Fattinger K, et al.: Fatal liver failure with atorvastatin. J Hepatol. 2003; 39(6):1095-7.
4. Kashani A, Phillips CO, Foody JM, et al. Risks associated with statin therapy: a systematic overview of randomized clinical trials. Circulation. 2006; 114(25):2788-97
5. Dolber PC, Spach MS: Conventional and confocal fluorescence microscopy of collagen fibers in the heart. J Histochem Cytochem. 1993; 41(3):465-9.
6. Ito DT, Molina HM, Andriolo A, et al.: The combination of atorvastatin and ethanol is not more hepatotoxic to rats than the administration of each drug alone. Braz J Med Biol Res. 2007; 40(3):343-8.
7. Ji G, Zhao X, Leng L, et al.: Comparison of dietary control and atorvastatin on high fat diet induced hepatic steatosis and hyperlipidemia in rats. Lipids Health Dis. 2011;10(1):23.
8. Hayashidani S, Tsutsui H, Shiomi T, et al.: Fluvastatin, a 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitor, attenuates left ventricular remodeling and failure after experimental myocardial infarction. Circulation. 2002;105(7):868-73.
9. Ichihara S, Noda A, Nagata K, et al.: Pravastatin increases survival and suppresses an increase in myocardial matrix metalloproteinase activity in a rat model of heart failure. Cardiovasc Res. 2006; 69(3):726-35.