Protective Effects of CYP2E1 Inhibitors on Metabolic Syndrome-induced Liver Injury in Guinea Pigs

Volodymyr V. Rushchak[1*], Ganna M. Shayakhmetova[2], Anatoliy V. Matvienko[2] and Mykola O. Chashchyn[1]

[1]*Department of Molecular Oncogenetics, Institute of Molecular Biology and Genetics, NAS of Ukraine, Zabolotnogo Str. 150, Kyiv, Ukraine.*
[2]*Department of General Toxicology, SI "Institute of Pharmacology and Toxicology, NAMS of Ukraine", Eugene Pottier 14, Kyiv, Ukraine.*

Authors' contributions

This work was carried out in collaboration between all authors. Author VVR managed the analyses of the study, performed the statistical analysis and wrote the first draft of the manuscript. Author GMS managed the analyses of the study, managed the literature searches and wrote part of the manuscript. Author AVM managed the histopathology studies. Author MOC designed the study and wrote the protocol. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

The present work reports the effects of CYP2E1-inhibitors (quercetin, 4- methylpyrazole and disulfiram) on the indices characterizing state of the liver in guinea pigs with metabolic syndrome (MS) induced by protamine sulfate repeated administrations.
The investigation of quercetin, 4-methylpyrazole and disulfiram effects on hepatic cytochrome P450 2E1 (CYP2E1) protein and activity changes was conducted. Simultaneously, the content of reactive oxygen species (ROS) and markers of liver damage were determined in experimental animals' blood. The link between increased hepatic expression of CYP2E1, prominent ROS generation and liver damage in animals with MS has been discovered. It has been demonstrated that CYP2E1 protein content and activity in guinea pigs with MS rose almost 3 times compared to intact animals. These events were accompanied by increase in ROS generation and metabolism

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Corresponding author: Email: v.v.rushchak@gmail.com;

and liver disturbances symptoms: increase in serum glucose and cholesterol contents (2 and 2.6 times respectively), alanine aminotransferase (2.8 times), aspartate aminotransferase (6.4 times), and alkaline phosphatase (1.8 times) elevations. Our investigation suggests that administration of quercetin, 4-methylpyrazole, and disulfiram in guinea pigs with MS caused decrease in this isoenzyme protein expression (2.5, 1.7 and 2.4 respectively) as well as its enzymatic activity in liver (6.6, 1.1, and 1.7 respectively). The content of blood ROS was partially restored or normalized by all three CYP2E1 inhibitors. In turn, suppression of CYP2E1 activity and ROS generation led to decrease in hepatic MS manifestation. It is apparent from the present observation that quercetin has the highest efficiency among the investigated substances. Further studies on various quercetin doses and administration regimens could provide relevant information for the development of MS-related nonalcoholic fatty liver disease treatment.

1. INTRODUCTION

The prevalence of metabolic syndrome (MS) is growing around the world at an alarming rate. MS is defined by a constellation of interconnected physiological, biochemical, clinical and metabolic factors that directly increases the risk of cardiovascular disease, type 2 diabetes mellitus and all cause mortality. Insulin resistance, visceral adiposity, atherogenic dyslipidemia, endothelial dysfunction, genetic susceptibility, elevated blood pressure, hypercoagulable state, and chronic stress are the several factors which constitute the syndrome [1].

Nonalcoholic fatty liver disease (NAFLD) is the hepatic expression of MS, which comprises a spectrum of clinical and histological events ranging from simple and benign fatty liver to steatohepatitis, which is characterized by the abnormal activation of pathways leading to an aggressive inflammatory condition [2,3]. NAFLD is usually clinically silent, and its impact has most likely been underestimated. Symptoms of NAFLD, if present, are minimal and non-specific, such as fatigue and right upper quadrant discomfort. The disease usually comes to medical attention incidentally when aminotransferases levels are found to be elevated or a radiographic study reveals that the liver is fatty [4]. This pathological state may progress to more severe damage known as cirrhosis, which endangers the anatomy and function of liver tissue. In addition, a small group of patients with end-stage liver disease may develop hepatocellular carcinoma and finally death [3].

It is generally assumed that NAFLD is commonly associated with insulin resistance, which is a major risk factor for the development of type 2 diabetes and a central feature of the MS [5]. Hyperinsulinemia, caused by an increased insulin secretion by the pancreatic β-cells and decreased insulin degradation by the liver, is a compensatory phenomenon to insulin resistance. Hyperinsulinemia leads to an increase in fat mass, lipogenesis and it is associated with increased concentrations of free fatty acids [6]. Most of the adverse effects induced by fatty acids accumulation are likely to be mediated by lipid intermediates, notably diacylglycerols and ceramides. Lipid intermediates can induce insulin resistance by activating different kinases such as mammalian target of rapamycin (mTOR), inhibitor of κB kinase (IKK), Jun N-terminal kinase (JNK) and novel protein kinase C (nPKC) that are known to exert negative feedback on proximal insulin signaling [5].

Indeed, insulin resistance and oxidative stress are major pathogenic mechanisms leading to chronic liver diseases in subjects with MS. The oxidative stress includes reactive oxygen species (ROS) production and lipid peroxidation eventually causing NAFLD [7]. The potential sources for the ROS in the liver include hepatic cytochrome P450 2E1 (CYP2E1), mitochondria and iron overload [7]. Insulin resistance and increased cytochrome CYP2E1 expression are both associated with and mechanistically implicated in the development of liver pathology. Although currently viewed as distinct factors, insulin resistance and CYP2E1 expression may be interrelated through the ability of CYP2E1-induced oxidant stress to impair hepatic insulin signaling [8]. On the other hand, CYP2E1 is normally suppressed by insulin but is invariably increased in the livers of patients with NAFLD [9]. Once liver disease is established, NAFLD-induced impairment of insulin signaling may then further promote the diabetic state. Findings of reduced suppression of hepatic glucose

production by insulin in NAFLD patients as compared with controls support this concept. There is suggestion that one mechanism of this effect can be mediated by the effects of CYP2E1 over-expression [8].

On the assumption of the above mentioned, the regulation of CYP2E1 expression level could be effective method to alleviate the extent of pathological processes in the liver accompanying MS. Recent findings, showing that severe liver injury associated with elevated oxidative stress was blunted by inhibitors of CYP2E1, also stimulated our interest in this problem [10]. Based on these facts and considering that MS has emerged as one of the major health care issues, the present work reports the effects of CYP2E1-inhibitors (quercetin, 4-methylpyrazole (4-MP), and disulfiram) on the indices characterizing state of the liver in guinea pigs with MS.

2. MATERIALS AND METHODS

2.1 Animals and Experimental Design

Male guinea pigs (n = 25) with initial mean body weight 370g (5 months old) were used in the study. They were kept under a controlled temperature (from 22°C to 24°C), relative humidity of 40% to 70%, lighting (12 h light-dark cycle) and on a standard pellet feed diet ("Phoenix" Ltd., Ukraine) and water *ad libitum*.

2.2 Chemicals

All reagents used in investigations were obtained from Sigma Aldrich and Serva.

The following CYP2E1 inhibitors were applied: protamine sulfate (Protamine sulfate salt from salmon) was from Serva; quercetin (3,5,7,3',4'-pentahydroxyflavone), 4-MP (4-Methyl-1*H*-pyrazole) and disulfiram (1,1',1'',1'''-[disulfanediylbis (carbonothioylnitrilo)] tetraethane) were from Sigma Aldrich.

2.3 Experimental Design

The guinea pigs were kept for acclimatization during 10 days, and then they were randomized into 5 groups. Each group contained 5 animals:

1. Control, n=5: intact animals.
2. MS, n=5: guinea pigs were injected intramuscularly with protamine sulfate solution in dose 15 mg/kg b.w., twice per day, daily during 5 weeks. After this animals were kept in usual conditions during 4 weeks till euthanasia [11].
3. MS with quercetin administration, n=5: Animals with MS were given quercetin (intramuscularly, 20 mg/kg b.w., daily, during 2 weeks). Quercetin administration was started in 2 weeks after the last protamine sulfate injection. Euthanasia of animals was performed in overnight after the last quercetin administration.
4. MS with 4-MP administration, n=5. Animals with MS were given 4-MP (intramuscularly, 20 mg/kg b.w., daily, during 2 weeks). Administration was started in 2 weeks after the last protamine sulfate injection, and euthanasia was performed in overnight after the last 4-MP administration.
5. MS with disulfiram administration, n=5. Animals with MS were given disulfiram (per os, 25 mg/kg b.w., daily, during 2 weeks). Administration was started in 2 weeks after the last protamine sulfate injection, and euthanasia was performed in overnight after the last disulfiram administration.

2.4 Blood and Tissue Collection

All overnight fasted animals were sacrificed in above mentioned terms under a mild ether anesthesia by decapitation. Euthanasia always was performed at the same time to avoid the daily changes in the activity of enzymes.

Blood for biochemical tests and flow cytofluorometry was withdrawn from the femoral vein prior to euthanasia. Blood with heparin was used for flow cytofluorometry analysis. Non-heparinized blood was centrifuged (1300 RCF, RT, and 10 minutes) in order to separate serum.

Liver was removed and processed for morphological studies.

Also samples of liver (100 mg) were collected, quickly frozen in liquid nitrogen, and stored at -80°C before examination.

2.5 Western Blot Analysis

The relative level of CYP2E1 protein in the liver was determined by Western blot analysis.

The extraction of total proteins was carried out according to a standard protocol for membrane proteins (Abcam, UK).

Proteins from the liver of each animal (50 µg per line) were separated using 12% polyacrylamide

gel with 0.1% sodium dodecyl sulfate (SDS). The semi-dry electro transfer of proteins to the nitrocellulose membranes was held at 200 mA for 40 minutes. Western blot analysis was held in the following way: nitrocellulose membranes (Biorad, USA) were pre-incubated in 2% nonfat milk (Sigma, USA), and then treated with polyclonal anti-CYP2E1 antibodies (obtained in Molecular Oncogenetics Department, Institute of Molecular Biology and Genetics NASU) at a 1:400 ratio v/v for 1 hour. After washing membranes were incubated with secondary anti-Rabbit IgG-HRP antibodies (Sigma, USA) at a 1:5000 ratio v/v during the same time. The β-actin (used as internal control) was identified using anti-β-actin antibodies (Sigma-Aldrich, USA). The treatment of membranes with secondary antibodies was followed by chemiluminescence detection according to manufacturers' instructions (Pierce). Membranes were exposed to autoradiography film (Agfa, Belgium) for 0.5 to 1 minutes. Digital images of immunoblots were analyzed using densitometric scanning analysis program Scion image 3.53.346.0 (http://www.scioncorp.com/).

The level of CYP2E1 protein was calculated as the ratio of protein values to β-actin on the same line, and presented as reference units.

2.6 Evaluation of CYP2E1 Activity in Liver Microsomes

Microsomes were obtained according to the protocol described by Jeong and Yun [12].

A spectrophotometric method for determination of CYP2E1 activity by monitoring of the *p*-nitrocatechol formation from *p*-nitrophenol (PNP) by isolated liver microsomes was used. This method is applicable to enzymatic studies for determination of P450-catalyzed *p*-nitrophenol hydroxylation activity [13]. The enzymatic product, *p*-nitrocatechol, is assayed at 546 nm after acidification of the reaction mixture with trichloroacetic acid followed by neutralization using 10 M NaOH.

2.7 Cytofluorometric Analysis of ROS

Studies were performed as it was described by Bhagwat et al. [14] using a Coulter Epics XL Flow cytometer (Beckman Coulter, US) with argon laser. The excitation wavelength was 488 nm. Detection channels were FL1 (515-535 nm) and FL3 (620-630 nm). The dyes used in this study (25 µmol/L 2,7-dichlorodihydrofluorescein

diacetate (2,7-DCFH-DA) and 10 µg/ml propidiun iodide) were from Molecular Probes (Leiden, the Netherlands).

The results were presented in reference units of dichlorodihydrofluorescein (oxidized product 2,7-DCFH-DA) fluorescence.

2.8 Clinical Chemistry

Glucose, and cholesterol contents, alanine aminotransferase (AlAT), aspartate aminotransferase (AsAT) and alkaline phosphatase (ALP) activities in blood serum were determined with fully automatic biochemistry analyzer (Prestige 24i, Tokyo Boeki, Japan).

2.9 Histopathology

Liver was fixed in 10% buffered neutral formalin, dehydrated in ethanol solutions and embedded in paraffin. Histologic sections (4 µm) were stained by McManus's method for glycogen detection [15].

Microscopic studies were carried out with microscope Cytophan (Leica Microsystems, Wetzlar GmbH).

2.10 Statistical Analysis

The obtained data were calculated by one-way analysis of variance (ANOVA) and expressed as mean ± standard error of mean (SEM). Data were compared using Tukey test. Differences were considered to be statistically significant at $P < 0.05$.

3. RESULTS

3.1 Evaluation of Liver CYP2E1 Protein Level and Enzymatic Activity

Western Blot analysis was performed to evaluate the effect of MS on CYP2E1 protein expression in the liver. We demonstrated that the CYP2E1 protein content in guinea pigs with MS increased more than 3 times in comparison with intact animals (Fig. 1A and 1C).

PNP hydroxylase activity as a selective enzyme marker for CYP2E1 was determined. The obtained results (Fig. 1B) suggested the significant increase in CYP2E1 enzymatic activity following MS development. It was also almost 3 times greater than in control (Fig. 1B).

As it is shown in Fig. 1A and 1B, CYP2E1 inhibitors in varying degrees reduced its expression and activity in the liver. The most effective inhibition was observed following quercetin administration, the least effective – following 4-MP treatment. It is interesting that 4-MP more effectively inhibited CYP2E1 protein expression, than its activity, whereas quercetin acted as the most powerful inhibitor of the enzyme activity.

3.2 Cytofluorometric Analysis of ROS

In order to explore the influence of MS-mediated CYP2E1 induction in liver on oxidative stress development, the cytofluorometric analysis of ROS level in experimental animals' leukocytes was performed.

It was shown that the development of MS in the experimental animals accompanied by a considerable increase in the number of blood ROS, which content was higher than in intact group almost 4 times (Fig. 2).

Data represented in Fig. 2 suggest that reducing of activity and CYP2E1 protein level by inhibitors led to decrease (2 times) in the intensity of ROS generation.

3.3 Clinical Biochemistry and Histopathology

Clinical biochemistry parameters and liver histology were studied in order to evaluate severity of MS signs in animals of different experimental groups.

In animals groups with MS we have revealed symptoms typical for this pathology, such as hyperglycemia and hyperlipidemia. Serum glucose and cholesterol contents were increased 2 and 2,6 times respectively, as compared with control (Table 1).

At the same time significant increases in liver damage marker enzymes activities were fixed (AlAT and AsAT – 2.8 and 6.4 times, ALP – 1.8 times).
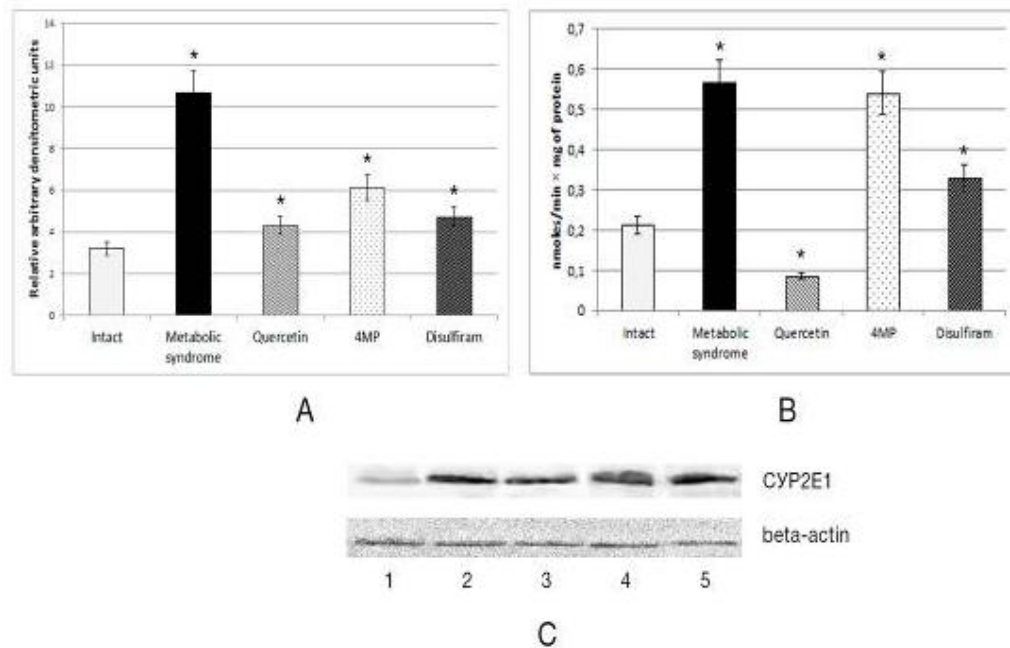
Fig. 1. Average rate of CYP2E1 protein expression, (M±S.E.M., n=5) (panel A) and CYP2E1 enzymatic (PNP hydroxylase) activity, M ± S.E.M., n=5 (panel B) in liver of guinea pigs with MS and CYP2E1 inhibitors administration. Representative Western Blot of CYP2E1 and reference-gene β-actin proteins are shown in panel C. Line 1 – intact animals, Line 2 – animals with MS, Line 3 – MS treated by quercetin; Line 4 – MS treated by 4MP; Line 5 – MS treated by disulfiram
* – P<0.05 in comparison with control

These results are in concordance with our data of liver histopathology. The main pathogenetic mechanism of MS development is carbohydrate metabolism disturbance. Normally liver cells contain a large amount of glycogen (Fig. 3A, intensive pink staining). Reducing of the livers slice color intensity in MS group (Fig. 3B) evidences the common for MS and DM 2 type [16,17] decrease of glycogen content due to glycogenolysis activation.
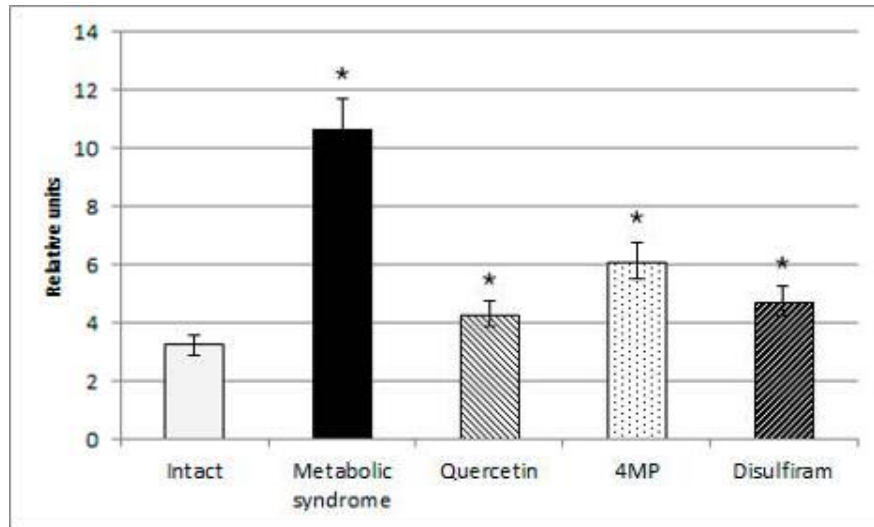

Fig. 2. Average content of ROS (M ± S.E.M., n=5) in leukocytes of guinea pigs with MS and CYP2E1 inhibitors administration
* – P<0.05 in comparison with control

Table 1. Clinical biochemistry parameters in guinea pigs with MS and CYP2E1 inhibitors administration

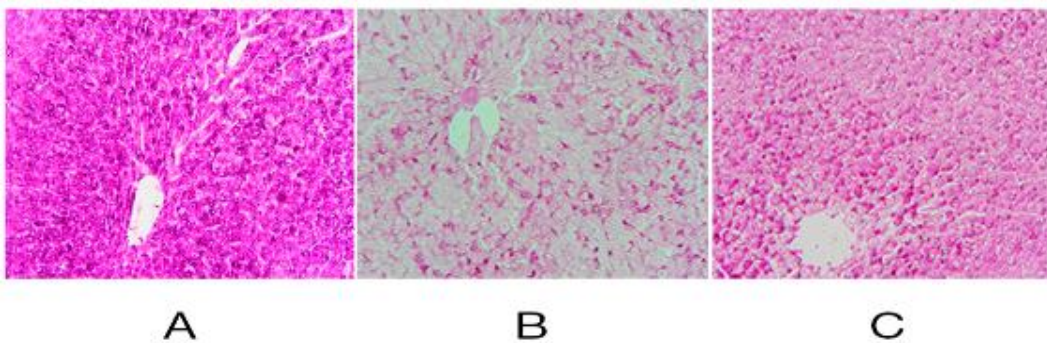| Groups of animals	Parameters				
	Glucose, mol/L	Cholesterol, mol/L	AlAT, IU/L	AsAT, IU/L	ALP, IU/L
Control	5.2±0.2	0.58± 0.03	37.5±2.6	39.5±2.7	68.75±1.2
MS	10.2±0.3*	1.5±0.09*	107.0±6.2*	255.0±15.1*	126.0±4.1*
MS + quercetin	8.65±0.3*	0.71±0.06*	59.5±3.5*	80.0±4.4*	81.0±2.8*
MS + 4-methyl-pyrazole	9.1±0.3*	0.72±0.07*	86.0±4.7*	176.0±12.7*	113.0±3.1*
MS + disulfiram	8.1±0.2*	0.79±0.09*	85.0±4.3*	201.0±11.2*	108.5±4.4*

* – P<0.05 in comparison with control

Fig. 3. Histochemical determination of glycogen content in liver cells (PAS-reaction by McManus). x1000, x400. A - control; B - MS; C - MS + quercetin

To identify links between CYP2E1-dependent processes at MS and liver state, we compared the serum biochemical parameters depending on the severity of CYP2E1 inhibition by disulfiram, 4-MP or quercetin. It was shown that CYP2E1 inhibitors alleviated hepatic manifestation of MS to varying degrees (Table 1). According to our findings all three compounds prevented hyperglycemia development. Serum glucose content in treated guinea pigs was almost 2 times lower than in MS group (Table 1). Furthermore, we have clearly indicated the most pronounced effect of the quercetin administration exhibiting marked reduction in AlAT, AsAT and ALP activities as compared with non-treated animals (Table 1). The partial restoration of glycogen content in liver cells was observed only following quercetin administration (Fig. 3.C). Disulfiram and 4-MP had no effects at this parameter (data are not shown).

4. DISCUSSION

It is known that cytochrome P450 system is a powerful source of ROS generation [18,19]. The contribution of isoform CYP2E1 is particularly noticeable, due to its high oxidase activity and ability to produce ROS even in the absence of the substrate [20]. Among them a special role belongs to superoxide anion (O_2^-), a byproduct of the CYP2E1-mediated metabolism [21], which can serve as part of the second hit to advance the severity of NAFLD. It is seems well documented that CYP2E1 protein expression and activity increases in obesity, fatty liver, and NASH in both humans and rodents, and this increase appears to correlate well with the severity of NAFLD [22]. On the other hand, the NAFLD has been described as the hepatic manifestation of MS. Main risk factors associated with MS are abdominal obesity, insulin resistance, diabetes and dyslipidemia, but it is of interest, that NAFLD can be described in non-obese and non-diabetic patients [23].

In our experiments the induction of CYP2E1 protein expression in MS-group guinea pigs' liver was accompanied by increasing in its enzymatic activity in this organ. Such results are in coincidences with other authors' data indicating the increase in CYP2E1 mRNA and protein levels in both obese and diabetic humans [24,25].

It should be noted that not only CYP2E1-dependent O_2^- production [21,26] contributes to liver injury. It has been reported recently that CYP2E1-mediated oxidative stress causes M1 macrophages polarization bias, which includes a significant increase in interleukin-1*β* (IL-1*β*) and IL-12 in experimental models of NASH, whereas CYP2E1-null mice or diallyl sulfide administration prevented it [27]. Our results on increase of ROS generation in organisms of guinea pigs with MS are in good accordance with above mentioned reports and suggest about oxidative stress occurrence, which could be one of critical factors in the development of NAFLD. Indeed, in serum of experimental animals we registered indicators of metabolic disturbances and liver injury, such as increases in glucose and total cholesterol contents, elevations of AlAT, AsAT, and ALP activities. Significant decrease in glycogen content in hepatocytes of MS-group also is clear evidence of MS-related liver pathology. As it has been established by Samuel et al. [28], at NAFLD, hepatic fat accumulation alone is insufficient to increase endogenous glucose production, but that it does cause hepatic insulin resistance. This can be attributed in part to decreased insulin-stimulated tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1) and IRS-2, which in turn blocks the ability of insulin to activate glycogen synthase and diminishes the ability of the liver to store glucose as glycogen.

Our attention was attracted to the report on protection from a high-fat diet-induced insulin resistance in *Cyp2e1*$^{-/-}$ mice [29,30]. Conversely, mice knocked in for the human *CYP2E1* transgene had higher fasting insulin level, greater hepatic fat accumulation, high level of oxidant stress, and liver injury [31]. The ability of insulin to decrease CYP2E1 expression has been proved, as well as the fact that insulin resistance lead to increase of CYP2E1 expression and activity [32]. In this case, the mechanism of CYP2E1 induction can be realized via the high concentration of ketone bodies produced from persistent mitochondrial fatty acid oxidation. Ketone bodies stabilize CYP2E1 and prevent its degradation. The increase in CYP2E1 and enhanced insulin resistance seem to promote each other by creating a positive feedback loop that may eventually make steatosis progress to steatohepatitis as oxidant stress increases [33]. Recently Leung and Nieto [33] have suggested that inhibiting of CYP2E1 may disrupt this feedback loop and reduce insulin resistance and liver injury. Moreover, recent work has shown that CYP2E1 activity correlates with ethanol-induced liver injury and lipid peroxidation [34]. The use of CYP2E1 inhibitors,

such as chlormethiazole and polyenylphosphatidylcholine, demonstrate partial but effective protection in ethanol-induced liver injury [35,36].

In this regard it was of our interest to investigate the different CYP2E1 inhibitors as tools for correction of MS liver's manifestations.

We have used three inhibitors of CYP2E1 (disulfiram, 4-MP and quercetin) to determine link between this isoenzyme expression and level of liver injury. Above mentioned substances have rather different inhibitory mechanisms toward CYP2E1.

There is report that disulfiram is not directly responsible for inactivation of CYP2E1, but its reduced form, diethyldithiocarbamate, reacts with the enzyme leading to its inactivation [37]. Authors have postulated that this metabolite could inactivate CYP2E1 by forming a disulfide bond with one of eight cysteines that are present in the apoprotein [37].

The CYP2E1 inhibitor 4-MP competed with PNP for the CYP2E1 catalytic site as shown through catalytic, binding, and docking studies [38]. Direct evidence for the high affinity interaction between the catalytic site and 4-MP has been shown through the generation of the type II binding spectra between CYP2E1 and the inhibitor. When bind to the catalytic site, 4-MP mediates van der Waals contacts with the same residues as observed for PNP. An additional interaction with Leu-210 and formation of the Fe–N bond likely plays a role in the higher selectivity for 4-MP over PNP hydroxylation [38].

As it has been shown by other authors, inhibition of CYP2E1 by 4-MP significantly decreases oxidative stress [39]. The property of 4-MP to inhibit CYP2E1 and alcohol dehydrogenase is widely used to prevent poisoning with ethanol, methanol and ethylene glycol [39- 41].

Quercetin, one of the most common flavonoids presents in various vegetables, fruits, herbs and red wine, and possesses broad bioactivity which is based on or implicated in its prominent antioxidative properties [42]. Growing experimental data have demonstrated that quercetin exhibits ability to suppress CYP2E1 activity [43-46]. *In vitro* experiments have shown that CYP2E1 suppression is concomitant with heme oxygenase-1 (HO-1) induction by quercetin and protects hepatocytes from ethanol-induced oxidative damage [47,48]. Further research of the same authors has demonstrated that HO-1 induction and CYP2E1 down-regulation is accompanied with decreased heme pool [44]. Depleted heme pool and CO release may contribute to the protective mechanism of quercetin by limiting the protein synthesis and directly inactivating of heme-containing CYP2E1 [44].

Our investigation suggests that administration of all three substances in guinea pigs with MS causes decrease of hepatic CYP2E1 protein expression, as well as its enzymatic activity. Our results, nevertheless, point to significant variability in their inhibitory effects toward PNP hydroxylation activity, while protein expression is suppressed approximately at the same level. The most pronounced effect on CYP2E1 activity was revealed in case of quercetin administration. Conversely, 4-MP administration practically was not effective. Such data are in agreement with results of other authors demonstrating relatively small impact of 4-MP on CYP2E1 activity [49,50]. These authors have shown also the 4-MP ability to decrease the content of ROS in hepatocytes cultures treated with ethanol [49,50].

The regulation of CYP2E1 expression and activity is of importance since CYP2E1 plays a central role in MS-related liver injury. CYP2E1 is triggered by both exogenous and endogenous substrates, and it induces disturbances in hepatocytes by generating ROS and lipid peroxidation reactions. The decline of endogenous antioxidants in such conditions may further enhance CYP2E1-induced lipid peroxidation, oxidant stress and cellular toxicity [8]. As expected, in our experiments down-regulation of CYP2E1 activity in liver of animals with MS led to reduce of oxidative stress in their organism. The number of blood ROS were partially restored or normalised by quercetin, 4-MP, and disulfiram treatment.

It is widely accepted that under pathologies accompanying by CYP2E1 induction, its inhibition and decrease in ROS generation are beneficial for maintenance of structure and function of liver state [51]. Indeed, our results on clinical chemistry parameters and liver histology confirm this theory. Decrease in CYP2E1 protein content and activity, as well the level of ROS led to reduction of liver cells damage. At least, following quercetin administration the liver state almost returned to normal. It is possible that such remarkable hepatoprotective activity of quercetin

can be realized not only due to its inhibitory effect on CYP2E1, but also because of its well-known antioxidant properties [42]. This assumption is confirmed by the fact that following 4-MP and disulfiram administration we have observed only tendency to AlAT, AsAT and ALP decrease and no effects on liver glycogen content.

5. CONCLUSION

Collectively our results suggest that liver damage in animals with MS may be reversible. The link between increased hepatic expression of CYP2E1, prominent ROS generation and liver damage in animals with MS has become clear. In turn, suppression of CYP2E1 activity using specific inhibitors leads to decrease in hepatic MS manifestation. However, further studies are warranted to understand the most effective ways to regulate hepatotoxic and hepatoprotective pathways by CYP2E1 inhibitors. From the present observation is apparent that quercetin has the highest efficiency among the investigated substances but its most effective doses remain unclear. Further studies on various quercetin doses and administration regimens could provide relevant information for the development of MS-related NAFLD treatments.

ETHICAL APPROVAL

ACKNOWLEDGEMENTS

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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