

MORPHOMETRIC ANALYSIS OF HEPATIC CHANGES IN EXPERIMENTAL DRUG-INDUCED CHOLESTASIS

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An experimental cholestasis was induced by Ethinylestradiol (EE) in a dosis of 1 mg/kg daily for 5 and 7 days in rats. By using a systemic network, a morphometric analysis of the liver of 18 animals was carried out. Rats were divided into three groups of 6 animals each as followed: 1st - controls; 2nd - EE-treated for 5 days, and 3rd - EE-treated for 7 days. With the treated animals, there was a changed ratio between hepatocytes and sinusoidal and perisinusoidal cells, respectively, from 10,36 : 1 for the controls down to 8,8 : 1 for the second group and 8,3 : 1 for the third one. There were also changes in the nuclear-cytoplasmic ratios in the hepatocytes themselves from 1 : 3,91 for the control group to 1,58 : 1 for the second and 1 : 5,29 for the third one. The changed nuclear-cytoplasmic ratios in the hepatocytes in the animals from the second and third group were due to the sharp increase of the count of hepatocytes with vacuolar degeneration from $0,667 \pm 0,42$ % for the first group up to $35 \pm 11,25$ % for the second and $29,65 \pm 9,08$ % for the third one. The vacuolar degeneration of hepatocytes and the proliferation of sinusoidal cells presented the most common processes established in the liver with the histological and morphometric assessment of this kind of cholestasis.

Key-words: Ethinylestradiol, hepatocytes, morphometry, female rats

Experimental ethinylestradiol-induced (EE-induced) cholestasis in rats possesses its own physiological, histochemical, and electron-microscopic characteristics (7,11,13-15). In a previous light-microscopical investigation of an experimental EE-induced cholestasis (7), we observed a series of unspecific changes in the liver such as vacuolar and eosino-

philic degeneration in hepatocytes, mitoses, slight cholangiolitis, proliferation of sinusoidal and perisinusoidal cells, dilatation of sinusoids, etc.

Aiming at objectifying our observation we applied a quantitative morphometric method and decided to solve the following tasks: (i) to establish the percentage ratio of the principal cell composition, sinusoids, and portal spaces in control and EE-treated animals; (ii) to reveal the percentage ratio between normal and pathologically altered hepatocytes, and (iii) to find out the percentage

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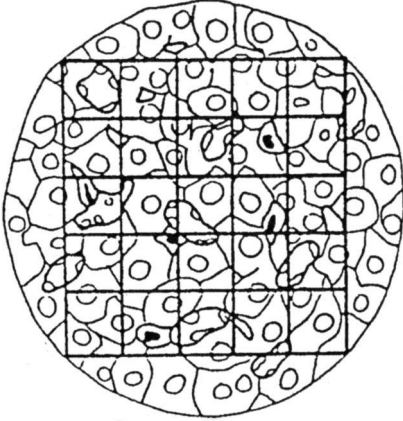


Fig. 1. A free network for morphometric investigation

ratio between nuclei and cytoplasm of hepatocytes in control and treated animals as well.

MATERIAL AND METHODS

We induced an experimental cholestasis in 12 female rats by treating them with 17- α -EE in a dosis of 1 mg/kg daily for 5 and 7 days (each group consisted of 6 animals).

The morphometric examination was carried out on 18 animals divided into the following three groups: 1st - controls; 2nd - EE-treated for 5 days, and 3rd - EE-treated for 7 days.

We used a free network to perform the morphometry (Fig. 1) (1). We read the hits of 16 points of the network on the histological section at a total microscope magnification of 640 (40 of the objective but 16 of the eye-lens). From each animal, one section was assessed by reading a

total of 1000 points. The hits in the nucleus and cytoplasm of the hepatocyte, the normal and pathologically altered hepatocytes, the sinusoids, as well as the sinusoidal and perisinusoidal cells, etc. were separately read. We compared and summarized the results by using the variation analysis. Student-Fisher's *t*-distribution was used to determine *p*-value.

RESULTS AND DISCUSSION

After performing the variation analysis and summarizing the data from the morphometric investigation it has been established that in control animals, hepatocytes amount to 74,38 per cent of liver volume, sinusoids to 11,55 per cent, sinusoidal and perisinusoidal cells both to 7,21 per cent, and portal spaces to 0,516 per cent. We demonstrate these results in Table 1.

There is an increase of the percentage of sinusoidal and perisinusoidal cells both up to $x = 8,25 \pm 0,27$ and $8,04 \pm 1,013$, respectively, as well as a statistically significant decrease of the percentage of hepatocytes down to $66,86 \pm 4,53$ ($p < 0,001$) in animals treated with EE for 5 and 7 says. The relative share of sinusoids increases significantly in treated animals up to $14,66 \pm 0,55$ in the second group and up to $14,24 \pm 1,52$ in the third one ($p < 0,001$) (Fig.

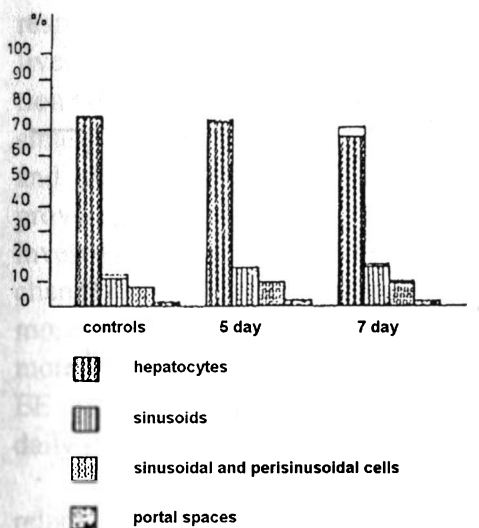


Fig. 2. Percentage ratio of the principal cell composition, sinusoids, and portal spaces in the liver of control and treated animals

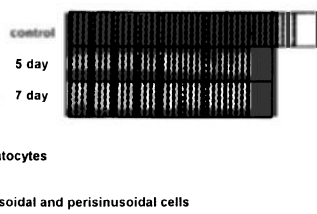


Fig. 3. Ratio between hepatocytes and sinusoidal and perisinusoidal cells

2). The reduction of the relative share of hepatocytes along with the enhancement of the relative share of sinusoidal and perisinusoidal cells both is most clearly reflected by the changed ratio between sinusoidal and perisinusoidal cells from 10,36 : 1 in the controls down to 8,8 : 1 and 8,31 : 1 in the two treated groups, respectively (Fig. 3). In the aggregate of hepatocytes, there exists a

statistically significant increase of the percentage of cells with vacuolar degeneration in animals with cholestasis as compared with that in control animals as follows: from $0,66 \pm 0,46$ up to $35 \pm 11,25$ (for the second group) and up to $29,65 \pm 9,08$ (for the third one) ($p < 0,001$) (Fig. 4). Probably, the sharp elevation of the percentage of the cells with vacuolar degeneration is due to the change of the ratio between the percentage hit in the nuclei and the percentage hit in the cytoplasm of hepatocytes: from 1 : 3,91 in the control group to 1 : 5,38 in the second and to 1 : 5,29 in the third one (Fig. 5).

The comparison with the control animals also reveals an increasing number of cells with acidophilic degeneration which is the highest in the animals from the third group ($2,983 \pm 2,07$ at $p = 0,05$).

No significant difference in the percentage share of binuclear cells and portal spaces has been detected. This method applied enables precise conclusions because of the large number of objects read for a single visual field (16 objects) and of points for one section (1000 points). The assessment of 6 animals per group provides the opportunity to read a total of 6000 points per group. This disturbed ratio between hepatocytes and both sinusoidal and perisinusoidal cells is most probably due to EE-stimulated proliferative activity of the

Table 1*Results from the morphometric investigation ($x \pm Sx$)*

No	Parameters	Controls	EE for 5 days	EE for 7 days
I.	Hepatocytes	74,38±0,967	72,96±1,06	66,86±4,53
	1. normal	69,67±1,55	34,43±1,55*	37,2±7,01*
	- nucleus	14,28±0,87	6,6±2,34*	5,38±1,35*
	- cytoplasm	55,4±1,43	27,83±8,67*	31,81±5,83*
	2. with vacuolar dystrophy	0,66±0,46	35±11,25*	29,65±9,08*
	- nucleus	0,067±0,046	4,317±1,505*	3,7±1,36*
	- cytoplasm	0,6±0,016	30,66±10,53*	25,95±8,07*
	3. with eosinophilic dystrophy	0	0,5±0,5	2,983±2,07**
	- nucleus	0	0,033±0,036	0,383±0,293
	- cytoplasm	0	0,467±0,111	2,96±1,79
II.	Sinusoids	11,55±2,71	14,66±0,55*	14,24±1,52
III.	Sinusoidal + perisinusoidal cells	7,21±0,61	8,25±0,27*	8,04±1,013
IV.	Central vein	0,283±0,19	0,35±0,149	0,283±0,14
V.	Portal spaces	0,516±0,36	1,216±0,103	0,58±0,388

* $p < 0,001$ ** $p < 0,05$

sinusoidal and perisinusoidal cells and degenerative processes in hepatocytes.

An increased number of sinusoidal cells in cholestasis has been reported in women (5), and more recently, in animals, too (10). Our results are complementary to the stereological analysis reported by

Hornstein et al. (8) who find out an enhancement of liver volume by 65 per cent, of hepatocytic volume by 35 per cent but of hepatocytic percentage by 23 per cent only in an experimental model of cholestasis induced with 5 mg/kg of EE. These results indicate that hepatocytes with their degenerative alterations are partially / in half

responsible for the augmentation of liver volume. Besides this augmentation is due to the proliferating sinusoidal and perisinusoidal cells and the dilatation of sinusoids as proved by our present morphometric investigation. The degenerative changes of the hepatocytes are much more disseminated and gradually more manifested under conditions of EE treatment in a dosis of 5 mg/kg daily than that of 1 mg/kg daily (2).

It seems that the increasing relative share of sinusoids established in this morphometric study presents an expression of the specific EE action on the cytoskeleton of endothelial cells and hepatocytes (2,13). Such a dilatation has been reported in rats treated with analogous doses of EE for 2 and 6 weeks (12). A dilatation of the periportal sinusoids has been established in females making long-lasting use of contraceptive steroids (3,5) as well as in estrogen-induced hepatomas (4,5).

The most clearly manifested pathological process consists in the vacuolar degeneration of the hepatocytes leading to changes of the nuclear-cytoplasmic ratios and to an enhanced volume of the hepatocytes. Electron microscopically, we detect a dilatation of the cisterns of smooth and rough endoplasmic reticulum, changes of the superficial hepatocytic membranes and intercellular contacts as well (2). The eosinophilic

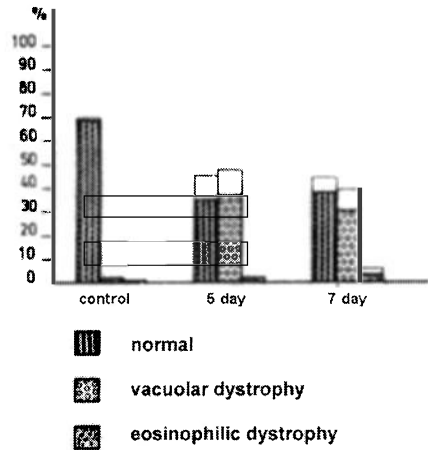


Fig. 4. Percentage ratio between normal and pathologically altered hepatocytes

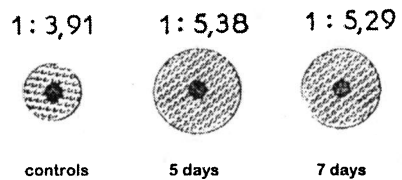


Fig. 5. Percentage hit in the nuclei of hepatocytes (Percentage hit in the cytoplasm)

degeneration and eosinophilic necroses are comparatively rare when our experimental model is concerned (in 2,9 per cent of the hepatocytes). They occur rather seldom in cholestasis described in females, too (5).

CONCLUSION

Our investigation allows us to establish essential changes in the ratio within the principal cellular composition, i. e. between hepatocytes, on the one hand, and sinusoidal

and perisinusoidal cells, on the other hand, in an EE-induced experimental cholestasis in rats. There are also changes of the ratios between normal hepatocytes and hepatocytes with vacuolar degeneration as well as of

the nuclear-cytoplasmic interrelations in the animals with cholestasis. These processes are accompanied by the enhancing relative share of the sinusoids, too.

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Морфометричен анализ на промените в черния дроб при експериментална лекарствена холестаза

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Резюме: Предизвикана е експериментална холестаза на плъхове с 1 mg/kg дневно етинилестрадиол (ЕЕ) в продължение на 5 и 7 дни. С помощта на системна мрежа е извършен морфометричен анализ на черния дроб на 18 животни, разделени в три групи по 6 животни: първа - контроли; втора - третирани с ЕЕ в продължение на 5

дни и трета - третираны с ЕЕ в продължение на 7 дни. При третираните животни се наблюдава промяна в съотношението между хепатоцитите и синусоидалните, респ. перисинуидалните клетки от 10,36 : 1 при контролите до 8,8 : 1 (за втора група) и 8,3 : 1 (за трета група). Установяват се промени и в ядрено-цитоплазмените съотношения в хепатоцитите - от 1 : 3,91 (за първа група) до 1 : 5,38 (за втора група) и 1 : 5,29 (за трета група). Промяната в ядрено-цитоплазмените съотношения в хепатоцитите при третираните животни се дължи на рязкото повишаване на хепатоцитите с вакуолерна дегенерация - от $0,667 \pm 0,42$ % (за първа група) до $35 \pm 11,25$ % (за втора група) и $29,65 \pm 9,08$ % (за трета група). Вакуолерната дегенерация на хепатоцитите и пролиферацията на синусоидалните клетки са най-честите процеси, установени в черния дроб при хистологичното и морфометрично изследване при този вид холестаза.