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QUARTERLY

The effects of galactosamine on UTP levels in the livers of young, adult and old rats★

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Galactosamine (GalN), a well-known hepatotoxin that depletes the cellular pool of uracil nucleotides, was previously shown to have greater impact on the inhibition of protein synthesis in hepatocytes of old rats as compared with young animals (Kmieć 1994, *Ann. N.Y. Ac. Sci.* 717, 216–225). In the present study we compared the effects of GalN on the nucleotide content (measured by ion-exchange HPLC) in the livers of young (4 months), adult (12 months), and old (24–26 months old) rats two hours after its intraperitoneal administration. UTP content of the livers of old control rats was significantly lower (by 28%) than that of young animals. GalN administration decreased the UTP content in the livers of young, adult and old rats by, respectively, 55%, 65% and 89%, and increased the content of UDP-sugars by 189%, 175% and 305%. The hepatic content of ATP, ADP, AMP, NAD, GTP except CTP did not differ significantly among the age groups of rats studied, and was not changed by GalN treatment. The content of CTP was significantly higher in old rats ($P < 0.03$) upon GalN treatment. The lower hepatic content of UTP may partially explain the increased sensitivity of hepatocytes and livers of old rats to the action of galactosamine, and possibly to other hepatotoxic compounds that decrease transcription in the liver.

Aging is associated with changes in cells and tissues that may lead to the deterioration of organ functions. Under basal conditions the majority of hepatic functions is well compensated during aging, however, under exogenous or endogenous stimulation an age-related decrease in liver functions may become evident. We found previously [1] that the well-known hepatotoxin galactosamine (GalN) suppressed protein synthesis in hepatocytes

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Abbreviations: GalN, galactosamine.

isolated from old rats almost twice as much as in young animals. The hepatotoxic action of GalN is mainly caused by the inhibition of transcription, and consequently, translation, due to the rapid decrease of the hepatic content of uracil nucleotides [2]. In our study [3] we found that under basal conditions hepatocytes isolated from old animals contained significantly smaller amounts of UTP than cells of young rats. However, it is possible that the process of cell isolation may decrease the content of nucleotides in parenchymal liver cells and thus the results obtained *in vitro* may not reflect the *in vivo* situation. Therefore the aim of the present investigation was to compare the effects of *in vivo* administered galactosamine on the content of UTP, UDP-sugars and other nucleotides in the livers of young, adult and old rats.

MATERIALS AND METHODS

D(+)Galactosamine hydrochloride was purchased from Sigma (Poznań, Poland).

Male Wistar rats aged 4, 12 and 24 months ("young", "adult", and "old", respectively) were used. The mean body mass of rats in each group was 325 ± 23 g (mean ± S.E., n = 12), 395 ± 35 g (n = 12) and 505 ± 41 g (n = 12), respectively. The mean and the maximal life span of this rat colony was 27 and 36 months, respectively [4]. The animals were housed 3 per cage and maintained on a controlled light schedule (light on 7:00–19:00) at 20 ± 1°C [5]. They were fed a standard diet containing (w/w) 13% protein, 55.5% carbohydrate, 2.5% lipid and 29% indigestible compounds (LSM, Motycz, Poland) and had a free access to water.

Rats were injected intraperitoneally with 0.2 g/kg of GalN (dissolved in 1 ml of 0.9% NaCl, pH was adjusted to 7.0 with 0.05 M NaOH and the solution was sterile filtered). Two hours later the animals were killed by decapitation. The abdomen cavity was opened and liver was immediately freeze-clamped with aluminium forceps prechilled in liquid nitrogen. The time between incising the abdomen and placing the liver in liquid nitrogen was usually less than 30 s. Blood was collected from the abdominal cavity, serum was separated by centrifugation and stored at –20°C until analysis. Blocks of liver were lyophilized, weighed, and extracted at 4°C with 12 M perchloric acid as described previously [6]. Precipitates were centrifuged, supernatants were neutralized with 2 M KOH to pH 6.6–8.0, and stored at –70°C until analysis. Samples were analyzed by ion-exchange HPLC using a Hitachi-Merck automated HPLC system [7]. Positions of nucleotides and UDP-sugars in chromatograms were established on the basis of the retention times of standards (150 pmoles).

Alanine aminotransferase activity in the sera of control and GalN-treated rats was assessed using a Hitachi 902 automatic spectrophotometer and Roche assay kit No. 851132 by the method of Bergmeyer *et al.* [8].

The results are expressed as mean ±S.E. Statistical significance was determined by the Student's *t*-test. The minimum level of significance was set at $P < 0.05$.

RESULTS

UTP and UDP-sugars content in the livers of control and GalN-treated rats

The data presented in Fig. 1A show that in the liver of old control rats the UTP content was significantly lower (by 28%) than in young animals, however, the hepatic content of UDP-sugars did not differ among age groups (Fig. 1B). Galactosamine decreased the UTP content in the livers of young, adult and old animals by 55%, 65% and 89%, respectively. The treatment of rats with galactosamine increased hepatic levels of UDP-sugars of young, adult and old rats by 189%, 175%, and 305%, respectively (Fig. 1B). In control animals UDP-glucose was the major UDP-sugar, whereas in the GalN-treated animals

UDP-GlcN, UDP-GalN, and UDP-glucuronic acid were present [3].

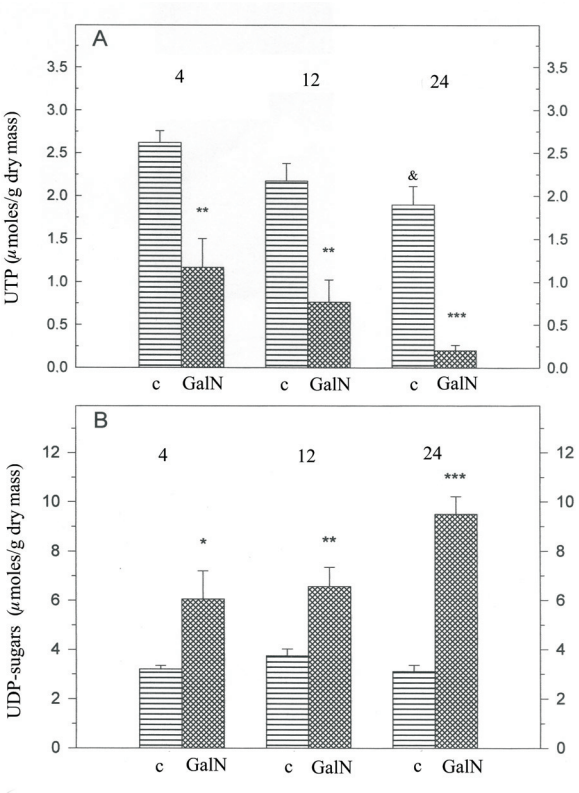

Figure 1. UTP (A) and UDP-sugars (B) content in the livers of galactosamine-treated young (4 mo), adult (12 mo) and old (24 mo) male Wistar rats.

The experiments and measurements were performed as described in the Legend to Table 1. & marks significant difference ($P < 0.05$) in comparison to 4 month old rats. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$, respectively.

The content of other nucleotides in livers of rats of different ages

The content of ATP was by 16% lower in the livers of adult and old rats compared with young animals (Table 1), however, the differences were statistically not significant.

After the treatment with galactosamine there was a tendency to a decrease in ATP content and the ATP/ADP ratio in the livers of young, adult and old rats, however, the differences between GalN and control values were statistically not significant (Table 1). The hepatic NAD, GTP and CTP contents were similar in all three age groups and did not change significantly after injection of GalN.

Serum alanine aminotransferase activities of GalN-treated rats

The activity of alanine aminotransferase was 24 ± 3.3 U/l, 19 ± 2.7 U/l and 26 ± 4.1 U/l in the sera of control young, adult and old rats, respectively. Two hours after GalN administration serum alanine aminotransferase activity did not change significantly in neither of the age-groups (26 ± 5.3 U/l, 23 ± 2.3 U/l and 28 ± 4.4 U/l in young, adult and old rats, respectively).

DISCUSSION

Galactosamine has been widely used as a model hepatotoxin because it produces in a dose-dependent manner a reversible liver damage which morphologically and biochemically resembles human hepatitis. Biochemical lesions induced in the liver by GalN involve a rapid depletion of uridine nucleotides and accumulation of UDP-sugars resulting in the inhibition of transcription and translation [9, 10], and suppression of glycogen and UDP-glucuronic acid synthesis [11]. The reduction of the hepatic UTP content after GalN administration has been generally considered the trigger of GalN-hepatitis characterized by a dose-dependent hepatocellular necrosis and compensatory hepatocyte proliferation [11].

Despite the frequent use of GalN for the induction of experimental hepatitis, the effect of aging on GalN hepatotoxicity has been investigated only by few authors. *In vivo* studies showed either increased hepatocellular damage by GalN in old female Wistar rats as compared to young animals [12] or no age dependence of response for GalN in male Fischer 344 rats [13]. For the observation of the age-dependency of GalN action on the hepatic content of UTP and UDP-sugars we administered

a relatively low dose of the aminosugar for a short period of time, so that the biochemical indices of hepatocellular damage, such as the rise of the serum activity of alanine aminotransferase, were not evident. We found previously that under *in vitro* conditions GalN was a much stronger inhibitor of protein synthesis in hepatocytes isolated from old than from young rats [1], and that this effect might be caused by the lowered cellular content of UTP [3]. The results of the present study indicate that also *in vivo* the liver of old rats is more sensitive to the UTP-depleting action of galactosamine than the liver of young or adult rats, probably due to the significantly lower basal hepatic UTP content. The cause of this phenomenon is at present not known, as the effect of aging on UTP synthesis and degradation in rat liver has not been investigated yet. However, it is tempting to speculate that the diminished basal level of UTP in livers of old rats may promote liver damage due to other factors. For instance, GalN was shown to sensitize rodents to small doses of endotoxin [14], mainly through the release of inflammatory mediators such as TNF-α [15] or leukotrienes [16] from hepatic macrophages, i.e. Kupffer cells. Our result suggest that the hypersensitivity of old rats to endotoxin [17] could be partially related to the lower hepatic UTP content.

In summary, we have demonstrated that the greater sensitivity of the liver of old rats to the UTP-depleting action of galactosamine may be caused by the lower hepatic content of UTP. The effect of aging on uracil nucleotide metabolism in rat liver has yet to be elucidated.

Table 1. Content of nucleotides in the livers of galactosamine-treated rats. Rats of various ages were injected intraperitoneally with galactosamine (0.2 g/kg)

Nucleotide	Young rats (4 months)		Adult rats (12 months)		Old rats (24 months)	
	Control	GalN	Control	GalN	Control	GalN
	μmoles/g liver dry mass					
ATP	14.97 ±0.68	14.95 ±0.93	14.31 ±0.29	12.04 ±1.45	12.52 ±0.94	11.74 ±1.87
ADP	5.04 ±0.28	5.91 ±0.44	5.83 ±0.55	7.63 ±0.61	5.89 ±0.77	6.63 ±1.07
AMP	1.23 ±0.10	1.35 ±0.09	1.55 ±0.28	3.06 ±0.74	1.79 ±0.46	1.82 ±0.63
ATP/ADP	2.97 ±0.30	2.53 ±0.13	2.45 ±0.29	1.58 ±0.15	2.13 ±0.38	1.77 ±0.21
NAD	7.21 ±0.15	6.31 ±0.26	6.40 ±0.52	6.72 ±0.84	6.51 ±0.30	5.55 ±0.27
GTP	3.42 ±0.42	3.03 ±0.43	2.78 ±0.49	2.95 ±0.44	3.29 ±0.42	2.75 ±0.43
CTP	0.71 ±0.10	0.87 ±0.26	0.47 ±0.06	0.50 ±0.13	0.36 ±0.16	0.69 ±0.14*

The nucleotide contents were determined by HPLC. For details see Materials and Methods. The data present means ±S.E., n = 6 in each control and GalN-treated group. *significantly different from control old rats $P < 0.05$.

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