QUANTITATIVE MORPHOMETRY ANALYSIS OF THE RAT EPIDIDYMIS AFTER A PERORAL ADMINISTRATION OF NICKEL

ANALIZA MORFOMETRICĂ A EPIDIDIMULUI DE ŞOBOLAN DUPĂ ADMINISTRAREA ORALĂ A NICHELULUI

BÁBIKOVÁ, L., TOMAN, R., HLUCHÝ, S., MASSÁNYI, P.
Slovak Agricultural University in Nitra, Slovak Republic

The aim of the study was to evaluate the changes of rat epididymis after a long term intake of nickel chloride using morphometry methods. The nickel was administered in a daily dose of 100 mg.l^{-1} $NiCl_2$ in drinking water to male rats during 3 months. The males were housed individually in a plastic cages. Ten males served as an untreated control group without nickel treatment. At the end of the experimental period (3 months), the animals from nickel–treated group and control group were killed and the samples of the epididymes were taken for morphometry evaluation. Three months after nickel treatment, no significant decrease in interstitial tissue volume from 61.23 ± 15.29 % to 59.44 ± 16.91 % in the epididymis of Ni- treated rats was observed. The tubule epithelium in nickel-treated group increased insignificantly to 25.97 ± 11.75 % and the tubule lumen volume to 14.58 ± 7.21 % in comparison with the control group (24.17 ± 11.08 % and 14.52 ± 8.40 %, respectively). The diameter of the epididymal tubule significantly (P<0.0001) increased from 168.31 ± 53.96 % to 218.85 ± 96.97 %. A significant (P<0.0001) decrease in the epithelium height from 33.55 ± 9.90 % to 28.18 ± 9.75 % was also noted. The findings show the slight effect of peroral nickel on the epididymal structures indicating the possibility of the male fertility depression caused by nickel.

Key words: epididymis, nickel, rat, morphometry, structure

Introduction

The toxicity of nickel has become of great interest because of its widespread environmental occurrence. Nickel has many industrial uses, including stainless steel, electroplating, pigments and ceramics.

Nickel enters the human body through food, air, water, objects for everyday use, and seldom in some other way. Nickel is partly excreted from the organism (through urine, feces, sweat or in some other way) and remains partly in the organism for some time manifesting its biological effects (Stojanovic and Dragana, 2005). Its excessive intake may cause the rise of an allergic reaction (Menne, 1994). The data on the effect of nickel on the male and female reproductive system are rare. The older information show the decrease amount of

the spontaneous contraction in the uterus after the nickel administration (Rubanyi and Balogh, 1982). In the testis, the administration of $NiCl_2$, in drinking water, induces the shrinkage of the seminiferrous tubules and decrease the number of basal spermatogonia (Käkelä, Käkelä and Hyvärinen, 1999). The application of nickel chloride to male rats causes some changes of testicular enzymes which took place in the metabolism of xenobiotics. The significant decrease of 7-ethoxyresorufin O-deethylase and glutathione S-transferase was displaced 16 hours after application of a single i.p. dose 59.5 mg $NiCl_2$ kg^{-1}. No other pathological effects on the testes were observed (Iscan et al., 2002). Some chelating agents have ability to protect the testis against the adverse effects of nickel and increase its excretion from the testis (Xie et al., 1995).
The aim of this study was to quantify the histological changes in the rat epididymis by the morphometry methods after the nickel administration in drinking water.

Materials and Methods

Twenty males Wistar rats were divided to two groups, nickel-treated group (B) and control group (A), each containing 10 males. The males were housed individual, in plastic cages. The animals had unlimited access to drinking water and feed. The rats in the nickel–exposed group were given drinking water treated with $NiCl_2$ at the concentration of 100 $mg.l^{-1}$ for three months. Ten males served as the untreated control group without nickel treatment. Average daily water consumption was measured. After the three months, the animals of both groups were killed and the samples of epididymes were collected for morphological analysis. The tissue samples were fixed in Bouin's solution and then processed by routine histology techniques. The relative volume (%) of the tubule epithelium, tubule lumen and interstitial tissue were determined by morphometric analysis modified by Uhrín and Kulíšek (1980) in at least 15 microscopic images of epididymis per animal. The measurements on the microphotographs were done using 540 point test net. The diameter of the epididymal tubule and the height of the epithelium (μm) were measured using the morphometric software M.I.S. quick photo. From final data, the basic statistical characteristics were calculated and the differences between groups were tested by Student's t - test.

Results and Discussions

The results of the morphometrical evaluation of the epididymis structures are summarized in Table 1.

Table 1.

Relative volume of structures, diameter and height of the epithelium of epididymis tubule.

Parameters	Group A	Group B
	Mean ± SD	Mean ± SD
Epithelium (%)	24.17 ± 11.08	25.97 ± 11.75
Lumen of tubule (%)	14.52 ± 8.40	14.58 ± 7.21
Interstitium (%)	61.23 ± 15.29	59.44 ± 16.91
Diameter of tubule (μm)	168.31 ± 53.96	218.85 ± 96.97****
Height of epithelium (μm)	33.55 ± 9.90	28.18 ± 9.75****

**** $P < 0.0001$

A morphometry analysis of the epididymis after 91 days of nickel chloride administration in drinking water revealed a highly significant ($P<0.0001$) decrease of the epithelium height to 28.18 μm in comparison with the control group (33.55 μm). Similar results found Toman et al. (2005) in his researches, but with the application of cadmium. In the experiment with mice, the height of the epididymis epithelium was higher in comparison to the control group.

The data on the influence of nickel on the epididymis are rare and morphometry evaluation of the epididymal tissues is completely missing in the literature. Toman et al. (2002) described the effects of an acute and chronic intake of cadmium on epididymis of rabbits. They observed significant increase in the tubule diameter from 199.20 μm in the control males to 342.34 μm in animals which were orally dosed with 1.0 mg Cd/kg body weight for a 5-month period. These results are comparable to our morphometric analysis of the tubular diameter. We have detected highly significant ($P<0.0001$) increase in the tubule diameter from 168.31 μm in group A to 218.85 μm in group B. The acute administration of cadmium causes the decrease in diameter of tubule by 18.19 μm (Toman et al., 2002).

Three months after the nickel administration, the decrease in the interstitial tissue volume of the epididymis from 61.23 % in group A to 59.44 % Ni-treated males was observed. Toman and Massányi (1997) noted enlargement of interstitial tissue in caput of epididymis which was caused by the increase of collagen fibers after 48 hours of cadmium intake. On the other hand, during the chronic experiment the volume of interstitial tissue decreased which corresponds to our results but the decrease was not significant. Toman et al. (2005) described increase of interstitial tissues in mice which were treated 12 weeks with $CdCl_2$ at a daily dose of 1 mg Cd^{2+}/kg b.w in diet.

The relative volume of the tubule epithelium covered 24.17 % of the epididymis in the control group and 25.97 % in the nickel-exposed group. The epithelial relative volume of the control rats was lower than that of the small rodents analyzed by Massányi et al. (2002). Authors found that the epithelial volume represent 54.3 % and 46.3 % in the epididymis of *Apodemus sylvaticus* and *Apodemus flavocollis*, respectively. Toman et al. (2002) note that long term intake

of cadmium causes the decrease of relative volume of epithelium. In our experiment with the chronic intake of nickel, the slight increase of the lumen from 14.52 % in the control group to 14.58 % in the nickel-treated group was found.

Conclusions

The findings show the slight effect of peroral nickel administration on the epididymal structures indicating the possibility of the male fertility depression caused by nickel. The diameter of the epididymal tubule significantly ($P<0.0001$) increased. A significant ($P<0.0001$) decrease in the epithelium height was also noted. The changes in the relative volume of tubule epithelium, lumen and interstitial tissue of the epididymis were insignificant.

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BÁBIKOVÁ, L., TOMAN, R., HLUCHÝ, S., MASSÁNYI, P.
Slovak Agricultural University in Nitra, Slovak Republic

Scopul acestui studiu a fost de a evalua prin metode morfometrice modificările care au loc în epididimul de şobolan după perioade lungi de administrare a cloruri de nichel. Nichelul a fost administrat, masculilor de şobolan, zilnic în apa de băut în doze de 100 mg^{-1} $NiCl_2$, timp de trei luni. Masculii au fost întreţinuţi individual în cuşti de plastic. Grupul de control a fost alcătuit din trei masculi netrataţi. La sfârşitul perioadei experimentale (3 luni), animalele din grupul tratat cu nichel au fost sacrificate şi au fost prelevate probe din epididim pentru efectuarea analizelor morfometrice. După trei luni de administrare a nichelului, nu a fost observată o scădere semnificativă a volumului interstiţial de la 61,23 ± 15,29 % la 59,44 ± 16,91 % la animalele din grupul tratat cu Nichel. Tubul epididimului la animalele tratate cu nichel a crescut semnificativ la 25,97 ± 11,75 % iar volumul lumenului la 14,58±7,21% în comparaţie cu grupul de control (24.17 ± 11.08 % şi respectiv 14.52 ± 8.40 %). Diametrul tubului epididimar a crescut semnificativ (P<0.0001) de la 168.31 ± 53,96 % la 218.85 ± 96.97 %. O scădere semnificativă a înălţimii epididimului a fost observată de la 33.55 ± 9.90 % la 28.18 ± 9.75 %. Aceste date au arătat că nichelul administrat oral o perioadă de trei luni afectează uşor structura epididimului ceea ce poate fi cauza depresiunii fertilităţii masculilor.

Cuvinte cheie: epididim, nichel, şobolani, morfometrie, structură