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The influence of natrium fluoride on serum protein and cholesterol levels in rats with adriblastin–induced nephrotic syndrome

Wpływ fluorku sodu na poziom białka i cholesterolu u szczurów
z zespołem nerczycowym wywołanym adriblastyną

Fluorine is a biochemically very active element, entering easily into the cells and interacting with a number of other ions and substances on various metabolic pathways. As an inhibitor of over 70 enzymes it can exert toxic effects on tissues. However, fluoric ions are known to increase the activity of some enzymes, e.g. adenylate cyclase and HMG reductase (1, 2, 3, 4). It has been experimentally stated that fluoride can interfere with protein, lipid and metabolism and its influence depends on the way and time of exposure, applied dose and even species and sex of animals used in the study (3, 5, 6, 7, 8, 9, 10,11). Toxic effects of action may be visible in kidneys, especially in the course of renal insufficiency causing accumulation in the body. It has been noticed, that only the long–term exposure to high of NaF led to structural changes within the nephrons – the kidney seemed to be less sensitive to fluoride action than other organs (4, 12, 13, 14).

As far as we know, there is a lack of information concerning fluoride influence on previously changed protein and lipid metabolism, and on kidneys without impaired function.

Nephrotic syndrome is a classic example of renal disease with coexisting disturbances of protein and lipid metabolism characterised by hypoproteinemia caused by massive losses of proteins in the urine and hypercholesterolemia. It may be complicated by renal insufficiency, but in the early

period of disease there are, as a role, no signs of uraemia. The purpose of this study was to attempt to evaluate the influence of fluoride natrium on the serum protein and cholesterol levels in rats with experimentally produced nephrotic syndrome.

MATERIAL AND METHODS

The experiment was performed on 30 male Wistar rats 1–month old, weighing 120–170 g at the start of the study. The animals were divided into 3 groups as follows: group I (ADR) consisted of 9 rats fed the regular laboratory rat chow and watered *ad libitum*. The animals were given Adriblastin (Farmitalia, Carlo Erba, Italy) intraperitoneally on the 1st day of the study and on the 56th day in the dose 5 mg/kg each time; group II (ADR + NaF) – consisted of 12 rats fed the same diet with addition of 10 mg NaF per kg/day supplied in drinking water. Adriblastin (ADR) was administered as described above; group III, consisting of 9 rats served as a control. The animals were fed the diet without NaF and given isotonic saline instead of adriblastin.

The rats were decapitated after 10 weeks. The kidneys were excised and prepared for light and electron microscopical investigation as described elsewere (15, 16).

Proteinuria was determined after 1, 3, 5 and 8 weeks of a study and on the day before decapitation. The total protein, cholesterol and creatinine levels in serum were determined in blood drawn from the heart immediately after decapitation (17, 18). Student's test and Cochrane–Cox test were used for statistics and the p–value = 0.05 was considered as significant. The data were expressed by mean ± SD.

RESULTS

Histological changes in kidneys of rats receiving adriblastin involved glomeruli and tubules. In the glomeruli fusion of foot processes and vacuolisation of podocytes were visible. The tubules contained homogenous protein casts. The histological picture resembled that described by others (5, 16, 19, 20, 21, 22, 23) and had some features in common with the minimal charge nephrotic syndrome in humans. No microscopical differences between group I and II were observed. All animals treated with adriblastin developed massive proteinuria and ascites from the second week of study. Proteinuria increased progressively approaching the main value of 4686 ± 471 mg/dl in group I and 4590 ± 397 mg/dl in group II ($p > 0.05$). In the control group its mean value varied from 72 mg/dl to 118 mg/dl through the time of the study. The results of chemical investigations of blond serum are summarized in Table 1.

As can be seen, the mean protein level in serum of nephrotic rats was significantly lower in comparision with the control group. No statistically evident difference was observed between group I and II. The serum concentration of cholesterol was significantly increased in rats given only adriblastin, while it was insignificantly elevated in nephrotic animals receiving NaF in the diet. The comparison between group I and II revealed the evidently lower cholesterolemia in rats receiving NaF ($p = 0.2$). No differences of creatinine levels amon the investigated groups were observed.

Table 1. Biochemical findings in serum of nephrotic rats

Group	Total proteins g/L	Cholesterol mmol/L	Creatinine μmol/L
I – ADR	52.2 ± 2.9 [1)]	3.14 ± 2.48 [3)]	77.8 ± 7.1
II – ADR + NaF	54.7 ± 2.9 [2)]	1.64 ± 0.88 [4)]	74.3 ± 6.2
III – Control	62.3 ± 2.0	1.26 ± 0.19	76.0 ± 3.5

1) $p < 0.001$ as compared to control, 2) $p < 0.002$ as compared to control,
3) $p < 0.01$ as compared to control, 4) $p < 0.02$ as compared to ADR

DISCUSSION

Adriblastine–induced nephropathy is a well–known model of the experimental nephrotic syndrome in rats (15, 16, 17, 18, 19, 20). It has been stated that in a short–term experiment the typical histological and biochemical changes developed without impairment of renal function (15). Our results are in accordance with this observation. In our previous work we observed no significant changes of serum protein and cholesterol levels in rats receiving NaF in diet (21), whereas other authors observed hypercholesterolemia or hypocholesterolemia in animals of different species exposed to fluoride (2, 3, 5, 6, 7). It is noteworthy that serum cholesterol level in nephrotic rats in our experiment was lower in animals fed the diet containing NaF. The nephrotic hypercholesterolemia is considered as secondary to pre–existing proteinuria and hypoproteinemia. As fluoride natrium did not alter the protein level in serum of rats in group II in comparison to group I, its direct influence on cholesterolemia should be taken into consideration. On the one hand fluoride increases the activity of HMG reductase – on enzyme taking part in cholesterol synthesis (2, 4, 7). On the other hand, however, fluoric ions inhibit the activation and oxidation of fatty acids and decrease the activity of pyruvate dehydrogenase (1, 2). This can lead to a reduction of the acetyl–CoA amount in the cells and impair in this way cholesterol synthesis from this substrate. It seems possible that such influence of fluoride on cholesterol metabolism is determined by pre–existing hypercholesterolemia in nephrotic rats. Our observations indicate that fluorine can exert some effects on cholesterol metabolism in nephrotic syndrome but considering our results as preliminary, we are of the opinion that further research is necessery to throw more light on this question.

CONCLUSIONS

1. Fluoride natrium in the diet diminishes cholesterol level in blood serum of rats with adriblastin–induced nephrotic syndrome.

2. No influence of fluoride on total protein level in serum of nephrotic rats was observed.

3. Fluoride did not impair renal function in nephrotic rats during 10 weeks of administration.

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STRESZCZENIE

Celem pracy było określenie poziomu białka i cholesterolu w surowicy krwi szczurów z zespołem nerczycowym wywołanym podawaniem adriblastyny. Badania przeprowadzono na trzydziestu szczurach, samcach rasy Wistar. Zwierzętom podawano adriblastynę dootrzewnowo, natomiast NaF był podawany w wodzie do picia. Adriblastyna wywoływała typowe dla zespołu nerczycowego zmiany w nerkach oraz hipercholesterolemię. Poziom cholesterolu w surowicy krwi szczurów z zespołem nerczycowym, którym jednocześnie podawano NaF, był znacząco niższy w porównaniu z grupą otrzymującą tylko adriblastynę. Wskazuje to, że fluor może zmniejszać hipercholesterolemię. Wyjaśnienie mechanizmu tego zjawiska wymaga dalszych badań.