CIRCADIAN RHYTHM OF URINARY IRON EXCRETION IN EXPERIMENTAL HEPATOSIS

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Proof of the circadian rhythmic nature of the iron concentration in the blood plasma and its excretion in healthy individuals [9] and data on a disturbance of iron metabolism and activation of cell membrane lipid peroxidation (LPO) [1] in hepatocellular lesions [7, 8] explain the urgency of the study of the temporal organization of iron metabolism and LPO.

The aim of this investigation was to study the principles governing the circadian rhythm of the excretion of iron and a product of LPO with the urine, and also their concentration in the blood plasma in experimental hepatosis, caused by carbon tetrachloride (CCl_4) poisoning.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 100-150 μg. The animals (group 1 – control; groups 2 and 3 – experimental: receiving 3 and 7 doses respectively of CCl_4) were kept under identical conditions of food and water supply and illumination.

Experimental hepatosis was induced by intragastric administration of a 10% oily solution of CCl_4 by means of a tube, at the rate of 1 ml of solution/100 g body weight. The frequency of administration was once each afternoon, the duration 3 and 7 days for groups 2 and 3 respectively. The experiments were carried out in January, 1990.

The plasma and urinary iron concentrations were determined by the diphenylphenanthroline method, followed by spectrophotometry of the test solutions at λ = 540 nm [5], 8 times at 3, 6, and 9 a.m., 12 noon, 3, 6, and 9 p.m., and midnight.

A parallel determination was made of LPO activity on the basis of the MDA concentration in the blood plasma and urine [4].

To obtain a sufficient quantity of urine, 3 h before the experiments were carried out the animals were given distilled water at a temperature of 37°C by gastric tube in a volume of 5 ml/100 g body weight [6]. The urine was collected in tubes with the aid of funnels, in which the animals were kept for 3 h. The quantity of iron excreted was calculated after measurement of the volume of urine in the 3-hourly portions by the equation: $X = A \times B \times 0.0187$, where A denotes the volume of urine in ml and B the iron concentration in a 3-hourly urine sample in μmoles/liter.

The results were analyzed by Student's test and by the use of rhythmometric parameters, calculated by the Cosinor program.

EXPERIMENTAL RESULTS

A statistically significant circadian rhythm of urinary iron excretion was found in intact animals (Table 1). The values of the circadian rhythm of iron excretion in the urine were independent of the concentration of the trace element in

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TABLE 1. Rhythmometric Parameters of Urinary Excretion of Iron and Malonic Dialdehyde and Their Plasma Concentrations in Healthy Rats and Rats with Experimental Hepatosis

Parameter	Intact animals			CCl_4 poisoning					
				3 times			7 times		
	period	mesor	acrophase	period	mesor	acrophase	period	mesor	acrophase
Volume of urine, ml	23.2±0.7	4.0±0.8	9.2 (6.5—11.0)	24.0±0.9	4.3±1.4	22.2 (20.0—23.4)	24.0±0.7	3.8±0.7	0.4 (23.4—5.4)
Excretion of iron with urine, μg	24.0±0.7	1.7±0.3	11.1 (10.0—11.2)	24.0±0.6	1.8±0.7	20.4 (19.0—22.5)	24.2±0.7	2.4±0.7	23.5 (22.1—1.3)
Excretion of malonic dialdehyde with urine, ng	24.1±0.7	1.3±0.2	11.5 (8.5—13.0)	24.0±0.7	1.9±0.6	19.6 (18.2—21.2)	24.0±0.9	2.2±0.7	0.4 (0.2—1.0)
Concen. of malonic dialdehyde in blood plasma, nmoles/liter	24.0±0.5	20.0±1.2	11.4 (10.4—12.4)	23.8±1.2	21.3±1.8	21.2 (20.1—22.2)	24.0±0.7	27.6±2.3	23.6 (23.2—0.3)
Iron concn. in blood plasma, μmoles/liter	24.0±0.7	22.5±1.2	19.0 (18.0—20.4)	27.7±0.9	23.0±1.4	16.6 (14.1—19.5)	23.9±0.9	27.7±2.9	7.4 (7.0—8.3)

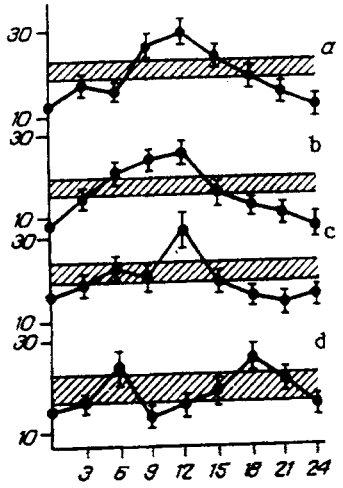

Fig. 1

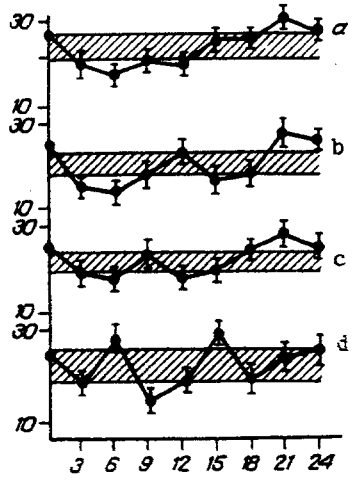

Fig. 2

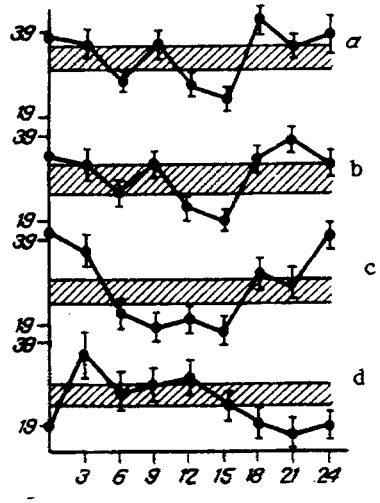

Fig. 3

Fig. 1. Circadian rhythm of iron and malonic dialdehyde concentrations in blood plasma and urine of intact rats. Abscissa, clock time, h. Ordinate: a) urinary iron concentration, μmoles/liter; b) urinary malonic dialdehyde concentration, nmoles/liter; c) blood plasma malonic dialdehyde concentration, nmoles/liter; d) blood plasma iron concentration, μmoles/liter.

Fig. 2. Circadian rhythm of iron and malonic dialdehyde concentrations in blood plasma and urine of rats after receiving 3 doses of CCl_4. Legend as to Fig. 1.

Fig. 3. Circadian rhythm of iron and malonic dialdehyde concentrations in blood plasma and urine of rats after receiving 7 doses of CCl_4. Legend as to Fig. 1.

the blood plasma and the circadian rhythm of urine formation, but they were synchronized with LPO activity (Fig. 1). The highest blood plasma MDA values and of excretion of MDA and iron with the urine were observed between 9 a.m. and 3 p.m., and minimal values between 6 p.m. and 3 a.m.

After administration of 3 doses of CCl_4 to the animals, although the increase in the mean 24-hourly values was not significant, a shift of the acrophase was observed and the lowest values were found within the interval from 6 to 9 a.m. (Fig. 2). An increase in iron excretion with the urine during the evening and night was synchronized with activation of the prooxidant system in the blood plasma; both the MDA concentration in the blood plasma and its excretion with the urine were maximal under these circumstances. The lowest values of the urinary iron concentration in the early morning hours

correspond to the lowest MDA concentration in the blood plasma and urine (Fig. 2). When the parameters of the circadian rhythms of urinary iron and plasma iron concentrations were analyzed, no correlation could be found (Table 1).

In animals receiving 7 doses of CCl_4 the circadian rhythm of urinary iron excretion was inverted. Minimal values from noon to 3 p.m. and maximal values from 6 p.m. to 3 a.m. correlate with the MDA level (Fig. 3). The mesor of water excretion was significantly reduced compared with its value in intact rats. The shift of the acrophase of the blood iron concentration by 7.4 h (Table 1) does not determine changes in the circadian rhythm of the urinary iron concentration.

The study of the chronobiological features of urinary excretion of iron in experimental hepatosis thus revealed correlation between the urinary iron concentration and the prooxidant system, independent of concentration of the trace element in the blood plasma. The change in its activity, evidently linked with activation of free-radical lipid oxidation and with overstrain of antiradical protection [3], determines the response of the urinary iron concentration circadian rhythm even in the early stages of damage to the organ, and the significant increase in the mesor of urinary iron excretion taking place subsequently.

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