CHANGES IN CHROMATIN DURING THE DEVELOPMENT OF LIVER CELL INJURY INDUCED BY GALACTOSAMINE

E. WEISS, V. GROSS and P. C. HEINRICH
Biochemisches Institut der Universität Freiburg im Breisgau West Germany

Received 19 February 1976

1. Introduction

The administration of D-galactosamine·HCl (GalN) to rats induces a biochemical and morphological liver cell injury similar to viral hepatitis [1]. The formation of GalN metabolites followed by a trapping of uridine phosphate is considered to be essential for the development of liver cell damage. The decrease in the concentration of UTP has been suggested as the cause for the observed inhibition of RNA synthesis [2]. In addition, a decrease in the rate of [^{14}C]leucine incorporation into protein has been found after GalN administration [3]. It has been shown that the hepatic UTP deficiency must persist for a minimum time period of 2.5 h in order to generate a hepatitis [4]. After this minimum metabolite deficit period liver cell damage is irreversible. The mechanism for the switch towards cell death is presently unknown. A different gene expression could be responsible for the pathogenesis of the liver cell injury. The mechanisms of the regulation of gene expression in eucaryotes are still largely unknown. However, it is now generally accepted that non-histone chromosomal proteins (NHP) play an important role in gene regulation [5]. In the present paper it is demonstrated that changes in chromosomal proteins after GalN administration have been found.

2. Materials and methods

Male Wistar rats (Ivanovas, Kisslegg, Germany) weighing between 180 and 200 g were used. D-Galactosamine·HCl was purchased from C. Roth OHG (Karlsruhe, Germany), [4,5-^{3}H]leucine (53 Ci/mmole) from the Radiochemical Centre (Amersham, England). Nuclei were prepared according to Blobel and Potter [6], chromatin was isolated from purified nuclei according to the procedure of Spelsberg and Hnilica [7]. Histones were extracted with 0.25 N H_2SO_4. Non-histone proteins were extracted from the pellet obtained after histone removal by use of 0.1 N NaOH. RNA was determined according to [8] using yeast RNA as standard (A_{260} = 1000, when 42 μg RNA/ml), DNA was estimated according to [9]. Protein determinations were performed according to Lowry [10] with bovine serum albumin as standard.

Chromatin was dissociated in 2 M NaCl–5 M urea. $PhMeSO_3F$ (1 mM) was added to inhibit proteinase activities. The dissociated chromatin was centrifuged for 15 h at 200 000 *g* in order to remove the DNA. The supernatant was dialyzed against 1% SDS, 1% 2-mercaptoethanol at room temperature. 60–100 μg of total chromosomal proteins were subjected to disc gel electrophoresis [11]. Protein-bound radioactivity was determined by the method of Mans and Novelli [12]. The sulfuric acid extract containing the histones and the alkaline solution of non-histone proteins were counted in a toluene–Triton X-100 (2:1) scintillation mixture.

3. Results

Chromatin has been isolated from rat liver nuclei, 1, 3 and 6 h after GalN injections (375 mg/kg). The components of chromatin: DNA, histones, non-histone proteins (NHP) and RNA have been analyzed (table 1). Since there was no change in the DNA content within 6 h after GalN administration the amounts of chromo-

Table 1
Composition of rat liver chromatin at different times after D-galactosamine administration

	Histones (mg/mg DNA)	Non-histone proteins (mg/mg DNA)	RNA (md/mg DNA)	Non-histones / histones
Controls	1.21 ± 0.02 (100)	0.80 ± 0.03 (100)	0.110 ± 0.010 (100)	0.66 ± 0.15 (100)
Galactosamine-treated				
1 h	1.18 ± 0.09 (97.5)	0.71 ± 0.04 (88.8)	0.102 (92.7)	0.59 ± 0.01 (89.3)
3 h	1.19 ± 0.16 (98.3)	0.52 ± 0.07 (65)	0.077 ± 0.008 (70.0)	0.52 ± 0.03 (78.8)
6 h	1.19 ± 0.07 (98.3)	0.59 ± 0.03 (73.8)	0.052 ± 0.014 (47.2)	0.54 ± 0.02 (81.8)

For each animal, which was intraperitoneally injected with D-galactosamine·HCl (375 mg/kg body weight) a control receiving 0.9% NaCl was used. The injections were given at 7:00 a.m. and the animals were killed after 1, 3 or 6 h. The experimental details for the determination of the chromatin components are given in Materials and methods. Values are means ± S.E.M. from 3 different animals with percentages of corresponding control values in parentheses.

somal proteins and RNA were referred to DNA. It can be seen from table 1 that the total histones remain unchanged after GalN injection. Chromosomal RNA, and the NHP, however, are significantly decreased 6 h after administration of GalN.

The change in the content of NHP was further examined by SDS-acrylamide gel electrophoresis (fig.1). A quantitative and qualitative difference in the pattern of protein bands can be detected 6 h after GalN administration. The decrease in NHP after GalN is evident from the comparison of the gels A and B, where on a DNA basis the same amounts have been added. The same amounts of protein were added to the gels B and C. Densitometer tracings revealed many qualitative and quantitative changes in the protein patterns, a few are marked by arrows. The protein pattern observed after SDS-gel electrophoresis was very reproducible. However, Bhorjee and

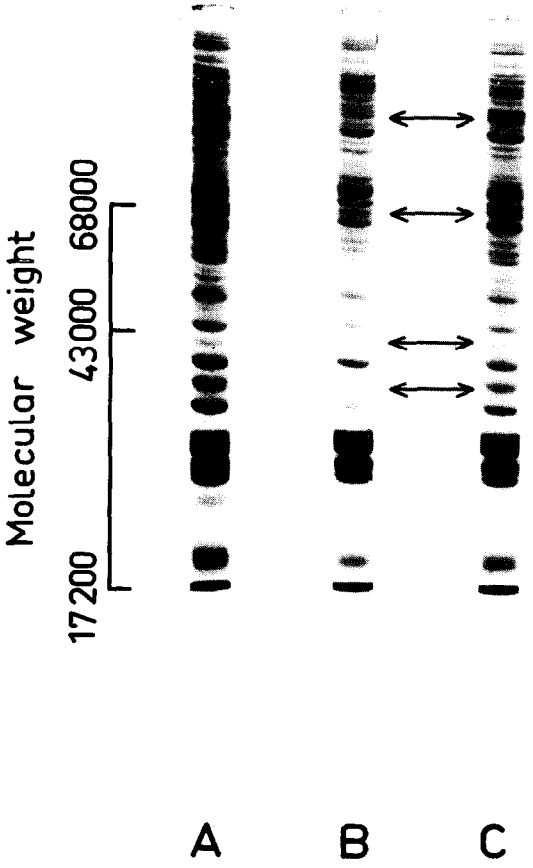

Fig.1. SDS-gel electrophoretic patterns of total chromosomal proteins, (A) Control (110 μg protein, ≃ 42 μg DNA). (B) 6 h after galactosamine injection (75 μg protein, ≃ 42 μg DNA). (C) Control (75 μg protein, ≃ 26 μg DNA). Electrophoresis was performed according to [11] for 3 h at 2 mA/gel at room temperature. Mol. wt. standards were serum albumin (68 000), ovalbumin (43 000), myoglobin (17 200) and cytochrome *c* (11 700).

Pederson [13] have demonstrated that NHP can be contaminated with proteins from heterogeneous nuclear ribonucleoprotein (HnRNP) particles. They have described a method for the preparation of chromatin which minimizes HnRNP contamination. Practically identical NHP patterns were obtained when chromatin was either prepared according to [13] or to Spelsberg and Hnilica [7]. Further evidence against a HnRNP effect on the composition of chromatin was obtained from the NHP pattern of chromatin isolated from rat liver 6 hours after the administration of actinomycin D (2 mg/kg), conditions where HnRNP synthesis is drastically decreased [14,15]. There was actually no difference in the NHP patterns of chromatin isolated from actinomycin D treated rat livers and controls (unpublished results).

Table 2 shows the marked decreases of [^{3}H]leucine incorporation into NHP and histones compared with total liver proteins.

4. Discussion

A large number of studies on the mechanisms of gene regulation in eucaryotes have been carried out with systems where the gene activity is stimulated. In such systems an increase in the NHP/DNA ratios is found [16]. After GalN administration to rats, particularly after the critical time of 3 h, the liver cells are irreversibly programmed towards cell death. It could be shown that this is reflected in a decreased NHP/DNA ratio (table 1). Although the decrease of NHP after GalN amounts only to 30% of normal controls, this portion of the NHP is probably the most important one in terms of regulation of gene activity.

From our data we propose a sequence of events towards cell death after GalN. The decrease in UTP levels leads to an impaired protein synthesis either due to the lack of mRNA synthesis or to blocked translation. After the critical time of 3 h the regulatory important NHP with a high turnover are lost (table 1 and fig.1) and the NHP/DNA ratio decreases. Even if at that time after GalN the UTP levels of the liver cells are restored by administration of uridine [4] the important genes on the DNA cannot be transcribed because of lacking NHP, necessary for gene activation.

Acknowledgements

The authors thank Drs. W. Reutter, D. Keppler and Professor Dr K. Decker for helpful discussions and Professor Dr H. Holzer for his interest in and support of this work.

References

[1] Keppler, D., Lesch, R., Reutter, W. and Decker, K. (1968) Exptl. Mol. Pathol. 9, 279–290.
[2] Keppler, D., Pausch, J. and Decker, K. (1974) J. Biol. Chem. 249, 211–216.

Table 2
Effect of D-galactosamine on the incorporation of [4,5-^{3}H]leucine

	Specific Radioactivity			
		Time after GalN (h)		
	Control	1	3	6
Total homogenate	57 520 ± 900	55 600	42 690	40 330
	(100)	(96.7)	(74.3)	(70.0)
Non-histone	23 818 ± 2695	21 040 ± 263	5532 ± 1918	4231 ± 1286
proteins	(100)	(88.3)	(23.2)	(17.8)
Histones	10 877 ± 722	10 545 ± 768	2760 ± 859	2482 ± 482
	(100)	(96.9)	(25.4)	(22.8)

At the different times after galactosamine administration (375 mg/kg body weight), the specific radioactivities of total liver proteins (dpm/mg protein), histones (dpm/mg DNA) and non-histone proteins (dpm/mg DNA) were determined. One hour before the animals were sacrificed 1.0 mCi [^{3}H]leucine/kg body weight were injected intraperitoneally. The data are means ± S.E.M. of determinations from 3 different animals with percentages of corresponding control values in parenthesis.