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Some Morphometric Evidence of Hepatoprotective Effects of (+)-Cyanidanol-3

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VARGA, M. AND L. BURIS. *Some morphometric evidence of hepatoprotective effects of (+)-cyanidanol-3.* PHARMACOL BIOCHEM BEHAV **33**(3) 523–526, 1989.—The (+)-cyanidanol-3 is used as an antihepatotoxic and hepatoprotective drug in both men and animals against alcoholic and experimental liver injury. Histologic staining techniques give mostly qualitative or semiquantitative description of liver damages. Experiments have been carried out to determine the hepatoprotective effects of (+)-cyanidanol-3 on alcoholic liver damage (i.e., fatty liver and hepatomegaly) by morphometric measurement of the liver tissue sections. Ethanol was administered ad lib to CFY rats to cause mild alcoholic liver damage together with 200 mg/kg/day (+)-cyanidanol-3 to prevent the tissue deterioration. The changes of hepatic lobule and hepatocytes were measured morphometrically. The chronic ethanol consumption results in hepatocellular hypertrophy, a significant increase in size of the hepatocytes and a mild increase of the intralobular extrahepatocytic space as well when compared with controls. The volume of cytoplasm was increased while the parameters of nuclei were unchanged. The (+)-cyanidanol-3 prevents changes and the morphometric parameters in the treated group were almost the same as in the controls. The treatment with (+)-cyanidanol-3 alone does not affect the hepatic tissue parameters. The results show the hepatoprotective effect of (+)-cyanidanol-3 and the suitability of the morphometric method for quantitative comparison of normal and experimentally-altered liver cells.

Ethylalcohol Liver damage Bioflavonoid Hepatoprotection

TWO of the earliest and most conspicuous features of the hepatic damage produced by alcohol are the deposition of fat and the enlargement of the liver (12). The number of hepatocytes and the hepatic content of DNA does not change after alcohol treatment, so hepatomegaly is entirely accounted for by the increased cell volume (1,2). There is an insignificant increase in the number of hepatic mesenchymal cells following ethanol feeding (2). In alcoholic liver disease the hepatocyte diameter increases 2–3 times, thereby its volume increases about 4–10-fold (13). Hepatomegaly is traditionally attributed to the accumulation of lipids but almost half of this enlargement is due to an increase in protein content (1). The gross distortion of the volume of the hepatocytes may result in severe impairment of key cellular functions. In clinical practice the measurement of hepatocyte surface area as part of assessment of apparently normal liver biopsies from patients with portal hypertension is recommended, as portal hypertension is due to enlarged hepatocytes rather than to regeneration nodules (11,15).

Morphological information is mostly restricted to semiquantitative descriptions. In recent years stereologic methods have become available permitting efficient and reliable measurement of structures by simple counting or measuring procedures applied to micrographs or sectioned tissues.

The stereological method is ideal for the quantification of some hepatic parameters because the hepatocytes are present in a large number. They are also homogeneously distributed, show no preferential orientation, and are rather isotropic regarding both shape and internal organization (27).

The (+)-cyanidanol-3 is a bioflavonoid and is used as an antihepatotoxic and hepatoprotective drug in men and animals (3). The influence of (+)-cyanidanol-3 on experimental and clinical data concerning hepatic enzymes or bilirubin is known (8, 9, 16, 17, 21, 22). Until very recently the biochemistry of bioflavonoids has not been clarified in every detail (10). The mechanism of the action of (+)-cyanidanol-3 against experimental liver injury consists of a strong radical scavanging function and an inhibition of

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TABLE 1

MORPHOMETRIC DATA OF THE EFFECT OF (+)-CYANIDANOL-3 ON ALCOHOL-INDUCED HEPATOMEGALY

Treatment	Group A Control	Group B Ethanol	Group C Ethanol and (+)-Cyanidanol-3	Group D (+)-Cyanidanol-3
Lobular parenchyma per 1 cm^3 of tissue	0.950 ± 0.005	0.971 ± 0.006	0.950 ± 0.006	0.950 ± 0.006
Extrahepatocytic space per 1 cm^3 of tissue	0.173 ± 0.005	0.140 ± 0.006	0.171 ± 0.005	0.172 ± 0.005
extralobular	0.050 ± 0.005	0.029 ± 0.006	0.050 ± 0.006	0.050 ± 0.003
intralobular	0.121 ± 0.003	0.142 ± 0.004	0.120 ± 0.004	0.121 ± 0.003
Hepatocyte volume μ^3	5230 ± 590	11325 ± 1213	5883 ± 664	5768 ± 482
surface μ^2	1810 ± 204	2662 ± 285	1919 ± 216	1900 ± 158
Nucleus volume μ^3	368.1 ± 29.4	349.2 ± 44.0	371.4 ± 41.7	375.4 ± 47.2
diameter μ	8.11 ± 0.65	8.08 ± 1.03	8.09 ± 0.91	8.10 ± 1.01
Cytoplasm volume μ^3 (calculated)	4862	10916	5512	5393
diameter μ	17.74 ± 2.01	21.52 ± 2.56	18.27 ± 2.05	18.18 ± 2.42
Nucleus per cytoplasm ratio	0.0703 ± 0.0061	0.0308 ± 0.0024	0.0631 ± 0.0060	0.0650 ± 0.0062

Four groups of ten CFY rats were fed a standard diet containing 20% ethanol ad lib in Group B and C for six weeks. (+)-Cyanidanol-3 was given once daily to the animals in Group C and D (200 mg/kg/day by gastric tube). Morphometric parameters of liver tissue sections given in Table 1. were measured at the conclusion of the study. Results are given as mean ± S.D. When the data of Groups A and B or B and C were compared, the p-value was of at least 0.05 or less in all cases. The differences between the data of Group A and C or A and D were not statistically significant.

intrahepatic lipid peroxidation (18,19). Its membrane-stabilizing function has also been suggested (16). It appears to prevent the reduction of glutathione levels, scavanges free radicals, reduces peroxidation, and may induce the microsoma (24). It has been stated that bioflavonoid (+)-cyanidanol-3 has a direct biochemical action on hepatic tissue (6) by increasing glycolysis which furnishes pyruvate (7).

Although biochemical considerations suggest such an effect, neither influence on the blood alcohol curve, nor detectable increase in elimination coefficient value were found in men (5).

Some histologic changes (4) and ultrastructural aspects of alcoholic liver damages were previously reported by us (23). In the present study, mild alcoholic liver damage has been induced in rats by administering ethanol ad lib. Hepatoprotective effect of simultaneous treatment with (+)-cyanidanol-3 was determined by morphometry of the liver tissue.

METHOD

Forty male adult CFY rats with an initial weight of 190 to 210 g were housed individually in wire bottomed cages at room temperature in 12-hr light/dark lighting cycles. The rats were randomly allocated into four groups as follows: Group A, Controls; Group B, LATY diet containing 20% ethanol ad lib; Group C, LATY diet containing 20% ethanol ad lib and (+)-cyanidanol-3; Group D, LATY diet and (+)-cyanidanol-3 treatment. The diet in Groups A and D was adjusted to reach isocaloric level with carbohydrates. The (+)-cyanidanol-3 (made as CATERGEN) was supplied by Zyma-Biogal Pharmaceutical Works, Debrecen, Hungary, at highest pharmaceutical grade. This drug was given as a suspension in water to the animals in Groups C and D once daily for 6 weeks through gastric tubes (200 mg/kg/day). The animals in Groups A and B received a similar volume of water daily by gastric tube. After 42 days the animals were sacrificed by cervical dislocation, their livers were removed, and formalin fixed for histologic examination. Blood samples were also taken for ethanol and drug level determination.

Random histological liver sections were stained with hematoxylin-eosine and methylene-blue for morphometric procedure. Subsequently, three liver sections of each animal were examined through a light microscope at a final magnification of ×80 with projection head carrying a test grid containing 81 points for the hepatic lobular. Then they were examined at ×800 with a quadratic lattice of 841 points for the volume and surface ratios measurements. Final image magnification was calibrated, the relative volumes of lobular and extralobular tissues and the mean nuclear-to-cytoplasmic ratio were determined for the treated and control groups. The extrahepatocytic compartment of intralobule space (Kupffer cells, Disse space, etc.) was not subdivided because this study related only to hepatocytes. The volume and number of bodies contained in the unit volume were calculated by counting the number of transsections on the unit area of the random sample (28,29). Hepatocyte nuclei were assumed to be spherical, and their true diameters were measured following Weibel's method (25, 26, 29). However, it appeared useless to measure individual profiles of structures with high precision for a relative, comparative study. Repeated measurements were reproducible within a range of 1.5–3.5%. All hepatocytes, regardless of their central, midzonal and peripherical positions, were considered "average" because of the structural homogeneity of more than 80% of normal liver parenchymal cells (14), so morphometric data could be comparable with biochemical ones obtained in liver homogenates. The data were expressed as mean ± S.E. When groups were compared, Student's t-test was employed. A p-value of <0.05 was considered to be statistically significant.

RESULTS

Morphometric data of the liver tissue as the conclusion of this study are shown in Table 1. Administration of (+)-cyanidanol-3

prevented all alterations of cell components of the hepatic lobule and caused a significant reduction in the extent of liver cell enlargement induced by chronic ethanol consumption in the animals. Besides the enlargement of hepatocytes, the relative space of lobular parenchyma (consisting of hepatocytes, sinusoids, Disse spaces, biliary capillaries) was increased in Group B, in spite of the widening of portal triads of the extralobular space, caused by an infiltration of eosinophils and lymphocytes. Compared to the control group, the intralobular extrahepatocytic space in Group B was only slightly increased due to the greater number of Kupffer cells and foamy macrophages although the vascular-sinusoidal compartments were compressed. The standard deviation in Group B was greater than in the other groups. The light microscope showed marked variations in size of the cells around the central veins. There were only very few binucleated cells in Group C as well, which appeared to have about twice the volume of mononuclear cells but the volume per nucleus very nearly approximated that of the average mononucleate cell. Administration of (+)-cyanidanol-3 did not cause any reduction in food intake when compared to that of animals given ethanol alone. Serum ethanol levels determined systematically by gas chromatography during the study were practically the same in the two groups consuming ethanol whether they were given (+)-cyanidanol-3 or not. Thus, the effect of (+)-cyanidanol-3 cannot be attributed to reduced ethanol intake or altered ethanol metabolism. In the case of (+)-cyanidanol-3 treatment alone there was a non-significant increase in diameter of average hepatocyte with a greater standard deviation without any visible signs of tissue deterioration. No significant differences were found among the results from individual animals.

DISCUSSION

Hepatomegaly is a nearly constant finding in alcoholic liver disease. An enlargement of the liver has also been observed in rats chronically fed by alcohol. This model has allowed the investigation of the mechanism leading to alcohol-induced hepatomegaly and the hepatoprotective influence of drugs on it. The hepatomegaly induced by chronic alcohol consumption is due to an increase in hepatocyte size rather than to an increase in the number of hepatocytes. Total liver DNA remains constant in this type of hepatomegaly, which observation supports the concept of liver hypertrophy rather than that of hyperplasia after chronic alcohol consumption (1,2).

For centuries, preparations containing bioflavonoids (different foods from plants, teas) as the principal physiologically-active constituent have been used in attempts to treat human diseases (10) such as alcohol-related liver disease. Recently, biochemical considerations also suggest such an effect. The (+)-cyanidanol-3 is effective at reversing the redox state disturbance following ethanol administration (18), and this correction appears to correlate with reduced disposal of fatty acids into hepatic triglycerides, and increased fatty acid oxidation rates.

Histologic examinations of alcohol-induced liver damage permit only restricted semiquantitative description and information. The stereological (morphometric) method can be useful for quantitative comparison of normal and experimentally-changed liver cells. Stereological methods are applicable only if the objects forming the structures are present in large numbers, are homogeneously distributed and show no preferential orientation. The liver cells fulfill these conditions.

We measured the ratio of cell compartment of hepatic lobule, the average hepatocyte surface and volume, the nuclei diameter and the cytoplasm to nuclei ratio. The intralobular extrahepatocytic space was not subdivided morphometrically but observed by light microscope. The term hepatocyte means average mononuclear hepatocyte.

The results of this study on the normal rat liver are in general agreement with other measurements (11, 12, 14, 25, 29). The small differences in the data may be due to differences in strain, age, weight of rats, the possible differences in fixation procedure, tissue shrinkage, section thickness or in the different sampling procedure employed. However, for a comparative work like this, a correction is unnecessary since it can be assumed that all experimental groups are affected by the same systemic errors.

Only one study came to different conclusions; namely that there was a significant shrinkage of the liver cells and their nuclei of the livers of alcoholics (20).

Our morphometric data demonstrated the hepatoprotective effects of bioflavonoid (+)-cyanidanol-3 on the alcohol-induced hepatic injury caused by chronic ethanol consumption in rats. These results show that there may be a morphometrical quantitative comparison of normal and experimentally-altered or treated liver cells in the studies on hepatoprotection or hepatic diseases.

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