PREVENTION OF THE HEPATOTOXIC EFFECTS OF D-GALACTOSAMINE BY D-GALACTOSE

K. MÉSZÁROS, F. ANTONI, A. HRABÁK, Ch. SZIKLA, T. GARZÓ, Anna TOMPA+ and K. LAPIS+

Institute of Medical Chemistry and +Ist Institute Pathology, Semmelweis Medical University, Budapest, Hungary

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1. Introduction

Galactosamine hepatitis [1] is accompanied by decreased incorporation of amino acid into protein [2,3] and of uridine into RNA in the liver [2]. Recently the inhibition of induction of rat liver tyrosine amino transferase by D-galactosamine has been reported [4]. The mechanism of liver damage is not known, and the involvement of unphysiological metabolites of galactosamine has been supposed [5–7].

In this report we describe the *in vitro* inhibition of amino acid, uridine and orotate incorporation into mouse liver slices caused by D-galactosamine, and the enhancement of inhibition in the presence of uridine and its reversion by D-galactose. Galactosamine hepatitis is prevented by D-galactose *in vivo*.

2. Materials and methods

L-[2,3-^{3}H]valine (279 mCi/mmol), [2-^{14}C]orotate (9 mCi/mmol) and [5-^{3}H]uridine (26 Ci/mmol) were purchased from the Isotope Institute of the Hungarian Academy of Sciences. D-[1-^{14}C]Galactosamine (52 mCi/mmol), [U-^{14}C]adenosine (307 mCi/mmol) and L-[1-^{3}H]fucose (1.8 Ci/mmol) were purchased from the Radiochemical Centre, Amersham. The unlabelled D-galactosamine-HCl was the product of Serva, Heidelberg.

Male white CFLP mice (weighing 25 g) were used. In each *in vitro* experiment liver slices from a single animal were prepared and incubated as described by Krebs et al. [8], with the modifications that the incubation medium also contained D-glucose (33 mM) and the amino acids necessary for protein synthesis (10 μM each) except valine. Incubations were carried out at 37°C for 120 min in the presence of radioactive precursors and additions.

In order to estimate the uptake of radioactive precursors by the slices, the incubated slices were washed with physiological saline and dried, then weighed and homogenized in 0.7 M perchloric acid; radioactivity was determined in the supernatant after centrifugation. For the estimation of the protein and nucleic acid bound radioactivity, the acid precipitable material of the slices was prepared [9] by using ice-cold 0.7 M perchloric acid. Weighed samples were dissolved in concentrated formic acid and counted in a Packard Tri-Carb (model 2425) liquid scintillation spectrometer.

3. Results and discussion

3.1. *Uptake and incorporation of radioactive precursor in vitro*

The uptake of [^{3}H]valine and [^{14}C]orotate into the perchloric acid soluble fraction of the slices remained unaltered in our experiments in the presence of 10 mM D-galactosamine, which however increased the uptake of [^{3}H]uridine to twice the control values (data not shown). Similar increase of uridine uptake into chick fibroblasts due to D-galactosamine treatment has been described [10]. The uptake of

Table 1
Incorporation of radioactive precursors into the perchloric acid insoluble material of mouse liver slices in the presence of D-galactosamine, D-galactose and uridine.

Precursor	Radioactivity (μCi/ml)	Control	D-galactosamine, (10 mM)	D-galactosamine, (10 mM) D-galactose (20 mM)	D-galactosamine, (10 mM) Uridine, (10 mM)	D-galactosamine, (10 mM) Uridine, (10 mM) D-galactose, (20 mM)
		dpm/mg Perchloric acid insoluble material				
Expt. A [^{3}H]valine	10	60 000	38 000	65 000	–	–
[^{14}C]orotate	2	300	40	360	–	–
Expt. B [^{3}H]valine	5	26 000	15 000	–	8 000	–
Expt. C [^{3}H]valine	5	30 000	–	–	13 000	25 000
Expt. D [^{3}H]uridine	10	18 000	8 300	20 000	–	–
Expt. E [^{14}C]adenosine	0,2	270	270	–	–	–
Expt. F [^{3}H]fucose	1	9 300	10 500	–	–	–

Incubation of slices and preparation of the radioactive material were performed as described in Materials and methods. One liver was used in each experiment. Values are means of the radioactivity of three samples.

[^{14}C]galactosamine was not influenced by the presence of 10 mM D-galactose under our experimental conditions.

The incorporation of [^{3}H]valine and [^{14}C]orotate into the perchloric acid insoluble material of liver slices was unaltered in the presence of 10 mM D-galactose. The incorporation of [^{3}H]valine was not influenced by 10 mM uridine.

D-Galactosamine at 10 mM concentration inhibited the incorporation of [^{3}H]valine into the perchloric acid insoluble material of liver slices by 41.5 ± 6.2% (mean ± S.D.; five experiments). Higher concentrations of D-galactosamine did not cause a significantly stronger inhibition. Our results are in accordance with the data obtained *in vivo*: decreased amino acid incorporation into liver proteins has been observed due to D-galactosamine treatment in the rat [2, 3], and it has been considered as a sign of decreased hepatic protein synthesis.

The inhibition of [^{3}H]uridine and [^{14}C]orotate incorporation by D-galactosamine (table 1) may be related to the increased synthesis of UDP-hexosamines and UDP-*N*-acetylhexosamines [5]. An inhibition of RNA synthesis does not seem probable, because the incorporation of [^{14}C]adenosine was unaltered.

3.2. *Effect of uridine and D-galactose*

The opposite effects of uridine (10 mM) and D-galactose (20 mM) on the inhibition of the incorporation of [^{3}H]valine, [^{3}H]uridine and [^{14}C]orotate by D-galactosamine (table 1) may be explained as follows: D-galactosamine is phosphorylated and *N*-acetylated in the liver, and gives rise to the synthesis of UDP-hexosamines and UDP-*N*-acetyl-hexosamines [5, 11]. Non-*N*-acetylated UDP hexosamines are aphysiological products and may be responsible for several toxic effects of D-galactosamine [5–7]. Galactose may competitively inhibit the phosphorylation of D-galactosamine by galactokinase, preventing thereby the formation of UDP-hexosamines. In our experiments with liver slices the formation of labelled nucleotides from [^{14}C]galactosamine and accordingly the incorporation of [^{14}C]galactosamine were strongly inhibited in the presence of D-galactose (fig. 1). The key role of galactokinase in D-galactosamine metabolism offers an explanation for the organ specificity of galactosamine damage. Large amounts of uridine in combination with D-galactosamine enhance the formation of non-*N*-acetylated UDP-hexosamines [4], which are probably responsible for the strong *in vitro* inhibition of [^{3}H]valine incorporation when both substances are present (table 1, Expt. B and C).

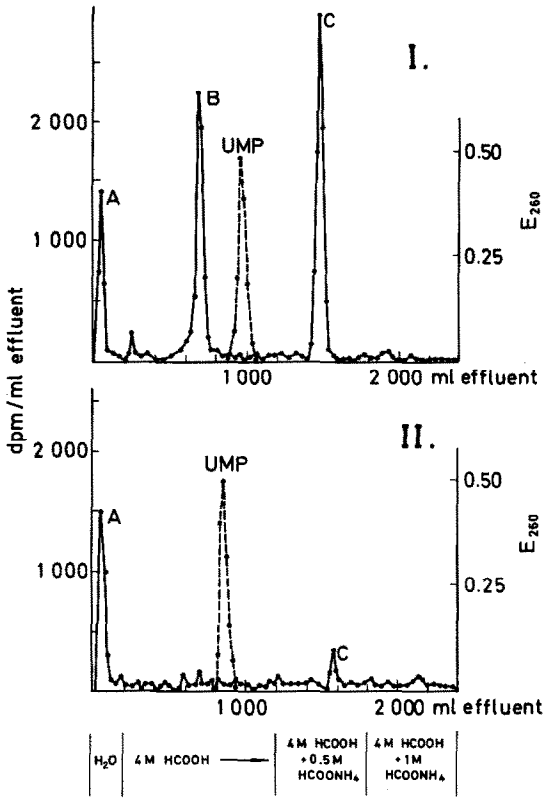

Fig. 1. Effect of D-galactose on the labelling of nucleotides in the presence of [^{14}C]galactosamine. Liver slices (300 mg) were incubated as described in Materials and methods with 0.5 mM unlabelled and 2.5 μCi/ml ^{14}C-labelled D-galactosamine in the absence (I) or presence (II) of 20 mM D-galactose. Nucleotides were isolated by charcoal absorption [12] from the perchloric acid soluble fraction of the slices. The material eluted from charcoal was dissolved in distilled water. UMP was added as marker and the mixture was applied to a Dowex 1 (formate form) column (15 × 1 cm) and chromatographed using the gradient elution technique [13, 14]: (●—●—●) Radioactivity; (●---●---●) absorbance; Peak A; [^{14}C]galactosamine absorbed unspecifically on charcoal. Peaks B and C; UDP-hexosamines and UDP-*N*-acetyl-hexosamines, respectively, identified on the basis of their elution position [11], their absorption on charcoal and their ^{14}C contents. In the same experiment incorporation of [^{14}C]galactosamine into the perchloric acid insoluble material (see Materials and methods) was 2 800 dpm/mg in the absence of D-galactose; addition of D-galactose to the incubation medium reduced this value to 60 dpm/mg.

Liver morphology was examined in mice starved for 12 hr. Physiological saline was injected into controls, 7 mice received D-galactosamine (1 g/kg body wt.) and another group of 7 mice received D-galactosamine (1 g/kg) and D-galactose (2 g/kg) intraperitoneally. The mice were sacrificed 18 hr after the injections. The light microscopic examination of liver sections stained with haematoxylin–eosin revealed necrotic changes [1] in the livers of the D-galactosamine treated rats. No necrosis was found in the livers of those animals which received both D-galactosamine and D-galactose.

It is concluded that D-galactose abolishes various toxic effects of D-galactosamine *in vitro* and *in vivo*. The significance of non-*N*-acetylated UDP-hexosamines in D-galactosamine toxicity is strongly supported by our results.

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