DOI 10.1007/s10517-017-3610-8

Evaluation of the Hepatoprotective Effect of L-Ascorbate 1-(2-Hydroxyethyl)-4,6-Dimethyl-1,2-Dihydropyrimidine-2-one upon Exposure to Carbon Tetrachloride

A. B. Vyshtakalyuk[1], N. G. Nazarov[1,3], V. V. Zobov[1,3], S. R. Abdulkhakov[3], O. A. Minnekhanova[1], V. E. Semenov[1], I. V. Galyametdinova[1], G. V. Cherepnev[1,2], and V. S. Reznik[1]

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 162, No. 9, pp. 322-325, September, 2016
Original article submitted November 23, 2015

Hepatoprotective properties of a new pyrimidine derivative — L-ascorbate 1-(2-hydroxyethyl)-4,6-dimethyl-1,2-dihydropyrimidine-2-one, synthesized on the basis Xymedon, were assessed in white rats exposed to CCl_4. The compound under study administered prior to exposure to CCl_4 reduced the deviation of biochemical parameters from reference values and severity of structural and morphological changes in liver, when compared to the control. Hepatoprotective properties of the studied compound were more pronounced than those of Xymedon.

Key Words: *pyrimidine derivatives; ascorbate; hepatoprotectors; toxic liver damage; CCl_4*

Pyrimidines are used as stimulants of protein synthesis and cellular recovery in liver upon its toxic or infectious damage. Hepatoprotective activity of a number of pyrimidine derivatives was revealed in case of experimental toxic hepatitis [5]. There is data on hepatoprotective effect of oxymethyluracyl [4]. Analogs of pyrimidine nucleobases, drugs Methyluracil and Pentoxyl, exerted weak hepatoprotective effect [8]. The group of 2,4-dioxo-5- arylidenimino-uracils possesses a broad spectrum of biological activities, including hepatoprotective properties [9]. The drug Xymedon (1-(β-hydroxyethyl)-4,6-dimethyl-1,2-dihydro-2-oxopirimidine), developed as tissue regeneration stimulator, enhanced activity of microsomal oxidases in human liver [6]. Studies on experimental toxic liver damage in rats demonstrated hepatoprotective effects of Xymedon and its stimulating effect on liver tissue recovery [1,2].

The aim of this study was to investigate hepatoprotective properties of the Xymedon derivative (XD) L-ascorbate 1- (2-hydroxyethyl)-4,6-dimethyl-1,2-dihydropyrimidine-2-one (Fig. 1), which exhibits high efficiency as actoprotector [8].

MATERIALS AND METHODS

The study was carried out on 68 male albino rats weighing 250-400 g using preventive exposure to the test preparation [7]. Effectiveness of XD win comparison with effectiveness of Xymedon (original substance) after administration of the compounds in identical doses. Maximum dose was 20 mg/kg. XD exerts pronounced actoprotective effect in this dose [3].

The animals orally received Xymedon or XD in doses of 10 and 20 mg/kg over 4 days. In 1-1.5 h after drug administration, CCl_4 in vegetable oil (1:1) in a dose of 2 ml/kg (calculated for the mixture). Controls received CCl_4 but without hepatoprotectors. Intact control group consisted of rats, not receiving CCl_4 or other compounds. The next day after the last administration of CCl_4 and compounds under study animals were dehematized under ether anesthesia, blood and liver samples were taken for biochemical and histological analysis.

[1]A. E. Arbuzov Institute of Organic and Physical Chemistry, Kazan Research Center of the Russian Academy of Sciences; [2]Medical Unit of Kazan (Volga Region) Federal University, Kazan, Russia; [3]Kazan (Volga Region) Federal University, Kazan, Russia. ***Address for correspondence:*** alex.vysh@mail.ru. A. B. Vyshtakalyuk

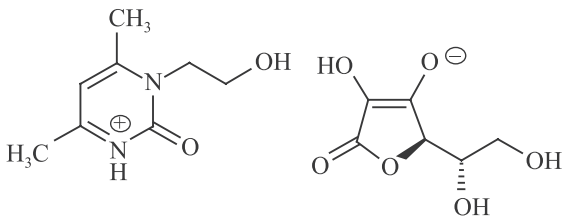

Fig. 1. Chemical structure of XD.

Blood serum biochemical parameters were determined using Daytona Randox biochemical analyzer and Randox kit. Standard biomarkers of toxic liver cell damage were measured: ALT, AST, AST/ALT ratio (coefficient of de Rytis), alkaline phosphatase (ALP). Liver synthetic function was determined from serum total protein level.

The data were processed statistically using nonparametric rank Mann—Whitney *U* test for comparison of two independent samples. Variable distribution is shown as median and upper and lower quartiles.

RESULTS

In control rats, ALT activity increased by 3.2 times, AST by 1.7 times, ALP by 27.2%, AST/ALT was reduced by 2.4 times in comparison with intact animals (Table 1). The revealed changes in biochemical parameters are indicative of toxic liver damage and hepatocyte cytolysis. CCl_4 produced a decrease in serum total protein concentration by 8.7%, which is indicative of a significant decrease in liver synthetic function.

In rats, treated with Xymedon or XD, there was observed a statistically insignificant trend to a decrease in ALT level, compared to control. AST level in the drug-treated groups was lower than in control. However, differences in the AST level between the groups, treated with different Xymedon doses, were only at the level of a trend.

In the groups, treated with 10 or 20 mg/kg of XD, AST activity was significantly lower than in control. Alkaline phosphatase level decreased under the influence of Xymedon in both groups, for the dose of 20 mg/kg the difference was statistically significant (Table 1). In contrast to Xymedon, XD did not significantly affect the alkaline phosphatase level.

In all experimental groups serum total protein level was higher than in control, which is indicative of recovery of protein synthesis in liver under the influence of the drugs under study. However, statistically significant differences from control were obtained only for the Xymedon dose of 20 mg/kg (Table 1).

Analysis of hematoxylin- and eosin-stained liver histological sections, obtained from the control group, revealed moderate fatty infiltration of parenchyma and necrosis of hepatocytes with prefedominant localization in the pericentral zone (Fig. 2). When Xymedon or XD was administered in doses of 10 and 20 mg/kg fatty liver dystrophy was less pronounced, compared to control (Fig. 3).

Thus, Xymedon and its derivative administered according to the preventice scheme, exerted hepatoprotective effects: Xymedon — in the dose of 20 mg/kg, XD — in doses of 10 and 20 mg/kg. The drugs under study had different effects on liver condition, affected by induction with CCl_4. Xymedon had a more pronounced effect both on the ALP level, which can be

TABLE 1. Biochemical Parameters of Rat Blood [median (lower quartile; upper quartile]

Group	ALT, U/liter	AST, U/liter	AST/ALT	Serum total protein, g/liter	ALP, U/liter
Intact (*n*=36)	36.37 (28.18; 43.3)	132.95 (118.24; 164.00)	4.26 (3.03; 5.23)	65.06^{+} (62.06; 68.98)	279.92 (183.51; 390.84)
CCl_4 (control) (*n*=12)	116.23** (76.96; 211.71)	230.08** (201.49; 290.03)	1.76** (1.47; 2.67)	59.62* (55.91; 63.55)	355.97 (224.37; 574.84)
Xymedon, 10 mg/kg+CCl_4 (*n*=6)	89.86 (87.06; 165.15)	211.19 (170.20; 250.16)	1.98 (1.51; 2.55)	56.92 (36.39; 72.14)	222.76 (216.35; 246.95)
Xymedon, 20 mg/kg+CCl_4 (*n*=3)	103.23 (38.19; 270.87)	193.61 (181.57; 274.69)	2.66 (0.71; 4.75)	72.00^{+} (69.63; 72.12)	130.65^{++} (75.66; 132.35)
XD, 10 mg/kg+CCl_4 (*n*=6)	80.28 (6.12; 141.82)	190.91^{+} (65.21; 198.65)	2.39 (1.89; 4.37)	64.41 (55.67; 65.50)	243.35 (126.84; 474.48)
XD, 20 mg/kg+CCl_4 (*n*=5)	100.33 (62.24; 144.64)	173.25 (135.50; 210.70)	1.51 (1.26; 2.74)	63.54 (57.24; 64.01)	390.15 (194.22; 545.91)

Note. *p<0.01, **p<0.001 in comparison with intact rats; ^{+}p<0.05, ^{++}p<0.01 in comparison with control.

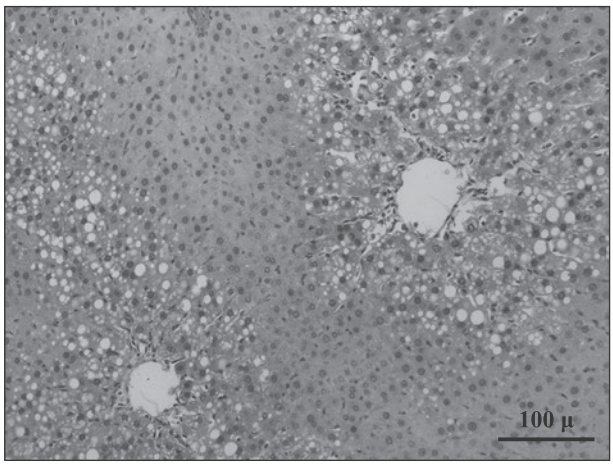

Fig. 2. Changes in liver structure (impaired lobular structure, narrowing of intercellular spaces, swelling, vast areas of fatty degeneration and necrosis of hepatocytes) in control rats. Staining with hematoxylin and eosin, ×300.

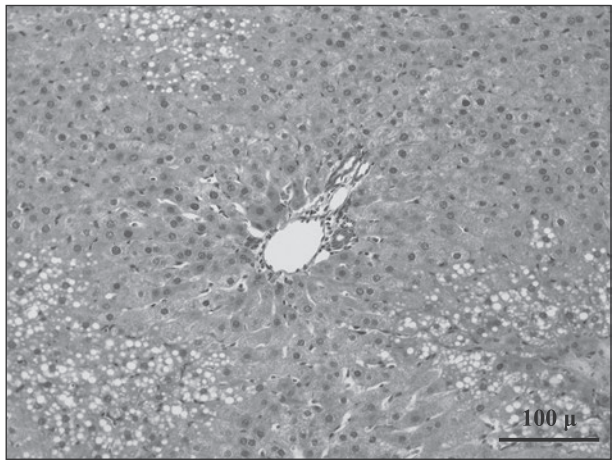

Fig. 3. Reduced pathological changes in liver tissue (preservation of the lobular pattern, reduced area of fatty degeneration and necrosis of hepatocytes) in rats, treated with 20 mg/kg of XD. Staining with hematoxylin and eosin, ×300.

elevated not only due to destructive changes in hepatocytes, but also as a cholestasis manifestation, and on the serum total protein level, which reflects protein synthesis in liver. XD had a more pronounced effect on AST level, which is one of the markers of hepatocyte damage (cytolysis). Lower effective dose of XD — 10 mg/kg, compared to Xymedon (20 mg/kg in this study and 50 mg/kg, effective according to [1,2]), and its pronounced effect on AST, the hepatocyte cytolysis marker, is indicative of its higher preventive effectiveness, compared to Xymedon.

The study was supported by Russian Science Foundation (grant No. 14-50-00014).

REFERENCES

1. Vyshtakalyuk AB, Nazarov NG, Zueva IV, Lantsova AV, Minnekhanova OA, Busygin DV, Porfiryev AG, Evtyugin VG, Reznik VS, Zobov VV. Study of hepatoprotective effects of xymedon.Bull. Exp. Biol. Med. 2013;155(5):643-646.
2. Vyshtakaliuk AB, Zueva IV, Minnechanova OA, Reznik VS, Zobov VV, Nicolskyi EE, Nazarov NG, Porfiriev AG, Mayatina OV. The influence of the Xymedon preparation (Hydroxyethyldimethyldihydropyrimidine) on the rat liver recovery under toxic damage induced by carbon tetrachloride. Doklady Biochem. Biophysics. 2015;462(1):143-146.
3. Zobov VV, Nazarov NG, Vyshtakalyuk AB, Galyametdinova IV, Semenov VE, Reznik VS. Efficiency of new pyrimidine derivativs influence on physical working capacity of rats in the test "swimming to failure". Ekol. Cheloveka. 2015(1):28-35. Russian.
4. Myshkin VA, Enikeyev DA. Oxymethyluracil and experimental pathology of the liver. Med. Vestn. Bashkortastana. 2009;4(2):147-151. Russian.
5. Novikov VE, Klimkina EI. Pharmacology of hepatoprotectors. Obzory po Klin. Farmakol. Lek. Ter. 2005;4(1):2-20. Russian.
6. Pogorel'tsev VI, Garmonov SJ, Reznik VS, Shitova NS, Jakovleva AV. Patent RF No. 2316327. Ximedon as inductor of human liver microsomal oxidase activity. Bull. No. 4. Published February 10, 2008.
7. Manual on Experimental (Preclinical) Study of New Pharmacological Substances, Khabriev RU, ed. Moscow, 2005. P. 685. Russian.
8. Taouk AN, Fiodorov VN, Kunitsa ZA, Smirnov NA, Kotshneva NV. Comparative efficacy of drugs of different pharmacotherapeutic groups in experimental toxic hepatitis. Ros. Med.-Biol. Vestn. 2010(1):66-73. Russian.
9. Krutikov VI, Ashkinazi RI. Patent US 6730787 B1. 2,4-Dioxo-5-arylidenimino-1,3-pyrimidines. Date of Patent May 04, 2004.