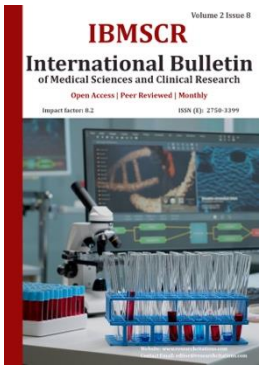

EFFECTIVENESS OF RUTAN IN THE CORRECTION OF XENOBIOTIC BIOTRANSFORMATION DISORDERS IN ACUTE TOXIC HEPATITIS IN PREPUBERTAL RATS

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Abstract. In prepubertal animals, the model of acute toxic hepatitis (ATH) was reproduced by intragastric administration of a 50% solution of carbon tetrachloride (CCL4) in olive oil at a dose of 2.0 ml/kg once daily for four days. Rutan was administered intragastrically at doses of 10.0, 25.0, and 50.0 mg/kg one hour prior to CCL4 administration on the first day and subsequent days, while the standard hepatoprotector Karsil was administered at 40 mg/kg. It was found that Rutan significantly prevents substantial disturbances in the biotransformation processes of xenobiotics during acute exposure to carbon tetrachloride in prepubertal rats. The shortening of chloral hydrate sleep duration under the influence of Rutan in prepubertal animals with acute toxic hepatitis indicates the stimulation of glucuronidation processes in hepatocytes. In terms of pharmacological activity in correcting liver detoxification dysfunction during acute toxic hepatitis in the growth period, Rutan is comparable to Karsil.

Keywords: Rutan, Karsil, acute toxic hepatitis, xenobiotic biotransformation, liver tests, prepubertal period.

Conflict of Interest

The authors declare no apparent or potential conflicts of interest related to the publication of this article.

Introduction

Improvement in the effectiveness of pharmacotherapy for the majority of human diseases is unthinkable without a detailed understanding of the pharmacodynamics (PD) and pharmacokinetics (PK) of medicinal products. As is known, the child's body, unlike an adult, has a number of peculiarities in the pharmacodynamics and pharmacokinetics of drugs [1-3]. The duration of action of many drugs depends on the functional state of the organs responsible for eliminating endogenous and exogenous biotics, particularly the liver and kidneys [4-6]. These organs are not fully matured in early childhood, and their damage can significantly affect the pharmacodynamics and pharmacokinetics of drugs [5,7]. It is known that many drugs are metabolized in the body by the monooxygenase system (MOS) enzymes, located in the endoplasmic reticulum of hepatocytes [8,9]. MOS plays a crucial role not only in the biotransformation of drugs but also in protecting the body from the harmful effects of various substances of endogenous and exogenous origin, ensuring chemical homeostasis [10]. Therefore, inhibition of MOS, particularly in children, is an unfavorable factor that plays a

negative pathogenic role in the course and outcome of many diseases, necessitating the search for effective ways and means to correct its dysfunction.

Viral hepatitis remains a serious issue worldwide, affecting millions of children, despite the availability of vaccines, preventive measures, and improved sanitary conditions [11-13]. Given the significant increase in the number of patients suffering from liver diseases of various etiologies (viral, toxic, drug-induced, and others), improving the effectiveness of pharmacotherapy for hepatobiliary diseases is a current issue in modern pediatric pharmacology.

Although the number of pharmacological agents used in the complex treatment of hepatopancreatobiliary diseases exceeds 1000 names, only a relatively small group of them have selective action [14, 15]. A comprehensive pharmacological study and the search for new affordable agents that restore hepatocyte function during pathological conditions of various etiologies in childhood were the basis for conducting this scientific research.

Objective of the Study The experimental study of the effectiveness of Rutan in the correction of xenobiotic biotransformation disorders in the liver during acute hepatitis in the prepubertal period.

Material and Methods. The studies were conducted on 80 two-month-old white rats, both sexes, with a body weight of 65-80 g, obtained from the vivarium of the Sanitary and Epidemiological Station of the Medical and Sanitary Union of the Ministry of Health of the Republic of Uzbekistan. The laboratory animals were kept under standard vivarium conditions with free access to food and water, natural light and dark cycles, and a room temperature of 20-24°C. The experiments were conducted in accordance with the “Rules for Conducting Experiments with the Use of Experimental Animals” and the regulations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123, Strasbourg, March 18, 1986). Each experimental group consisted of 8 animals. The acute carbon tetrachloride hepatitis (ATH) model was induced by intragastric administration of a 50% solution of carbon tetrachloride (CCL4) in olive oil at a dose of 2.0 ml/kg once a day for six days [16]. On the first day and subsequent days, Rutan was administered intragastrically at doses of 10.0, 25.0, and 50.0 mg/kg, and the reference hepatoprotector Karsil was administered at 40 mg/kg. Control animals received olive oil in the same volume. All the studied drugs were administered intragastrically using a metal olive-tipped probe once daily for six days. A day after the last procedure, the pharmacodynamics of ethyminal sodium and chloral hydrate were studied. These tests were conducted as follows: freshly prepared aqueous solutions of ethyminal sodium and chloral hydrate were administered intraperitoneally at doses of 30 and 150 mg/kg. The pharmacological activity of the test substances was judged by the duration of time the rats spent in the "lateral" position after administering the drugs, as well as by the absence of the "turn-over" reflex, and the results were expressed in minutes.

The described pharmacological method is simple, does not require specialized equipment and expensive reagents, which significantly reduces the time required for the study of drug biotransformation in the liver and allows for the investigation of liver function in vivo. It may find wide application in hygiene, pathophysiological, and toxicological research. The hepatoprotective properties of Rutan and Karsil under toxic liver damage conditions were assessed by determining various biochemical markers characterizing liver functions: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase

(ALP), and gamma-glutamyltransferase (GGT) levels in the serum. Biochemical studies were conducted by the photometric method using a semi-automatic biochemical analyzer Mindray (China, 2014) with reagent kits from Human (Germany) and Cypress Diagnostics (Belgium).

The obtained results were statistically processed using the Biostat 2009 software package. The data are presented as the mean value (M) and standard error of the mean (m). To check the statistical hypotheses of differences between the studied groups, the Student's t-test was used. Differences with a probability level of 95% and above ($p < 0.05$) were considered statistically significant. The indicators of the control (untreated) group were compared with the indicators of the intact (healthy) animals; the indicators of the group receiving Rutan and Karsil were compared with those of the control and intact animals.

Results and Discussion

In experimental pharmacology, in vivo methods are widely used to assess the state of xenobiotic biotransformation processes in the liver, particularly by utilizing test drugs whose metabolism occurs in hepatocytes involving enzymes localized in the endoplasmic reticulum [15, 16, 17-20].

In our research, the duration of the hypnotic action of ethyminal sodium and chloral hydrate was also used to assess the intensity of xenobiotic biotransformation processes in the liver. The results of experiments using this barbiturate in rats with acute toxic hepatitis (ATH) showed a significant increase in sleep duration compared to intact animals (more than 2.4 times). In contrast, in animals treated with the known hepatoprotector Karsil, the sleep duration was reduced by 50.0% compared to the control group, but it remained slightly extended by 18.0% compared to healthy rats. A similar effect was observed in animals that received Rutan preventively. As seen from the data in Table 1, the best effect was observed when using this compound at a dose of 50 mg/kg. Notably, under the influence of this dose of Rutan, the reduction in sleep duration was statistically indistinguishable from that of healthy rats.

Therefore, Rutan eliminates the biochemical disturbances responsible for the biotransformation of ethyminal sodium in acute toxic hepatitis. As noted, the metabolism of ethyminal sodium occurs in hepatocytes with the participation of enzymes from the monooxygenase system, localized in the endoplasmic reticulum. It is logical to assume that Rutan restores the functional activity of the monooxygenase enzyme system in hepatocytes. In the same hepatocytes, the second phase of xenobiotic biotransformation processes — conjugation — also takes place. It was noted that pharmacotherapy with Rutan in sexually mature rats with acute toxic hepatitis increases the excretion of bilirubin in bile [21, 22].

Table 1

Comparative study of the effects of Rutan and Karsil on the pharmacodynamics of ethyminal sodium and chloral hydrate in rats with carbon tetrachloride hepatitis during the prepubertal period (M±m, n=8).

Indicators / Groups	Prodolzhitelnost sna(minute)		
	Dose, mg/kg	sodium ethamin	chloral hydrate
Healthy	-	98,83 ± 4,54	119,17 ± 11,67
Control	-	233,31 ± 9,09	259,33 ± 19,80

P		< 0,001	< 0,002
Rutan + CCL4P P_1	10	190,16 ± 10,13 < 0,001 < 0,05	203,01 ± 16,88 <0,01 > 0,05
Rutan + CCL_4 P P_1	25	131,50 ± 8,14 <0,02 < 0,001	170,83 ± 13,94 <0,05 < 0,02
Rutan + CCL_4 P P_1	50	123,34 ± 11,17 > 0,05 < 0,001	158,67 ± 12,48 > 0,05 < 0,01
Carsil + CCL_4 P P_1	40	116,66 ± 10,48 > 0,05 < 0,001	144,33 ± 21,41 > 0,05 < 0,05

Note: *P* — significance of data relative to intact animals; *P1* — significance of data for control animals compared to control groups.

Since bilirubin is exclusively excreted in its conjugated form with glucuronic acid [23, 24], it can be assumed that Rutan stimulates the activity of the enzyme uridine diphosphate glucuronosyltransferase (UDP-glucuronosyltransferase) during acute toxic hepatitis (ATH).

To test this assumption, a separate series of experiments was conducted to study the duration of chloral hydrate-induced sleep, as the pharmacological effect of this drug mainly depends on the intensity of glucuronidation processes [18].

The results of this series of experiments showed that in rats with ATH, there was a significant increase (by 117.6%) in the duration of chloral hydrate-induced sleep compared to healthy animals. After preventive administration of Karsil, the sleep duration was reduced by 44.3% compared to the control group. Similar changes were observed in animals that received Rutan preventively. Notably, in terms of pharmacological activity, Rutan did not differ from the standard hepatoprotector — Karsil.

It is known that the liver in children plays a vital role in metabolism, detoxifying, and eliminating toxic products [25, 26]. Liver damage due to various diseases may not directly affect its functional activity, as the liver has significant functional reserves. Consequently, assessing liver function by a single indicator is not always possible. Traditionally, liver function is assessed using a set of laboratory indicators, which include studies of total protein, albumin, bilirubin levels, and the activity of ALT, AST, GGT, and ALP. Despite their differences in structure and function, these indicators should be considered together, as they are all used to establish the degree and type of liver damage. Each of these indicators primarily reflects a disruption in one of the liver's functions, but together, these tests provide a comprehensive view of the liver's overall condition, which is why they are traditionally referred to as liver function tests. Considering this information, we studied several liver function test indicators.

The results of this series of experiments demonstrated a clear hepatoprotective effect of the tested drugs in animals during the prepubertal period. In rats with acute hepatitis, there was an increase in the activity of ALT by 245.4%, AST by 53.7%, ALP by 109.6%, and GGT by 117.4%, respectively, compared to healthy animals (Table 2).

Therefore, acute toxic hepatitis induced by carbon tetrachloride in the prepubertal period is significantly associated with the development of cytolytic and cholestatic syndromes.

As mentioned, Rutan had a distinct positive effect in this model of hepatitis in rats, and this effect largely depended on the dose of the drug. For instance, at a dose of 10 mg/kg, the activity of ALT decreased by 25.6%, whereas at a dose of 50 mg/kg, it decreased by 49.2%, and at 100 mg/kg, by 54.8%. In comparison with the control group, the reduction in AST activity at the specified doses of the drug was 25.0%, 28.2%, and 41.1%, respectively. On this background, the reduction in ALP and GGT activities was 9.2% and 13.3%, 38.3% and 40.0%, and 44.5% and 47.0%, respectively. It is evident that Rutan has the best therapeutic effect when administered at a dose of 50 mg/kg. Notably, the therapeutic effect of the drug at this dose is not significantly different from the pharmacotherapeutic effect of Karsil. It should be noted that under the influence of Karsil, ALT activity decreased by 58.7%, AST by 37.7%, ALP by 48.3%, and GGT by 48.9% compared to the control group values.

Table 2

Study of the influence of different prophylactic doses of Rutan and Karsil on some biochemical blood indicators in carbon tetrachloride-induced hepatitis during the prepubertal period (M±m, n=8).

Indicator / Groupss	Dose, mg/kg	ALT, IU/L	AsAT, U/l	FFA, U/l	GGT, IU/L
Intact		61,18 ± 3,31	89,96 ± 3,82	278,26 ± 15,21	2,98 ± 0,31
ControlP	-	211,35 ± 14,09 < 0,001	138,26 ± 10,38 < 0,05	583,23 ± 35,69 < 0,001	6,48 ± 0,37 < 0,001
Rutan +OTG P P_1	10	157,28 ± 12,45 < 0,002 < 0,05	103,62 ± 7,02 > 0,05 > 0,05	529,63 ± 38,25 < 0,002 > 0,05	5,62 ± 0,41 < 0,002 > 0,05
Rutan +OTG P P_1	25	107,45 ± 9,11 <0,05 < 0,001	99,23 ± 6,41 > 0,05 < 0,05	359,56 ± 21,47 > 0,05 < 0,002	3,89 ± 0,32 > 0,05 < 0,002
Rutan +OTG P P_1	50	95,52 ± 9,08 <0,05 < 0,002	81,37 ± 7,23 > 0,05 < 0,05	323,72 ± 27,9 > 0,05 < 0,01	3,43 ± 0,29 > 0,05 < 0,002
Karsil+OTG P P_1	40	87,17 ± 9,24 <0,05 < 0,01	86,01 ± 4,78 > 0,05 < 0,05	301,37 ± 28,56 > 0,05 < 0,01	3,31 ± 0,32 > 0,05 < 0,002

Note: *P* — differences statistically significantly different from intact indicators; *P1* — differences statistically significantly different from the control group.

Based on the analysis of the results of the experimental studies presented above, it can be concluded that Rutan has a distinct hepatoprotective effect in animals during the prepubertal period, which is manifested in the clear elimination of cytolytic and cholestatic syndromes. Notably, the drug is as effective as Karsil.

Thus, Rutan clearly protects the liver cells' functional state during the growing period in animals, particularly the monooxygenase enzyme system, which is reflected in the enhancement of biotransformation and conjugation of xenobiotics. Given that the main criterion for the severity of hepatitis is the toxemia syndrome, which is related to detoxification processes in the liver, it can be asserted that the use of Rutan as a hepatoprotector, like Karsil, will improve the effectiveness of therapeutic measures for children with acute hepatitis and reduce complications.

Conclusions:

1. Rutan clearly prevents significant disruptions in the biotransformation processes of xenobiotics in animals during the prepubertal period after acute exposure to carbon tetrachloride.
2. The shortening of chloral hydrate-induced sleep under the influence of Rutan in prepubertal animals with acute toxic hepatitis indicates its stimulation of glucuronidation processes in hepatocytes.

In terms of its pharmacological activity in correcting the detoxifying function of the liver in acute toxic hepatitis during the growing period, Rutan is comparable to the classical hepatoprotector, Karsil.

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