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EFFECT OF REDUCED GLUTATHIONE (GSH) ON PHARMACOKINETICS AND DISTRIBUTION OF RIFAMYCIN SV IN RATS

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Abstract—Changes of the pharmacokinetic parameters of rifamycin SV in the blood and changes of the antibiotic concentration in rat organs after its coadministration with glutathione in reduced form (GSH), in oxidized form (GSSG), and with the individual amino acids entering the tripedtide were studied. The use of reduced glutathione together with rifamycin was found to accelerate the distribution phase in the kinetics of the antibiotic in the blood by acceleration of transport of the drug from central compartment (blood) to tissue compartment (tissues), this being reflected by increased K_{12} and K_{12}/K_{21} values. Studies of the distribution of rifamycin in individual rat organs have shown GSH to significantly elevate the concentration of the antibiotic in the lungs with concomitant reduction of its level in the liver. The blocking of free sulfhydryl groups of glutathione by its use in oxidized form completely abolished this effect. The administration of rifamycin together with L-cysteine resulted in increased concentration of the antibiotic in both lungs and liver. The two other amino acids entering GSH — glycine and L-glutamic acid — did not cause any changes of rifamycin concentration in the organs. From these results it may be concluded that the mechanism of the observed effect of glutathione on rifamycin concentration differs in the lungs and liver though both effects are mediated by the free sulfhydryl groups of glutathione.

The foremost limitation of pharmacotherapy is the toxic effect of a drug on the one hand and its side effects on the other. These result from the fact that most drugs introduced into the organism penetrate similarly to both the sick (target) organ and other tissues and organs, often resulting in impaired functioning of the latter. In view of the above, studies are being made in order to find an efficient way of controlling the distribution of a drug within the organism with its accumulation mainly in the target organ. Three trends in these studies can be currently distinguished: (1) modification of the chemical structure of a drug increasing its affinity to a particular tissue [1, 2]; (2) use of liposomes which transport enclosed drugs to their destination [3–6]; and (3) administration of a drug together with compounds activating its transport to particular tissues. The studies of Danysz and Wiśniewski [7–10] have shown that such properties are demonstrated by certain sulfhydryl peptide hormones, e.g. insulin, hormones of the anterior and posterior lobes of the hypophysis and certain other natural polypeptides such as bradykinin and angiotensin. However, the possibility of the use of these polypeptides for combined therapy, particularly in chemotherapy, is very problematic due to their strong effect on basic body processes which may result in various side effects and interfere with the interpretation of the results. It thus seemed that low molecular weight oligopeptides with low toxicity and relatively low pharmacological activity could be better suited for control of the distribution of drugs within the body. The compound of our choice was reduced glutathione (GSH) due to its properties of activating many transport processes in the body [11–15], and because of known examples of its interaction with various drugs which resulted in changes in certain phases of the kinetics of the drug (acetazolamide) in the organism [16], or in its reduced toxic effect of drug, e.g. bleomycin [17, 18]. In our earlier studies [19] we demonstrated that the use of GSH together with rifamycin SV in rabbits causes changes in the kinetics of the antibiotic in the blood which indicate an acceleration of the transport of the drug from the blood to the tissue compartment. It seemed interesting, therefore, to find whether the observed effect in rabbits of GSH on the kinetics of rifamycin would be confirmed to a similar extent in a different species of animals (rats). In the event of positive results, we decided to see whether glutathione altered the distribution of rifamycin in the different rat organs, and to investigate the structure of active groups of glutathione to which the effect could be attributed.

MATERIALS AND METHODS

Five hundred male Wistar rats weighing 190–250 g were used. Two types of studies were made:

(1) *Effect of reduced glutathione (GSH) on rifamycin SV pharmacokinetics in blood serum.* Rifamycin SV (TZF, POLFA, Poland) was injected intravenously in dose 10 mg/kg alone (control group) or together with reduced glutathione (A.G. Fluka, Switzerland) in doses of 2, 4, or 8 mg/kg. Blood samples for the determination of rifamycin concentration were taken from a pulsating heart in shallow

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atherization 2, 5, 10, 15, 20, 30, and 45 min after injection of rifamycin. Each group studied at a particular time consisted of 10 animals.

(2) *Effect of glutathione and its components on rifamycin distribution in rat organs.* Rifamycin SV was injected intravenously in doses of 5 or 10 mg/kg alone (control groups), and together with one of the following preparations: reduced glutathione in dose 8 mg/kg; oxidized glutathione (Reanal, Hungary) in dose 8 mg/kg; L-cysteine (A.G. FLuka, Switzerland) in dose 3 mg/kg; glycine (S.F.Ch., Poland) in dose 2 mg/kg, and L-glutamic acid (B. Braun, West Germany) in dose 3.5 mg/kg. Organs for determination of antibiotic concentration (lungs, liver, kidneys, section of triceps muscle of the thigh) were removed 2, 5, 10, 15, and 30 min after the injection of rifamycin. Each group consisted of 6 animals.

The organs or fragments thereof were completely freed of fat, washed in saline solution to free them of any blood or other organic fluids, and homogenized in phosphate buffer (pH 7.38). The homogenized mass was centrifuged and the supernatant layer used for microbiological assay.

The concentration of rifamycin in blood serum and in organs was determined microbiologically by the Petri dish method using Difco Penassey seed agar (pH 6.6 after sterilization) as a culture medium, and *Sarcina lutea* ATCC 9341 as the test microorganism [20, 21]. According to this method, the concentration of rifamycin in the serum and organs of treated animals was calculated by comparing the figure obtained with those referring to solutions in normal animal serum and organs of known antibiotic content (standard curve method). To make sure that compounds administered together with rifamycin do not influence the microbiological assay of rifamycin, the standard curve was compared to this, referring to antibiotic solutions in serum and organs of animals which had been previously treated with each of the used preparations alone, in the same dose that was administered together with rifamycin (no influence of the used preparations on the microbiological assay was found).

The rifamycin pharmacokinetics were evaluated based on the course of the kinetics in the blood serum according to Wagner [22] with the use of two-compartment open model for rapid intravenous injection. For such a model, the serum concentration at any time can be expressed by the following equation:

$$C_P = Ae^{-\alpha t} + Be^{-\beta t},$$

when A and α are coefficients representing the distribution phase; B and β are coefficients representing the elimination phase.

All pharmacokinetic parameters of rifamycin were calculated by mini computer (Wang, USA) using the adequate pharmacokinetic computer program in BASIC language.

The results of the studies on the distribution of rifamycin in organs were statistically evaluated with the use of Student's t-test for the difference between means from two compared groups (rifamycin alone and with each of the listed preparations).

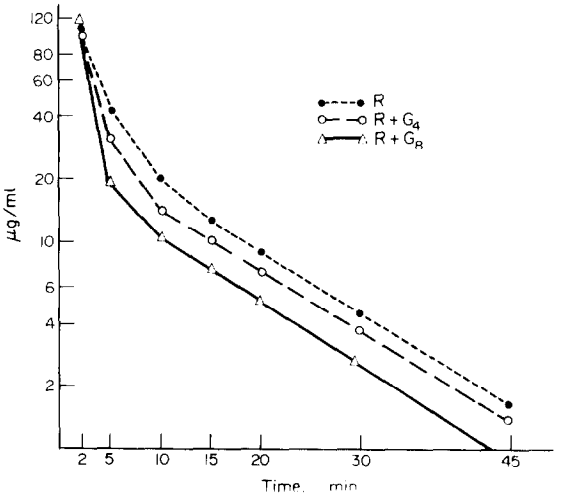

Fig. 1. The rifamycin concentration in the blood serum after administration of 10 mg/kg alone (R) and together with GSH in the doses: 4 mg/kg (R + G_4) and 8 mg/kg (R + G_8). (Semilogarithmic scale.)

RESULTS

The kinetic of rifamycin SV in rat blood corresponds to two-compartment open model with distribution and elimination phase (Fig. 1), and is defined by the following equation:

$$C_P = 173.15\, e^{-27.10\, t} + 33.71\, e^{-4.07\, t}$$

The distribution phase of rifamycin is very short, and thus the elimination phase is reached very soon.

Effect of GSH on rifamycin SV pharmacokinetics. The coadministration of rifamycin and GSH was found to decrease the antibiotic level in serum (especially during the distribution phase), depending on the dose of glutathione (Fig. 1). The analysis of the pharmacokinetic parameters of rifamycin, presented in Table 1, shows that GSH increases the apparent first-order rate constant of distribution processes (α) by 25.9 and 87.4 per cent for GSH doses of 4 and 8 mg/kg, respectively. At the same time the drug elimination rate, reflected by: first-order rate constant of drug elimination from the organism (β), half life (T_{50}) and plasmatic clearance (Cl. plas.) is not significantly altered. Acceleration of the distribution phase of the antibiotic is limited solely to the processes of its movement from the blood to tissues, as indicated by two and two and a half fold increases of first-order rate constant of transport of drug from blood to tissue compartment (K_{12}) after glutathione doses of 4 and 8 mg/kg, respectively (Fig. 2), with no concomitant change in rate constant of drug transport from tissue compartment to blood (K_{21})—Table 1. Ratio K_{12}/K_{21}, which reflects the degree of penetration of rifamycin to the tissues, thus increases to the same extent as the value of constant K_{12} (Fig. 2). This increase depended on GSH dose over the dose range studied.

Effect of glutathione on rifamycin distribution in rat organs. GSH strongly affected the concentration of rifamycin in the rat organs. The concentration of

Table 1. Pharmacokinetic parameters of rifamycin SV after i.v. administration of 10 mg/kg alone and together with glutathione (reduced GSH)

Pharmacokinetic parameters	Rifamycin alone	Rifamycin + GSH doses (mg/kg) 2	4	8
α (hr^{-1})	27.10	24.04	34.12	50.69
K_{12} (hr^{-1})	7.25	6.35	13.62	16.04
K_{21} (hr^{-1})	7.83	6.66	6.99	6.82
K_{12}/K_{21}	0.92	0.95	1.95	2.36
β (hr^{-1})	4.07	4.23	3.48	5.10
T_{50} (hr)	0.17	0.16	0.20	0.14
Cl. plas. (ml/hr)	156.60	162.12	141.88	191.45

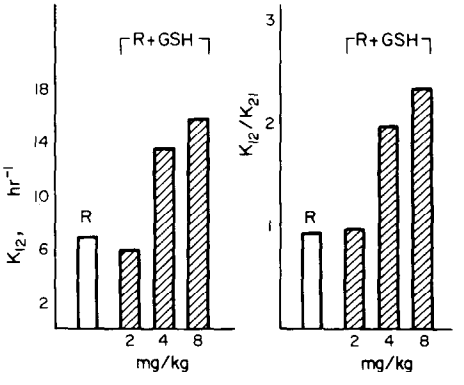

Fig. 2. Changes of rate constant of rifamycin transport from blood to tissue compartment (K_{12}) and of degree of rifamycin penetration to tissues (K_{12}/K_{21}) caused by GSH. Key: R = rifamycin administered alone; R + GSH = rifamycin administered together with GSH.

the antibiotic in the lungs strongly increased but in the liver and skeletal muscle showed a sharp drop. This phenomenon occurred after the use of GSH together with both tested rifamycin doses, i.e. 5 and 10 mg/kg (Figs. 3 and 4) although the effect of glutathione was stronger with lower rifamycin content, particularly in the liver. In the kidneys a small, though statistically significant, increase in rifamycin concentration in the initial period after coadministration of GSH and higher rifamycin dose was observed (Fig. 3). No such changes were observed when the lower dose of rifamycin was employed (Fig. 4).

The use of oxidized form of glutathione (GSSG) did not cause any changes in rifamycin concentration in the examined organs, irrespective of the dose (Figs. 3 and 4).

Cysteine in dose 3 mg/kg, which corresponds to its content in the employed GSH dose, caused a statistically significant increase of rifamycin content in the lungs, kidneys, and liver, similar in each of these organs. However, the concentration of the antibiotic in the skeletal muscle showed a similar decrease in the presence of either cysteine or GSH (Fig. 5).

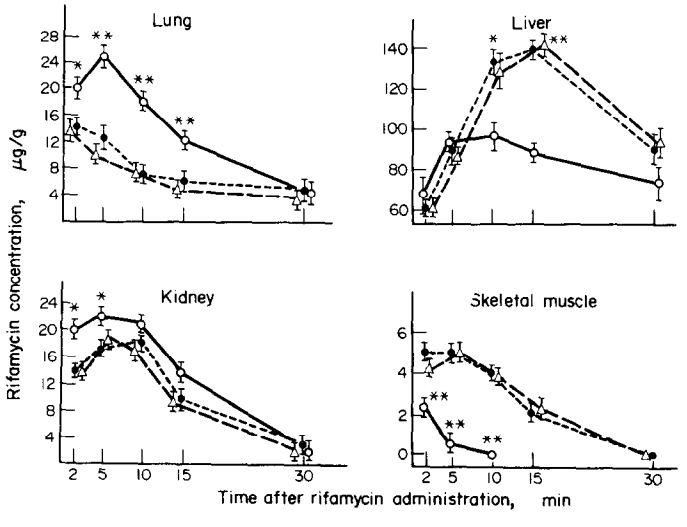

Fig. 3. Rifamycin level in rat organs after administration of 10 mg/kg alone (single administration—●) and together with glutathione in reduced (○) and oxidized (△) form. Key: *P = 0.02 – 0.005; **P < 0.005.

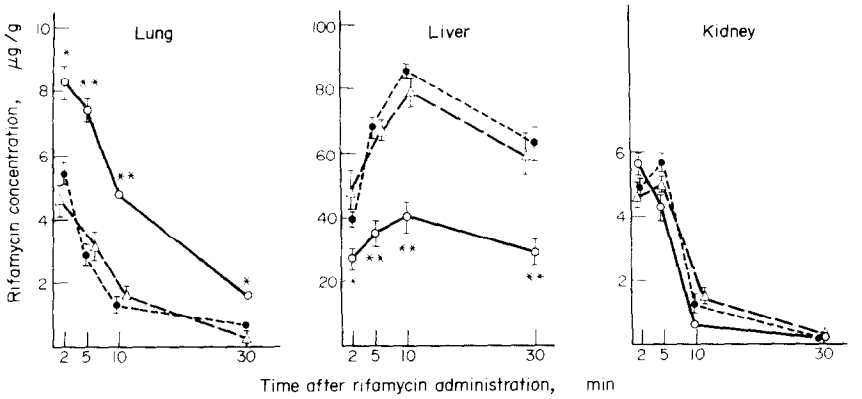

Fig. 4. Rifamycin level in rat organs after administration of 5 mg/kg alone and together with GSH and GSSG. Determination as in Fig. 3.

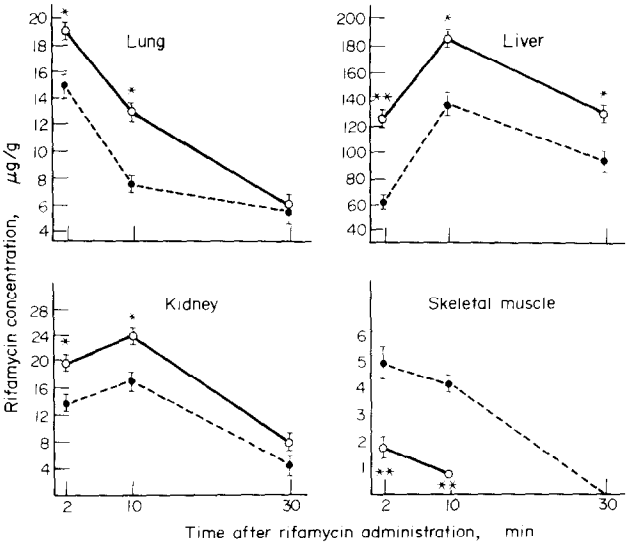

Fig. 5. Changes of rifamycin level in rat organs caused by cysteine. Key: ● rifamycin; ○ rifamycin + cysteine. *$P < 0.05$; **$P < 0.005$.

Table 2. Rifamycin concentration (in μg/g) in rats organs after administration of 10 mg/kg alone and together with glycine and L-glutamic acid

Organs and time after rifamycin administration	Rifamycin alone	Rifamycin + glycine	Rifamycin + L-glutamic acid
Lungs			
2 min	14.78 ± 0.88	13.95 ± 0.92	14.24 ± 0.72
10 min	7.60 ± 0.61	7.80 ± 0.48	7.00 ± 0.65
30 min	5.56 ± 0.95	5.74 ± 0.60	4.98 ± 0.85
Liver			
2 min	62.24 ± 3.85	70.00 ± 8.15	65.20 ± 5.10
10 min	136.50 ± 7.50	128.40 ± 10.20	130.50 ± 6.20
30 min	92.75 ± 7.25	88.25 ± 5.12	86.85 ± 7.50
Kidneys			
2 min	14.50 ± 0.75	14.00 ± 0.68	13.85 ± 0.78
10 min	17.55 ± 0.82	16.98 ± 0.95	17.00 ± 0.89
30 min	4.25 ± 0.92	4.80 ± 0.65	5.00 ± 0.56
Skeletal muscle			
2 min	5.02 ± 0.60	5.85 ± 0.85	4.80 ± 0.35
10 min	4.20 ± 0.30	4.00 ± 0.23	3.85 ± 0.48
30 min	0.00	0.00	0.00

The administration of rifamycin together with glycine or L-glutamic acid in an amount corresponding to the content of the amino acids in employed GSH dose (2 mg/kg glycine and 3.5 mg/kg L-glutamic acid) did not result in any changes of the distribution of the antibiotic in the rat organs (Table 2).

DISCUSSION

The results of pharmacokinetic studies point to the strong activation by GSH of processes of the penetration of the antibiotic from the blood to tissues and are compatible with similar results obtained earlier by us in rabbits [19]. It thus seems that the observed effect of glutathione is independent of animal species.

Studies of changes of the concentration of rifamycin in rat organs effected by GSH dose causing strongest acceleration of penetration of the drug to the tissue compartment (in pharmacokinetic studies) demonstrated a visible increase of the antibiotic concentration in the lungs. However, no such effect of GSH was observed in the other organs examined. On the contrary, the concentration of rifamycin in the liver and skeletal muscle strongly decreased. Consequently, GSH can be assumed to increase or reduce the concentration of rifamycin, depending on the type of tissue.

The blocking of free sulfhydryl groups of glutathione through its used in oxidized form completely abolished this effect, which points to the role of free SH groups in the activity of the component. Hence the assumption that a similar effect might be caused by cysteine alone which is the source of SH groups in glutathione. However, the coadministration of cysteine and rifamycin did not confirm this hypothesis since an increased concentration of the antibiotic observed in all organs except for the skeletal muscle. Thus the characteristic tissue specificity of GSH, particularly towards the liver, was not observed. On the other hand, the results of studies on the rifamycin content in the organs after its administration together with glycine and L-glutamic acid preclude the participation of other components of glutathione, besides the sulfhydryl group, in its activity.

We can therefore conclude that the mechanism of the action of GSH on rifamycin differs in the lungs and liver, although in both cases free sulfhydryl groups of glutathione play an essential role. The effect of GSH on rifamycin in the lungs can be compared with the effect of cysteine on rifamycin in the lungs, kidneys, and liver (increased rifamycin level in these organs), and indicates that these compounds activate the transport of the antibiotic from the blood to those organs. This observation is compatible with the results of pharmacokinetic studies. This is probably connected with increased permeability of cell membranes to rifamycin, which effect is determined by the sulfhydryl group [10, 23, 24].

The question arises why GSH does not bring about enhanced penetration of rifamycin in the liver and causes but a very poor effect in the kidneys. It seems that a possible explanation for the obtained results is that the determined level of rifamycin in the liver (and also kidneys) does not reflect the full amount of the antibiotic penetrating to these organs, due to GSH. The method for determination of rifamycin content used in the present study allows detection of only biologically very active rifamycin. Thus if the antibiotic is somehow deactivated in certain organs, its level, determined by microbiological methods, will always be reduced.

In the liver, and to a lesser extent in the kidneys, the deactivation of rifamycin by GSH may occur through a specific interaction in which the sulfhydryl group of GSH participates, with the help of glutathione-dependent enzymes [25–27]. The presence of these enzymes has been detected mainly in the liver, and the lower concentration in the kidneys; they have not, however, been detected in lungs.

The possibility of the deactivation of the drug by exogenous GSH is reinforced by the results of the studies of Kaneda and Ichikawa [17, 18], who showed that the use of exogenous glutathione together with bleomycin results in the interaction of the antibiotic and GSH with subsequent loss of biological activity by the former.

However, irrespective of the mechanism responsible for the effect of GSH on rifamycin, demonstrated in this study, the consequences of its activity may be of practical use. On the one hand, one can expect the increased therapeutic effect of the antibiotic due to its increased concentration in the lungs, which are the target organs in most of the rifamycin-treated infections. On the other hand, the reduced concentration of rifamycin in the liver brought about by GSH may result in lower hepatotoxic effect of rifamycin. It has been reported [28, 29] that the hepatotoxic effect of antibiotics of the rifamycin group is connected with inhibition of protein synthesis in the hepatocytes. This inhibition is therefore a certain type of side-effect of the antibacterial properties of rifamycins, whose loss also results in abolished hepatotoxic effect. Since the normal metabolism of rifamycins in the liver does not result in loss of their activity, the possibility of the selective deactivation of the antibiotic in the liver by GSH creates a chance for the considerable reduction of one of the major toxic effects of the rifamycins.

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