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THE INFLUENCE OF SELENIUM ON RAT BRAIN CHROMATIN DURING CARCINOGENESIS

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Chromatin was isolated from control rat brains and from the brains of animals treated with carcinogen, or selenium alone and carcinogen tagether with selenium. The relative content of some chromatin constituents (proteins, RNA and DNA) was determined. The amount of nonhistone proteins and histone was higher in all treated groups when compared to control rats. Differences between protein species assessed on the basis of their electrophoretic mobility mobility were also evident. The obtained results were in correlation with extinctional and melting profiles of chromatin. Namely, higher values of Tm for chromatin in the animals treated with selenium alone, selenium with carcinogen and especially carcinogen alone were found and its susceptibility to degradation with DNase was lower compared to controls. Enzymatic degradation of chromatin produced well differentiated nucleosomal fractions. There was increased release of polynucleosomes in all experimental groups. The obtained results indicate that after treatment of the animals with carcinogen and selenium, the chromatin under went some structural rearrangement.

Key words: Brain chromatin, carcinogenesis, selenium.

INTRODUCTION

Selenium is an ultratrace element that is essential to the human diet (Burk, 1989; Golczewski et al. 1989; Kumpulainen, 1989; Shamberger, 1986). The soil of several Serbian towns and regions has a low concentration of selenium, and its residents have a lower selenium status than do most other populations (Maksimović, et al. 1992).

Some biological functions are thought to depend on the tissue concentration of that trace element. Selenium occurs in all cells and tissues of the body at levels that vary with the tissue as well as the level of selenium. For each trace element there is a range between the minimum reginement and maximal non-toxic level, but range every trace element is potentially toxic when the

upper limit is exceeded. Selenium componds have demonstrated toxicity in humans, as well as in human tissues in culture. As early as 1956. selenium was used as an antioneoplastic agent in man with some demonstrated activity. Recently, evidence in both tumor-bearing animals and human tumor-cells in culture has confirmed an antitumor effect of potential clinical benefit (Batist, 1988).

In assessing the biological role of proteins associated with DNA in chromatin, an important consideration is the effect of DNA-protein interaction on the structure of genetic material, since there is a close correlation between the structure of chromatin and its biosynthetic activity (Davie et al, 1981; Weisbrod, 1982). There fore, the aim of this paper was to find out whether selenium expresses any effect on chromatin structure and function which might be influenced by a carcinogen.

MATERIALS AND METHODS

The present investigation was carried out on the brains of male Wistar rats treated with a carcinogen and selenium for 120 days. The animals were divided into four groups. Eight rats were fed a diet supplemented with 40/mg/L 9,10-dimethyl-1,2-benzanthracene (II); eight more rats were fed a diet supplemented with 0.01 mg/L sodium selenite (III) and eight more rats were (IV) given a selenium supplemented diet with carcinogen. Five control rats (I) and all the experimental animals (II,III and IV) were housed in suspended stainless steel wire cages in a temperature controlled room with a 12-h light and dark cycle, and maintained on the pelleted diets with tap water supplied ad libitum. The rats were then sacrificed and the brains were rapidly removed and washed in ice cold physiological saline solution. The investigations were carried out with a pool of brain tissues for each croup.

From the isolated nuclei (McEwen et al, 1972), chromatin was prepared by the modified method of Vujović et al. (1989). Two groups of nonhistone proteins were extracted from purified chromatin using different procedures. The first group of nonhistone proteins ("Extract A") was extracted with a solution containing 0,14 mol/l NaCl-0,01 mol/l Na-citrate (Vujović et al, 1989), and the second one was extracted with a solution containing 0,35 mol/l NaCl, adjusted to pH 7.0 ("Extract B"), according to Goodwin and Johns (1973). After separation of these protein species, the histones ("Extract C"), RNA and DNA were extracted (Vujović et al, 1989).

The protein concentration was assayed by the method of Lowry et al. (1951). The amount of DNA was measured by the orcinol method (Hutchison, and Munro, 1961), and DNA according to Burton (1956). The isolated chromatin proteins (histones and nonhistones) were separated by NaDodecylSO$_4$-polyacrylamide gel electrophoresis (Lemmly, 1973).

The absorption spectrum of chromatin was obtained by determint the extinctions at different wavelengths (from 220-300 nm). The thermal denaturation profiles were obtained by determining the extinction at 260 nm by continuously increasing the temperature from 50°C to 100°C (Vujović et al, 1989). The chromatin was first digested by incubation with DNase I (Vujović et al, 1989), then the product of digestion was separated on a sucrose gradient in a L5-L6 Beckman centrifuge at 27000 rpm for 24 h. The tubes were emptied using an LKB fraction collector. The absorption of each fraction was measured at 260 nm.

RESULTS

Table 1. shows the relative content of structural proteins which was higher in the groups of animals treated with carcinogen alone, selenium alone and carcinogen with selenium than in the control group. In the chromatin of all examined groups the ratio of nonhistone proteins ("Extract A") and histones ("Extract C") was close to 1, which indicates the same amount of nunhistone proteins and histones. These results were correlated with the values for RNA in all examined groups.

Table 1. Stehiometric ratio of some protein species and DNA. (I controls, II carcinogen, III selenium, IV carcinogen with selenium).

EXPERIMENTAL GROUPS	"EXTRACT A" DNA	"EXTRACT B" DNA	"EXTRACT C" DNA	RNA DNA
I	3,6	1,1	3,2	0,9
II	7,4	2,8	6,7	1,4
III	7,8	3,9	7,7	1,0
IV	7,4	3,1	7,3	0,9

Diffferences between protein species extracted were evident from the distribution and amount of some fractions obtained after electrophoresis in polyacrylamide gel, as shown in figure 1.

The characterization of chromatin by hyperchromicity (Figure 2.) revealed some differences in thermal stability of these macromolecules between the examined groups of animals. It seems that thermal susceptibility of chromatin depends on the relative content of structural proteins (nonhistones and histones). A higher content of proteins was associated with higher values for Tm, which was evident for chromatin from all the experimental groups (Figure 3.).

The degradation of chromatin with DNase I produced well differentiated nucleosomal fractions (Figure 4). The chromatin isolated from the experimental groups was more resistant to degradation with DNase I compared to the controls.

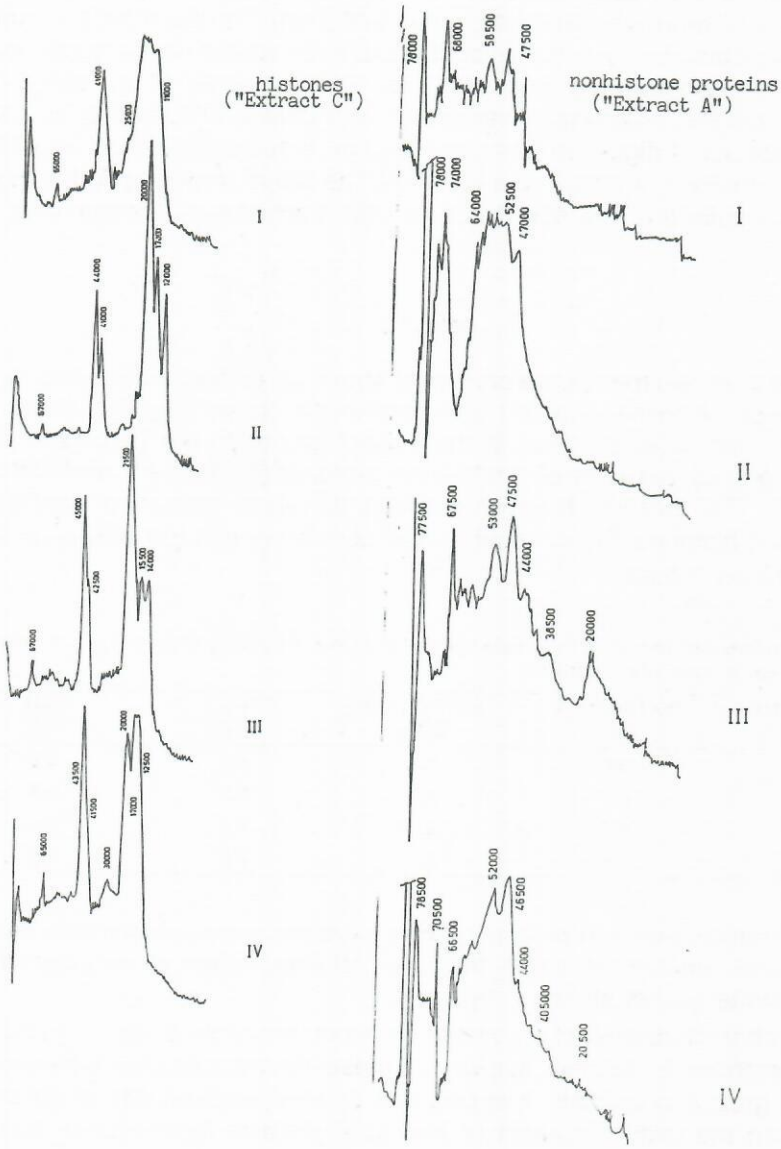

Figure 1. Electrophoretic profiles of histones and nonhistone proteins (I control, II carcinogen, III selenium, IV selenium with carcinogen)

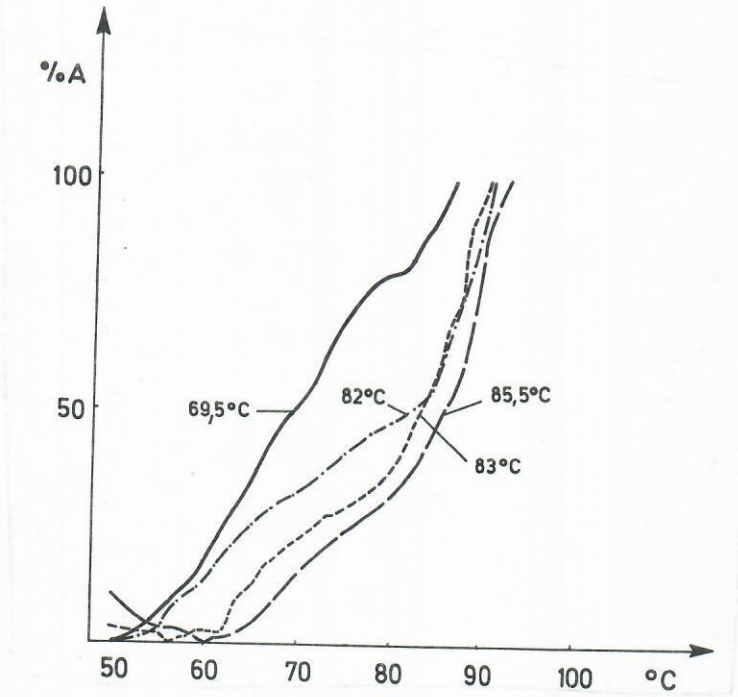

Figure 2. UV spectar of examined chromatins. (control—, carcinogen – –, selenium – – –, and carcinogen with selenium – · – · – ·).

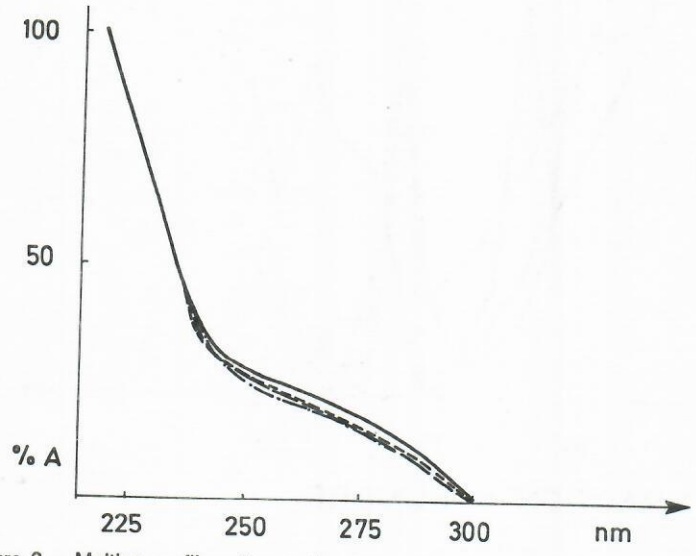

Figure 3. Melting profiles of control and experimental groups (control—, carcinogen – –, selenium – – –, and carcinogen with selenium – · – · – ·).

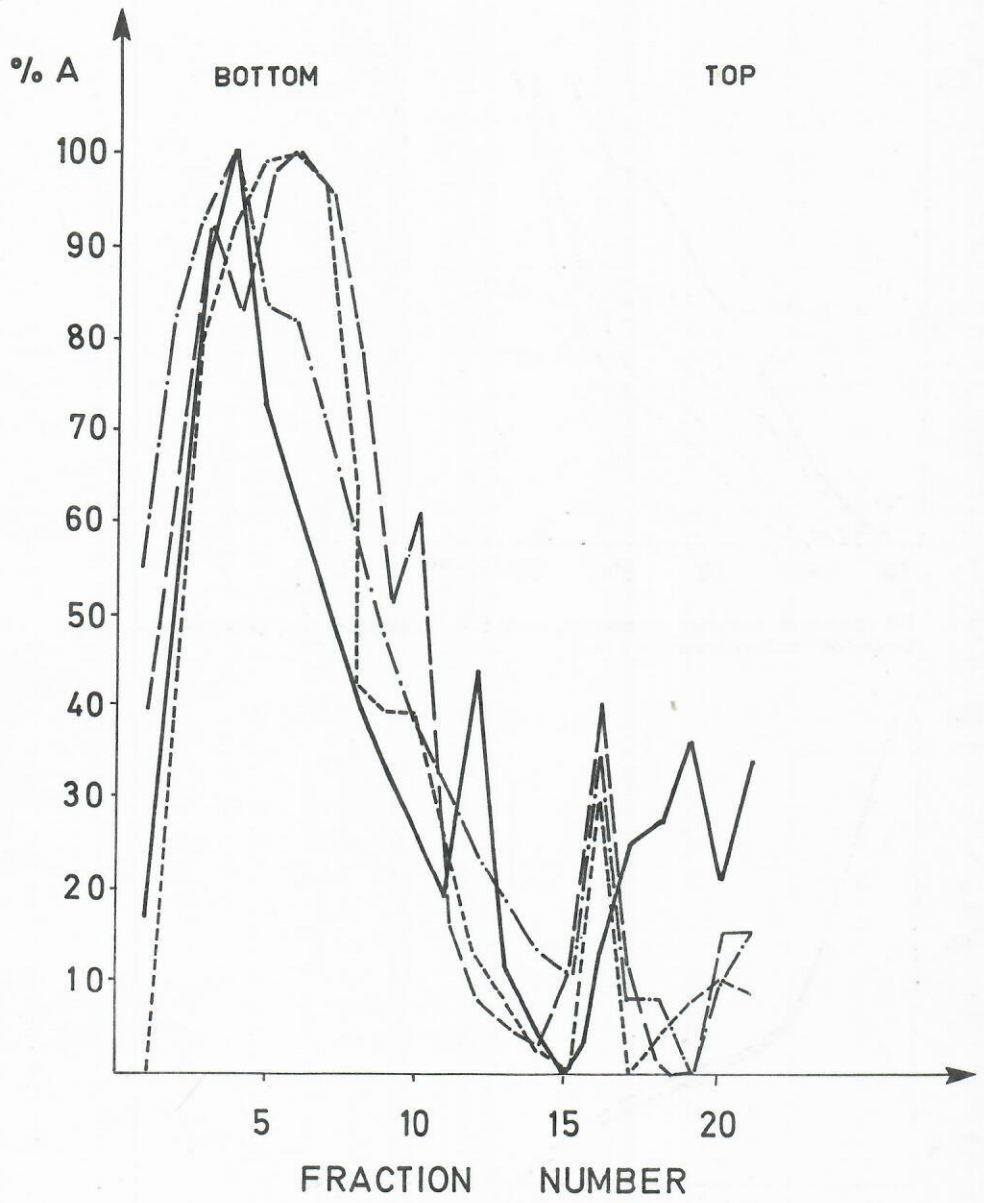

Figure 4. Distribution of nucleosomal fractions control and experimental groups (control —, carcinogen – –, selenium – – –, carcinogen with selenium – · – · –).

DISCUSSION

The only well established physiological function of selenium in humans is the antioxidant activity of selenoproteins (Lockitch, 1989; Christeinsen and Pusey, 1994). In humans, low intakes of selenium have been correlated with increased risk of cancer at certain sites (Batist, 1988; Ganther, 1990; Bratakos et al, 1990; Yang et al, 1990;Combs, 1989).

The characterisation of proteins associated with DNA in chromatin is a difficult problem which has not been solved yet. Contrary to the small number of histones, there are apparently hundreds of different nonhistone proteins present. Among them, high mobility proteins are the best characterised (Mirnow et al, 1986; Jose, et al. 1987). The group of nonhistone proteins is very complex. Some are the structural proteins of chromatin, but the way they interact with DNA in nucleosomes is not known. Although there has been considerable progress with regard to determining chromatin structure (Davie, 1981; Jose, et al. 1987), detailed aspects of the structure and mechanism of gene expression have not been verified yet.

After analysing the relative content of chromatin protein species, a higher content of histone and nonhistone proteins was found in all experimental groups compared with the control. Differences in protein species were evident from their distribution and the amount of some fractions. The differentiation of chromatin isolated from rat brain treated with carcinogen and selenium was confirmed by determining the melting temperature. Differences in the thermal denaturation of chromatin is probably due to protein/DNA difference. It seems that the thermal susceptibility of chromatin depends on histone and nonhistone protein content. The stabilisation of DNA by a high content of structural proteins was demonstrated in the experimental groups.

The differences found in protein species were corelated to the enzymatic degradation of chromatin by DNase I.

Furthermore, chromatin from the experimental groups showed less expressed susceptibility to DNase I degradation as compared to the control, which was evident from the distribution of nucleosomal fractions. These findings are consistent with the stability to thermal denaturation of chromatin in the observed groups.

The results obtained point to differences in the degradability of chromatin isolated from the brains of animals treated with carcinogen and selenium comparing to control rats, which is probably in related to the presence of some structural protein which might contribute organizationally to the compactness of the chromatin structure.

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UTICAJ SELENA NA KARCINOGENEZU I PROMENE U STRUKTURI HROMATINA MOZGA PACOVA

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SADRŽAJ

Ispitivan je uticaj selena na hromatin mozga kontrolnih pacova i pacova tretiranih karcinogenom (9,10-dimethyl-1,2-benzantracene), selenom i karcinogenom sa selenom istovremeno. Stehiometrijski odnosi konstituenata hromatina (proteina, RNA i DNA) ukazuju na veću količinu nehistonskih proteina i histona u grupama tretiranih životinja u odnosu na kontrole. Mada nema velike razlike u sadržaju ukupnih proteina između ispitivanih grupa, očigledne su razlike u zastupljenosti pojedinih proteinskih vrsta dobijene razdvajanjem elektroforezom na poliakrilamidnom gelu. Utvrđene razlike u proteinskim vrstama su u korelaciji sa "ekstinkcionim" i "melting" profilima hromatina (veće Tm vrednosti za hromatin pacova tretiranih selenom, selenom sa karcinogenom i posebno karcinogenom u odnosu na kontrole), kao i njegovoj podložnosti degradaciji DNazom I. Hromatin eksperimentalnih grupa je manje podložan enzomskoj degradaciji u odnosu na hromatin kontrolne grupe, što se i vidi iz veće zastupljenosti polinukleozomalnih frakcija. Ovi rezultati su u saglasnosti sa višim temperaturama denaturacije struktura hromatina, kao i većom količinom strukturnih proteina čvršće vezanih za DNA eksperimentalnih grupa u odnosu na kontrolu. Dobijeni rezultati ukazuju na promene u strukturi hromatina posle administracije karcinogena i selena.