The Impact of 5-Azacytidine on Placental Weight, Glycoprotein Pattern and Proliferating Cell Nuclear Antigen Expression in Rat Placenta

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Abstract

During the placentalization process, the expression of various glycoproteins plays an important role in embryonal development. Alterations in DNA methylation caused by 5-azacytidine (5azaC) can disturb normal glycoprotein expression as well as the proliferative ability of trophoblast cells. In order to assess this, a single dose of 5azaC was injected intraperitoneally into pregnant rats during days 1—19 of gestation. Animals were euthanised on day 20 and placental weight, as well as glycoprotein composition, was analysed together with immunohistological assessment of the degree of proliferation of the trophoblast cells. The placental weight was found to be significantly smaller in animals treated by 5azaC during days 4 to 14 of gestation (p < 0.01, Student’s t-test). The treatment on days 4, 5, and 6 resulted in a lack of labyrinth with the strong proliferative activity of the cells in the basal layer. Expression of glycoproteins with molecular mass smaller than 60 kDa was reduced with treatment on day 6. The 5azaC administered from days 7 to 10 completely disturbed the placental structure and the proliferation of trophoblast cells was poor. During these days GP70 exhibited stronger expression in treated animals, contrary to GP40, which was stronger in controls. A natural border between the labyrinth and the basal layer was established on days 11 and 12. The basal layer was dominant with a lower proliferation of trophoblast cells compared with the controls. With the establishment of the labyrinth on day 13, the expression of GP40 was restored. Proliferation of the trophoblast cells from days 13 to 15 was higher compared with the controls. The changes in placental mass and the proliferative ability of trophoblast cells in rat placenta exposed to 5azaC represent more proof of the importance of epigenetics in the regulation of placental development.

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1. Introduction

Normal embryonic human development is considered as one of contemporary medicine’s hottest topics. It is impossible to imagine such development without the synchronised cooperation of the embryo and placenta [1].

The rat placenta represents one of the most convenient models for study of the molecules and their interactions in the processes of human implantation and placentation. Rat placenta resembles the human one in many respects [2]. While the human placenta is villous, the rodent placenta is of the labyrinth type, but both of them are haemochorial [3].

Any deviation in gene expression can bring about significant changes in the placenta, which are potentially important for ongoing pregnancy [4]. Considering mammals, gene
expression in most cases is epigenetically regulated [5]. The modification of the mammalian DNA molecule may consequently change the gene expression at the level of transcription and is called DNA methylation [6]. Presently, numerous genes whose methylation appears to be crucial to the normal development of mammalian placenta are known. Mash2 is one of them. It is responsible for coding the transcription factor and it is indispensable for the maintenance of trophoblast stem cells [7]. The methylation process is of significant importance for the development of the rat placenta. The placental basal layer displays different patterns of methylation from the labyrinth layer, suggesting that the normal differentiation of placenta is regulated by precise DNA methylation mechanisms [8].

In order to investigate the influence of hypomethylation on the development of rat placenta we used demethylating agent 5-azacytidine (5azaC). 5azaC inhibits post-replication methylation by its incorporation into DNA causing subsequent inhibition of DNA methyltransferase and loss of methylation followed by the change in gene expression. In this study we analysed possible changes in placental weight, the proliferative ability of trophoblast cells and glycoprotein expression in the rat. All of this was performed while bearing in mind that the glycoprotein expression pattern in human placenta is consistent with a potential role in implantation and placenta- tion [9].

2. Materials and methods

During the study period (2000–2005), we analysed 1278 rat placentas. The study was approved by the Ethics Committee, School of Medicine, University of Zagreb. All placentas were analysed in the laboratory of the Department of Biology.

2.1. Placental samples

Adult Fischer female rats (3 months old) were mated overnight with males of the same age. Vaginal plug designated day 0 of pregnancy. Two groups of animals (three animals in each group) were established for each day of gestation (from days 1 to 19); one group was treated with 5azaC (Sigma-Chemie, Deisenhofen, Germany) (study group), while the second group was treated only with phosphate-buffered saline (PBS; control group). 5azaC dissolved in PBS was administered to rats in the study group by a single intraperitoneal injection at a concentration of 5 mg/kg of body weight. Animals were euthanised on day 20 of gestation. Immediately after isolation, placentas were weighed and frozen in liquid nitrogen for storage at −80 °C for further analysis.

2.2. Western blotting

The glycoprotein pattern was analysed by Western blotting with SNA, UEA-I, PHA-E, and DBA lectins (Vector, Burlingame, CA, USA). This was done on gestational days when placental weight differences between treated and control animals were found, but also in placentas whose growth was not influenced by 5azaC (i.e., days 3, 15, and 17). Placentas were mechanically homogenised (at least two placentas from the same animal in each sample) in homogenisation buffer (50 mM Tris−HCl, pH 7.5; 100 mM NaCl; 1 mM EDTA) containing 1 mM phenylmethylsulfonyl fluoride. The homogenates were centrifuged at 15,000 g for 10 min (at 4 °C) and supernatants were stored at −80 °C. Measuring of the protein content was performed according to the Lowry assay method. Gel electrophoresis of glycoproteins was performed in concentration gradient polyacrylamide gels (5–20%) containing 0.1% sodium dodecyl sulphate (SDS; Sigma, St. Louis, MO, USA) according to Laemmli (SDS−PAGE). The protein samples were dissolved in sample buffer (pH 6.8, Tris−HCl 50 mM, glycerol 10%, β-mercaptoethanol 5%, SDS 2%, bromphenol blue 0.1% in distilled H2O) at a ratio of 1:1. Samples were denatured for 5 min at 95 °C before further analysis. Forty micrograms of total proteins per slot was used, with a constant current of 40 mA. Molecular weight protein markers were loaded simultaneously.

After electrophoresis, proteins were transferred to the PVDF Immobilon membrane (Millipore, Bedford, MA, USA) by a semidyblotting system (Pharmacia, Uppsala, Sweden) in semidyblotting buffer (Tris−HCl 48 mM, glycine 39 mM, SDS 1.4 mM, methanol 20%) [10]. Blotting was carried out at 0.8 mA/cm2 for 60 min. After blotting, the part of the PVDF membrane with protein markers was separated (SDS−PAGE, SDTS, low range, BioRad Lab, Hercules, CA, USA) and the rest of the membrane was blocked overnight with 3% bovine serum albumin (pH 7.5). The membrane was incubated with biotinylated lectins (PHA-E, UEA-I, SNA and DBA) in lectin buffer (MgCl2 1 mM, CaCl2 1 mM in TBS). Lectin−glycoprotein complexes were detected with streptavidin−alkaline phosphatase conjugate and visualised with BCIP (5-bromo-chloro-3-indoly] phosphate) and nitro blue tetrazolium (NBT; Sigma) [11].

2.3. Histology and immunohistochemistry

Placental samples were immersed in a fixative solution containing 4% paraformaldehyde at 4 °C. After fixation and dehydration, the specimens were transferred to paraffin, sectioned in 5 μm slices, deparaffinised, and stained with haematoxylin and eosin. Serial sections were put on silanised slides (S 3003; Dako, Glostrup, Denmark) and air-dried for 24 h at room temperature. Sections were routinely deparaffinised and placed in a jar filled with PBS (pH 7.4). Prior to application to the specimens, primary antibody was diluted with 0.05 mol/dm3 Tris−HCl buffer, pH 7.6, containing 1% bovine serum albumin and labelled by mixing for 15 min in a solution with the biotinylation reagent—a modified biotinylated anti-mouse immunoglobulin. The blocking reagent (normal mouse serum in Tris−HCl buffer containing carrier proteins, 50% w/v bovine serum albumin, and 15 mmol/dm3 sodium azide) was added for 5 min to the mixture to inhibit the appearance of endogenous signal. Monoclonal Mouse Anti-PCNA, Clone PC10 (M 0879, DAKO) was diluted to 1:100. Negative control was performed by omitting the incubation with primary antibodies step. The DAKO Animal Research kit (Peroxidase) was used for primary antibody visualisation according to the manufacturer’s instructions. Haematoxylin was used for counterstaining. The slides were covered with 50% glycerol in PBS.

2.4. Quantitative stereological analysis of numerical density

Randomly selected paraffin blocks of the placenta were used for stereological analysis. In order to evaluate the immunohistochemistry of proliferating cell nuclear antigen (PCNA)-positive cells, five consecutive sections of the placenta were taken in a random fashion from each series. Quantitative stereological analysis of numerical density was performed using a Nikon Alphaphot binocular light microscope (Nikon, Vienna, Austria) using Weibel’s multipurpose test system with 42 points (M 42) at a magnification of ×400 [12]. The area tested (A1) was 0.0837 mm2. For each investigated group the orientation/ pilot stereological measurement was carried out in order to define the number of fields to be tested [12]. The numerical density of PCNA-positive cells was determined according to the point counting method [12]. Numerical density (Nv) was calculated by the formula $N_v = N_A1 / dD$, where $N$ is the number of PCNA-positive cells on the tested area [13,14]. The mean tangential diameter (D) calculated by light microscopy at a magnification of ×400 and for 300 cells was 0.015 mm. The giant trophoblast cells were not counted.

2.5. Outcome measure and data analysis

The primary outcome measure was the placental weight difference between the control group and the group treated by 5azaC on different days of gestation from days 1 to 19. Secondary outcome measures were the comparison of the glycosylation pattern of the placental proteins between the treated placentas (study group) and the controls, and finally correlation and immunohistochemical analysis of PCNA-positive trophoblast cells. The statistical analysis of placental
weight comparing study and control group was performed using the Student t-test. The stereological data for PCNA-positive cells were evaluated by descriptive statistics. Distribution of the data was assessed by the Kolomogorov—Smirnov test, Lilliefors test, and the Shapiro—Wilks W-test. The homogeneity of the variance was tested by the Levene test. Differences in the numerical density of PCNA-positive cells in the groups investigated were analyzed using the multiple analysis of variance (MANOVA) with the post hoc LSD test. Statistical significance was set at \( p < 0.05 \). Statistical analyses were performed using STATISTICA 6.0 software (Stat Soft, Tulsa, OK, USA).

3. Results

In total, 117 rats were included in the study. Among them 58 were treated by 5azaC (49.6%) and 59 were used as a control group (50.4%). The final results were based on 610 placentas treated by 5azaC and 668 controls.

3.1. Placental growth after treatment with 5-azacytidine

Significantly smaller placentas were found when 5azaC was administered from day 4 to 14 (inclusive) of gestation (Fig. 1; \( p < 0.01 \), Student’s t test). Before day 4 and after day 14 there were no significant weight differences between treated and control placentas (Student’s t-test, \( p > 0.01 \)). Placental weights of female rats treated on day 5 of pregnancy equaled 59.5% of the weight of the untreated control placentas; all those treated on day 8 of pregnancy equaled 25.4% of the weight of controls. Those treated on day 9 of pregnancy were only 13.2% of the weight of control placentas, while placentas from animals treated on day 11 amounted to 15.7% of the weight of controls. Finally, placentas from animals treated on day 13 of pregnancy regained 80.3% of the weight of untreated controls.

3.2. Comparison of the glycosylation pattern of placental proteins between treated placentas and controls

No differences in placental glycoprotein pattern/composition were found when animals were treated with 5azaC on days 5 and 14; however, significantly smaller placentas were found in this group. On the other hand, in placentas treated with 5azaC on day 6, the absence of glycoproteins with molecular mass under 60 kDa was found. The only exception was the presence of GP34, showing the same intensity in treated and control placentas (Fig. 2A). Using lectin SNA a novel glycoprotein GP70 was detected in placentas treated with 5azaC on day 7 of gestation, as well as glycoprotein GP40 with stronger expression in control samples (Fig. 2B). Analyses carried out on placentas treated on day 8 of gestation displayed a similar GP70 pattern as that observed on day 7 (Fig. 2C). 5azaC treatment on day 9 of gestation resulted in GP40 expression only in control samples, while GP70 was present in those treated by 5azaC (Fig. 2D). After administration of 5azaC on day 10, GP40 was still restricted to controls (Fig. 2E). However, after administration on day 11, expression of GP40 was stronger in controls and GP70 in treated animals (Fig. 2F). A similar pattern of GP70 was found on day 12 of gestation (Fig. 2G). Finally, administration of 5azaC on day 13 resulted in nearly complete absence of GP40 in controls. However, it was found in treated placentas (Fig. 2H).

3.3. Histological, immunohistochemical, and stereological analysis of placentas treated with 5azaC

The fifth degree polynomial created by using the least squares method was found to be the best way of describing the changes in the mean values of \( N_v \) by days of gestation assessed (Fig. 3a). Multivariate analysis of variance (MANOVA) presented that the difference between the mean \( N_v \) values is highly significant (\( F = 250.76, p < 0.0001 \)).

The significance between the mean \( N_v \) values of the analysed groups was tested using the LSD test (post hoc analysis). The differences between \( N_v \) of all investigated groups compared with the controls are statistically highly significant (LSD, \( p < 0.0001 \)). The mean values of \( N_v \) after the application of 5azaC between days 4 and 6 are statistically significant (LSD, \( p = 0.045 \)), while between days 5 and 14, between days 5 and 15, and between days 8 and 11 those values were not statistically significant. The differences between mean \( N_v \) are statistically highly significant among all other groups (LSD, \( p < 0.0001 \)).

A clearly visible border between the two main parts of the rat placenta (the basal layer and the labyrinth) was found in the control group. The labyrinth layer was prominent (see Fig. 5D). However, in placentas treated with 5azaC on days 4, 5, and 6, no labyrinth layer was identified (Fig. 4). Although significantly smaller, those placentas showed a significantly higher proliferation rate compared with controls (positive PCNA nuclei found in numerous trophoblast cells; LSD, \( p < 0.0001 \)). The same demethylating agent applied to animals from days 7 to 10 of gestation completely disturbs the placental structure, with no recognisable labyrinth layer. Intranuclear PCNA signal was significantly very poor compared with controls (LSD, \( p < 0.0001 \)). The determination of numerical density was performed on day 8 on treated animals only. Placentas treated on days 7 and 10 had a completely disturbed structure.

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![Fig. 1. The placental weight difference between the control group and the group treated with 5azaC on different days of gestation (20-day-old rat placentas).](image-url)
and there was no PCNA signal at all, while on day 9 there was only one PCNA-positive placenta. Therefore, statistical analysis was impossible (Fig. 5A–C).

Application of 5azaC on days 11 and 12 of gestation demonstrated recurrent establishment of a natural border between these two layers, although compared with controls, the natural border of the labyrinth was significantly reduced with a predominant basal layer. Proliferation detected with PCNA was significantly higher compared with the previous days, but still not comparable to controls (LSD, \( p < 0.0001 \); Fig. 6A,B).

Fig. 2. Western blot analysis of placentas treated with 5azaC (group 2) compared with control (group 1) detected by lectins SNA, DBA, PHA-E, and UEA-I. Placental homogenates (40 \( \mu \)g/lane) were loaded onto concentration gradient SDS polyacrylamide gels (5–15%). The glycoprotein pattern of placenta treated with 5azaC on day 6 (A), day 7 (B), day 8 (C), day 9 (D), day 10 (E), day 11 (F), day 12 (G), and day 13 (H) of gestation, obtained by electrophoresis and detection with lectins (group 2) in comparison with control placenta (group 1).

Fig. 3. (a) Separate values of numerical density \( (N_v) \) for proliferating cell nuclear antigen (PCNA) in 20-day-old rat placentas presented graphically depending on the day of 5azaC application. The red lines represent the fifth degree polynomial. (b) The mean values with 95% confidence intervals (CI) of the groups investigated. The red lines represent the mean value and 95% CI for the control group. Based on this analysis a relationship can be found between 5azaC application and the trophoblast proliferation ability of the basal layer cells in rat placenta.

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Fig. 4. Proliferating cell nuclear antigen expression in the placental tissue after the application of 5azaC on day 4 (A), day 5 (B), day 6 (C), and negative control (D). Signal found in trophoblast cells (arrow). b, basal layer; d, decidua. *Giant trophoblast cell. DAB/haemalaun contrast.

Fig. 5. Proliferating cell nuclear antigen expression in placental tissue after 5azaC application on day 8 (A), day 9 (B), and negative control (C) as well as normal placenta (D). Signal found in trophoblast cells (arrow). DAB/haemalaun contrast.
characteristic relationship of the basal and the labyrinth layer appears to be present only when 5azaC was applied on days 13, 14, and 15, with positive intranuclear PCNA signal present in both trophoblasts and giant cells (Fig. 6C). However, numerical density ($N_v$) of PCNA-positive cells was significantly higher than in the control group (LSD; $p < 0.0001$).

4. Discussion

The role of DNA methylation in growth and differentiation of the rat placenta, with special emphasis on glycoprotein expression, is opening a new field in reproductive biology. The glycoproteins are crucial molecules during the process of placentation. In this work we wanted to emphasise the necessity of the combination of two different areas of molecular biology, epigenetics and glycoproteomics, which have both been of particular interest to scientists in recent years.

The pioneering experiments of McGrath and Solter [15] significantly contributed to the positioning of epigenetics, making it specifically interesting to many of the scientists involved in the technology of cloning and methods of assisted reproduction. Also, upon the discovery of lectins, the proteins that recognise specific groups of sugars, glycoproteomics flourished as the special branch of glycobiology, which is well documented by original research papers in this field [16].

The lack of knowledge of the development of human placenta [17], together with the fact that placentation disturbances represent the origin of many diseases related to pregnancy, may potentially explain the relatively low fertility in humans compared with some other mammals. The trophoblast cells, being crucial for placentation development, display a very specific behavioural pattern of invasiveness resembling the tumour cells in some of their life characteristics. The key difference between the two of them is defined by the spatial and temporal limitations of trophoblast cells [18].

By interruption of the normal methylation pattern using the demethylating agent 5azaC, we followed several parameters of rat placenta development including placental weight, expression of glycoproteins, appearance of the two main layers (the basal and the labyrinth), and the degree of cell proliferation. In order to assess the importance of methylation during gestation, 5azaC was applied in animals in the study group on different days, covering day 1 to day 19 of the pregnancy.

The analysis of placental weight after the application of 5azaC in pregnant rats at different stages of gestation showed a significantly reduced weight in the study group compared with the control group. The weights of placenta from pregnant rats treated on days 4–14 of pregnancy were significantly smaller compared with those of the untreated control group ($p < 0.01$). It has been shown that experimentally induced hypomethylation has a far more serious impact on embryonic tissue development compared with the extraembryonically induced one. However, the impact of hypermethylation is directly opposite to that [19]. The development of the placenta is less affected by experimentally induced mutations in the <i>Dmnt1</i> gene, compared with serious damage in the embryonic
tissues in carriers of those mutations [20]. Hypomethylation of the imprinted genes does not lead necessarily to their reactivation because they are additionally silenced through the histone modification. Therefore, it was postulated that the imprinting mechanism is much more stable, particularly when the extra-embryonic tissues are involved [21].

Treatment with 5azaC in the study group from day 1 to day 3 of gestation did not influence the growth of placenta as assessed by placental weight. Before day 4 the pre-implantation embryo shows the lowest degree of methylation. The process of de novo methylation and simultaneous epigenetic reprogramming of the genome starts from day 5 [22]. As a consequence of 5azaC treatment from day 4 a statistically significant reduction in placental weight was found; however, no difference in glycoprotein composition was noted with any of the lectins.

Application of 5azaC on day 6 results in many differences between placentas in the study group compared with control group. The Western blot analysis found that the glycoproteins with a molecular mass of less than 60 kDa displayed reduced glycosylation in study group samples. They were barely recognisable with all lectins observed. PHA-E lectin reacts with endothelial cells of the fetal blood vessels and it is particularly strong in the labyrinthine layer [23]. The histology of the analysed placentas was almost totally devoid of labyrinth with a dominant basal layer if 5azaC was administered on day 6. Therefore, we can conclude that the glycoprotein pattern appears to be poor. The degree of proliferation of these cells in the basal layer was exceedingly high, pointing to a question about gene affection during our application of 5azaC resulting in the absence of labyrinth development. We believe that inactivation of the gene Gcm1 is involved in this reaction. Gcm1 codes the transcription factor responsible for the initiation of chorioallantoic ramification in healthy individuals and it is a precondition for the establishment of the blood circulation of the mother and the fetus. It was found that Gcm1 mutants do not form the labyrinth at all [24]. Also, Gcm1 and its protein gene products are responsible for the differentiation of syncytiotrophoblasts. The expression of Gcm1 mRNA is visible in mouse chorion cells before the establishment of the contact with allantois [25]. In humans, reduced expression of the GCM1 gene analogue is observed in a pathologic condition called pre-eclampsia [26].

The application of 5azaC from days 7 to 10 of gestation displays a different pattern of glycosylation. The expression of the new glycoprotein GP70, which was completely absent or only slightly expressed in control samples, was clearly detected in placentas treated by 5azaC. This is opposite to glycoprotein GP40 being found only in the controls. By application of 5azaC we probably changed the expression of genes at that very moment when the eutocplacental cone, as a primordium for the development of the final placenta, was developing [27]. This explains the histology findings of barely detectable labyrinth in 5azaC-treated placentas.

Days 11 and 12 of rat pregnancy represent the period of particularly pronounced endovascular invasion of giant trophoblast cells into the maternal vascular system and differentiation of spongioblasts into the glycogen-rich trophoblast cells responsible for interstitial infiltration [28]. 5azaC-treated placentas showed a clear border between the labyrinth and basal layer at this stage of pregnancy. Schreiber and collaborators constructed mutants for the gene Fasl, a member of the gene family that codes for synthesis of AP-1 (Activator Protein 1) crucial for embryonic development and carcinogenesis. Embryos lacking this gene die in mid-gestation with the reduced labyrinth, while the major part of the placenta is avascular. On the contrary, the basal layer remains without changes [29].

Hemberger and Cross quote a series of genes (i.e., Sos1 and Gab1) whose mutations can result in reduced labyrinth. The expression of the Mash2 gene, which is epigenetically regulated by the process of DNA methylation, is responsible for the maintenance of the trophoblast stem cells. The Mash2 gene is intensively present in the placenta up to day 12 of gestation, while in the later stages of pregnancy its expression diminishes [30]. Although 5azaC probably activates the Mash2 gene when applied after day 12 of gestation, its activity does not disturb the development of the labyrinth layer. Western blot analysis using PHA-E lectin shows stronger expression of GP70 in treated placentas. The stronger expression of this glycoprotein was found in treated rats from day 7 to day 12 of pregnancy, but was not or was only slightly visible in control placentas.

Day 13 of rat gestation is the period during placental development when glycogen-rich cells continue decidual invasion and concentrate around the central maternal arteries [2]. In 5azaC-treated specimens the labyrinth occupies more than two-thirds of the placenta, resembling the normal pregnancy. Moreover, a positive intranuclear signal is visible in trophoblast and giant cells. Intensive cellular division is in concordance with endovascular invasion of giant cells and interstitial invasion of glycogen-rich cells. Although the historical picture resembles that of the normal, healthy placenta, the expression of glycoproteins in the 5azaC-treated animals still does not match the expression pattern in controls. Before day 13 of gestation GP40 showed elevated expression in controls compared with 5azaC-treated placentas. When 5azaC was applied to pregnant female rats on this very day it caused elevated expression of GP40 in treated placentas. Therefore, it appears that its expression is important for the establishment of the normal placental structure.

We assumed that the preconditions for normal placental structure are established before day 13 of gestation. Consequently, there was no difference in glycoprotein composition in placentas treated by 5azaC on days 14, 15, and 17, compared with untreated placentas. However, a statistically significant difference in placental weight was found in animals treated by 5azaC on day 14 compared with controls. As this is the period when the endovascular invasion of giant cells and interstitial invasion of glycogen-rich trophoblast cells are not completely finished, we can still interfere with the establishment of flow through the labyrinth [31].

The proliferative capacity of placental cells was determined by studying the expression of PCNA. This is a non-histone nuclear protein, which functions in the system of DNA polymerase δ and serves as an excellent marker for cell proliferation [32]. Many researchers use it in oncology not only because
it has been proven that its elevated expression indicates cancer invasiveness, but also because it indicates the prognosis of the malignant disease [33].

It is clear that PCNA is included in the process of DNA methylation. The DNA methyltransferase (Dnmt1) is associated with PCNA in the region of replication forks [34]. Dnmt1 demonstrates much stronger affinity for DNA with already bound PCNA, compared with a free DNA molecule. Therefore, the interaction of PCNA and Dnmt1 enhances the process of methylation [35]. The application of 5azaC influences the development of the placenta and the degree of trophoblast proliferation in their basal layer. A statistically significant difference in the expression of PCNA-positive cells is clearly evident in all analysed placentas, starting from day 4 and continuing to day 15 of 5azaC application. The most prominent changes occurred in placenta exposed to 5azaC from days 7 to 12. The expression of PCNA-positive cells is lower during that period and the difference is statistically significant compared with the controls. If 5azaC was applied on other days of the rat pregnancy the PCNA expression would be higher than that of the controls. The PCNA protein, which is included in the replication of DNA, is also involved in repair of a damaged DNA molecule [36]. However, tumour suppressor protein p53 also participates in the regulation of its activity. The low concentration of protein p53 in cells leads to the activation of the PCNA promoter, while its high concentration results in its inactivation [37]. Based on our results we believe that 5azaC administered on days 4, 5, and 6 of gestation was so teratogenic that the reaction against its application has mobilised the whole cell machinery for damaged DNA repair.

However, there is still an open question as to why there were no differences found in the glycoprotein content of the placenta exposed to 5azaC on day 5. Also, in this group of analysed placentas, no clear labyrinth was found. Therefore, in our next research project we intend to expand the number of lectins in order to recognise different glycoproteins. The results regarding animal outcome were presented in another of our papers [38] and are not further discussed here.

Our results prove the theory that all placentation research projects on a molecular level should include morphological assessment. This can have a significant impact on designing further studies indicating the importance of the exact identification of detected glycoproteins. According to methylation patterns their expression is a prerequisite for normal placentation and fetal development.

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References


