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Glycoprofiling in rats as a possible tool to model different pharmacological approaches

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Abstract

Glycosylation is one of the most common posttranslational modifications of proteins. Glycomic studies on rat serum have revealed that variations in the *N*-glycans of glycoproteins correlated with disease progression. The goal of our study was to describe the glycoprofiles of different rats strains. The 15 weeks old male Wistar (W) and Spontaneously hypertensive rats (SHR) fed 5 weeks standard diet were used. The analysis of serum N-glycoprofile was done by mass spectrometry analytics on MALDI-TOF/TOF instrumentation. The cluster of 22 N-glycans was appointed and sorted with special impact on their structural type. The changes in relative intensities of N-glycans were not significat, however, there were observed some trends in its remodelation within different rats strains: either higher percentage of high-manose N-glycan type (W) or higher portion of complex-bi-antennary N-glycans with fucose (SHR). This preliminary glycoprofiling might assume as a possible tool for basic research to test various therapeutic perspectives with further impact on clinical studies tendencies.

Keywords: *rats;* Wistar*; SHR; glycomics; N-glycans; mass spectrometry; MALDI-TOF/TOF*

Introduction and Objectives

Glycosylation is the enzymatic addition of oligosaccharides (also known as glycans) to proteins and lipids. This is one of the most common posttranslational modifications of proteins. Most of the human secreted and membrane-bound proteins are glycosylated, suggesting a determinant role of carbohydrates in protein function [1]. Moreover, altered glycosylation characterized by different number (macroheterogeneity) and nature of glycans (microheterogeneity) is present in many pathophysiological conditions such as cancer, inflammation, autoimmune and aging [2]. Thus, it is a dynamic equilibrium. Within an individual, the glycan signature is highly reproducible [3], however, during aging or pregnancy or when a disease occurs the glycan pattern can change dramatically [4-5].

Many important biological roles of glycoproteins are modulated by N-linked oligosaccharides: e.g. by influencing the functions of glycoprotein [2] involved in various cellular recognition signals and/or involved in pathological situations such as cancer and inflammation [6-7]. Testa *et al*. [8] found significant changes in N-glycan composition in the sera of type 2 diebetes mellitus (T2DM) patients compared to healthy controls.

The rat is an important alternative for studying human pathology owing to certain similarities to humans. Glycomic studies on rat serum have revealed that variations in the *N*-glycans of glycoproteins correlated with disease progression, which is consistent with the findings in human serum [9]. For our observation we used Wistar rats (W), the general multipurpose model strain and Spontaneously hypertensive rats (SHR) that have been developed as animal models for human essential (idiopathic or primary) hypertensions [10-11].

The main goal of our study was to describe the glycoprofiles of different rats strains (W vs SHR) and to evaluate their differences according to the N-glycan type.

Materials and methods

Animals and diet. All experimental procedures involving animals were approved by the Ethical Committee of the Institute of Experimental Pharmacology and Toxicology, Animal Health and Animal Welfare Division of the State Veterinary and Food Diet-induced neuronal dysfunction 621 Administration of the Slovak Republic (the number of the permit 3635/14-221) and they conformed to Directive 2010/63/EU on protection of animals used for scientific purposes. Adult male W and SHR rats aged 15 weeks (weight 248 ± 56 g at the onset of the experiment) from the Breeding Station of the Institute of Experimental Pharmacology and Toxicology (Dobra Voda, Slovakia) were used. The rats had free access to water and food and were kept on 12h/12h light/dark cycle and housed 5 animals per cage. Animals in each experimental group (n=10) were fed 5 weeks with standard diet (SD).

Analysis of serum N-glycoprofile by MALDI-TOF/TOF. 10 µl of serum was premixed with 40 µl 10 mM Tris, pH 7.5 + 0.1% SDS and incubated with DTT and IAA according to standard protein reduction and alkylation protocols [12]. To release the N-glycans, serum was incubated with 1 U of PNGase F (peptide-N-glycosidase F, Roche) at 37°C overnight. Isolation of N-glycans was performed by PGC SPE (100 mg Supelclean ENVI-Carb, Supelco) as described previously [13] by 60% ACN + 0.1% TFA. To increase the signal intensities and stabilize the sialic acid, N-glycans were subjected to permethylation [14]. Permethylated N-glycans were analyzed by UltrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics) in reflectron positive ion mode with 20 mg/ml DHB in 30% ACN + 1 mM NaOH as the matrix solution. Analyzed data were processed by FlexAnalysis (Bruker Daltonics) and GlycoWork Bench [15] software. Obtained MS and representative MS/MS spectra of free and permethylated N-glycans were compared and evaluated with special focus on N-glycan type.

Statistical evaluation. The data were statistically evaluated using GraphPad Prism 6 Software (La Jolla, U.S.A). Data were expressed as means ± SEM. One-way analysis of

variance (ANOVA) was used to evaluate the difference among all experimental groups (using the Bonferroni multiple comparison test). The level of $p < 0.05$ was considered as statistically significant difference.

Results and discussion

In general, chronic civilization diseases are multifactorial. The key role in their development act not only the environment factors (diet, physical activity, stress, life style) but also genetic predispositions. However, up to this date, little is known regarding the changes in N-glycans during metabolic disturbances in rodents, but observed similarities between the glycomic profile of rat and human sera provided important selection criteria for choosing an appropriate animal model for pathological and/or further pharmacological studies [9].

N-glycosylation of proteins is a complex process, reflecting the physiological state of individual and changes thereof [16]. Thus, characterisation of glycosylation status is an important aspect of understanding the molecular basis of these diseases. As many of the pathways associated with metabolic disturbances involve the production of inflammatory cells, glycosylation profiles may prove to be important in providing insight into the metabolic state of productive cells. Recent studies demonstrated that high levels of circulating glucose, diabetes and diabetic complications are linked with this type of post-translational modification [17].

The 15 weeks old male W and SHR rats (n=10/per group) fed 5 weeks SD were used. The glycoconjugates were isolated and purified from blood sera, then analyzed by means of mass spectrometry MALDI-TOF/MS. The representative glycoprofile is depicted on Fig. 1.

The cluster of 22 N-glycans was appointed and sorted with special impact on their structural type (Tab. 1.).

The changes in relative intensities of N-glycans were not significat, however, there were observed some trends in its remodelation within different rats strains (Fig. 2.). In W group there was detected higher percentage of high-manose N-glycan type. In SHR group was higher portion of complex-bi-antennary N-glycans with fucose.

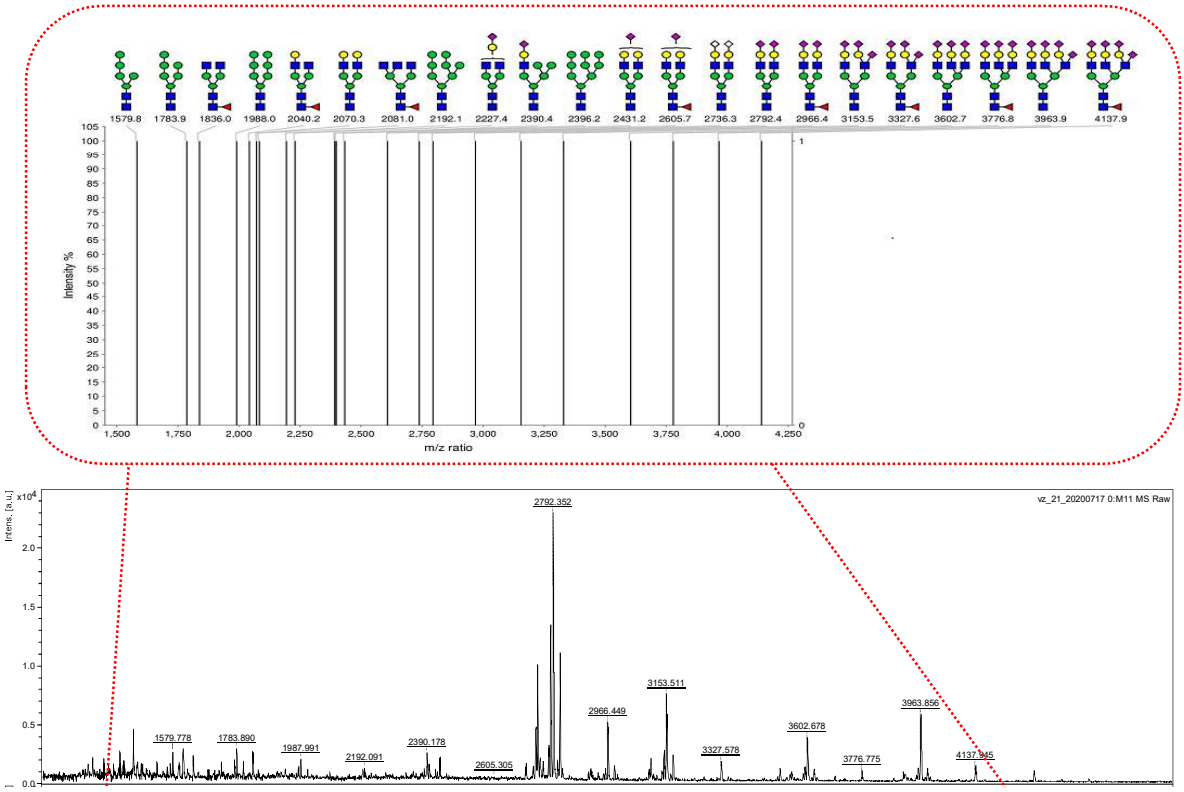

Fig. 1. Representative mass spectrum of N-glycans derived from blood sera of Wistar rats
Legend: green circle – Mannose; blue rectangle – N-acetylglucosamine; yellow circle – Galactose; red triangle – Fucose; purple diamond – Sialic acid; white diamond – Neuraminic acid; Ac - A cetyl.

Tab. 1. The list of detected N-glycans sorted according to their structural type

Glycan type	m/z
High -Man	1579.8; 1783.5; 1988.1; 2192.2; 2396.4
Complex – Bi	2070.3; 2227.4; 2431.5; 2736.8; 2792.7
Complex – Bi – Fuc	1836.1; 2040.2; 2605.7; 2966.9
Complex - Tri	3154.1; 3603.2
Complex – Tri - Fuc	2081.2; 3328.1; 3777.4
Complex - Tetra	3964.3
Complex – Tetra -Fuc	4138.4
Hybrid	2390.4

Similarly, Itoh *et al.* [18] when conducted study on mice T2DM has demonstrated an increase of core-fucosylated serum N-glycans. It has been demonstrated that modifications of fucose content in serum glycoproteins occur also in T2DM patients [19].

The protective association of higher levels of core fucosylated N-glycans was mentioned when the negative correlation changes in level of core fucosylation of plasma N-glycans was found to be associated with metabolic syndrome [20]. However, these studies were

performed on the human population, where many secondary factors, other health issues and complex lifestyle can influence the obtained results.

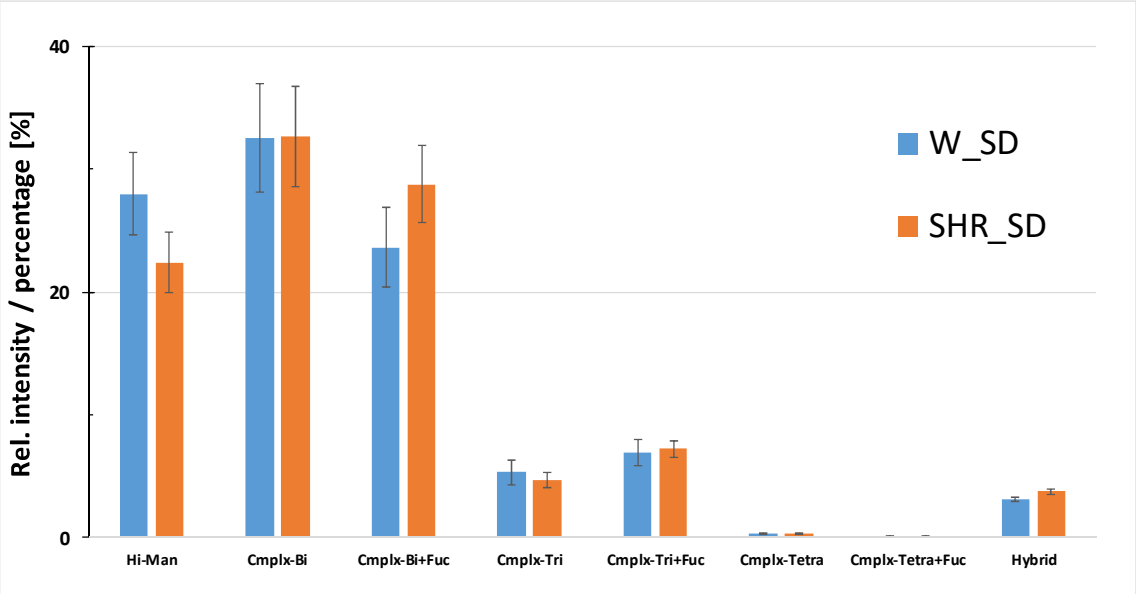

Fig. 2. The relative intensity of N-glycans of Wistar and SHR rats according to their glycan type
W- Wistar rats; SHR - Spontaneously hypertensive rats; SD - standard diet; Statistics: ANOVA One-way analysis of variance with Bonferroni multiple comparison test: W_SD *vs* SHR _SD – NS.

Conclusion

The analysis of serum N-glycoprofile was done by mass spectrometry analytics on MALDI-TOF/TOF instrumentation. The changes in relative intensities of N-glycans were not significat, however, there were observed some trends in its remodelation within different rats strains. In W group there was detected higher percentage of high-manose N-glycan type. In SHR group was higher portion of complex-bi-antennary N-glycans with fucose.

This preliminary output of blood sera glycoprofiling in different rats strains might assume as a possible tool for basic research to test various therapeutic perspectives with further impact on clinical studies tendencies.

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