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Il Farmaco 56 (2001) 175–180

IL FARMACO

Glutathione uptake after intraperitoneal administration and glutathione radiopharmacology after rectal administration, in mice

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Received 12 June 2000; accepted 30 November 2000

Abstract

Glutathione is a biologic aminothiol radioprotector. Hydrolysis of exogenous glutathione takes place in the extracellular compartment and leads to two metabolites: γ-glutamylcysteine and glycine. In healthy mice, after an intraperitoneal administration of glutathione, all organs absorb the γ-glutamylcysteine and the glycine with variable kinetics according to their enzymatic equipment. The rectal administration of glutathione in mice previously irradiated at the pelvic region, increases the availability of glutathione in the rectum and in other organs at a distant from the irradiation site. This contribution could be used to protect the rectum and the uterus during therapeutic irradiation.

Keywords: Glutathione; Radioprotector; Plasmatic hydrolysis

1. Introduction

Only a small number of biogenic compounds demonstrates radioprotective activities. These are essentially polyamines and aminothiols or their derivatives. Among the latter, glutathione (L-γ-glutamyl-L-cysteinyl-glycine) is the most abundant in mammalian cells. This radioprotector exists in all tissues [1]. It protects the cells by reducing free radicals or peroxyded lipids, by preserving the protein–thiol groups as well as repairing radio-induced damage [2]. Exogenous glutathione exhibits a very weak acute toxicity; its administration could be a method to reinforce the radioprotective metabolism. Indeed, during the irradiation of a tumour, all the tissues essentially situated in the cone of irradiation are submitted to the formation of free radicals. Thus, the irradiation of a uterine tumour can affect the rectal region. The aim of this work is to verify if the rectal region can be locally protected by the preventive administration of glutathione-containing suppositories. The diffusion of glutathione labelled with ^{35}S and glutathione marked with a tritium atom on the glycine part (both administered intraperitoneally), were initially compared in healthy mice. Secondly, a survey of the diffusion of ^{35}S glutathione administered rectally was undertaken to determine its distribution in mice having a healthy rectum and in mice with an irradiated rectal mucous membrane.

2. Materials and methods

2.1. Animals

Animals were pure race female mice of the lineage 10 PS C57 B2S/6 JICOS, aged 10 weeks and weighing about 20 g.

2.2. Chemical compounds

Both ^{35}S glutathione and ^{3}H glutathione present a radiochemical purity of 99%. Labelling with ^{35}S allows us to follow the thiol part of the molecule, which is the essential component of the radioprotection. The ^{3}H of the methylene grouping of glycine allows us to follow the terminal part of the molecule.

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0014-827X/01/$ - see front matter
PII: S0014-827X(01)01073-4

2.3. Administration of the suppositories

Animals fasting for 48 h received a rectal injection of a 0.30 ml preparation maintained at 37°C. The mixture, homogenised by permanent agitation, contains 0.02 mM of cold glutathione (about 6 mg), 7.4 MBq of ^{35}S glutathione, 0.06 ml of glycerin and 0.24 ml of wax. During anaesthesia with ether, the administration was achieved using an adequate diameter polyethylene catheter. In order to avoid a possible leakage, the anus was closed up by depositing some drops of paraffin at 37°C, that quickly solidified on contact with a test tube full of crushed ice.

2.4. Administration of ^{35}S glutathione and ^{3}H glutathione

Animals fasting for 24 h received an intraperitoneal injection of a physiological serum solution containing cold glutathione at a concentration of 1 mM/kg and into which the marked glutathione was added. Each animal received 0.30 ml of solution having an activity of 0.12 MBq.

2.5. Irradiation of the animals

The pelvis of each animal was irradiated 72 h before the administration of the ^{35}S glutathione suppository. Fifteen minutes before irradiation, the mice received an intraperitoneal injection of anaesthetic (thiopental) at a dose of 40 mg/kg. During the narcosis, the small intestine was driven back up the abdomen by massage. It was maintained free of the rectum by means of an elastic ribbon placed around the body of the animal at the iliac crest level, according to the protocol of Ito et al. [3]. The animals were then spread on the back, disposed in circle; their members and their tail were fixed on a plate of altuglas with adhesive ribbons. Eight mice were simultaneously irradiated. A unique γ-irradiation of 12 Gy with a debit of 1.86 Gy/min was delivered by a ^{60}Co irradiator. A 5 cm thick plate of lead bored with eight holes, 20 mm in diameter disposed in circle constitutes the collimator. It is placed 15 cm above the animals so that each field of irradiation only covers the pelvic region of each mouse.

2.6. Measures of the radioactivity of the tissues

Animals were sacrificed by section of the carotid arteries 15 min, 1, 2 or 4 h after the administration of glutathione. Blood was collected in heparined test tubes. The heart, the kidneys, the liver and the uterus were removed at once; lastly, the rectum was excised to avoid radioactive contamination. The latter was carefully emptied and cleaned with physiological serum in order to eliminate the non-absorbed content. The radioactivity of each organ was measured on fractions of 50–100 mg after congealment in liquid nitrogen and grinding in a mortar until homogeneity. Each sample was degraded by the addition of a 1 ml mixture of toluene–propanol (1:1), then discoloured by the addition of 0.6 ml of oxygenated water at 110 volumes. After about 6 h, 0.6 ml of 1 N HCl was added to re-establish a neutral pH and 10 ml of scintillating mixture to determine the radioactivity in a scintillation counter.

3. Results

3.1. Diffusion of ^{35}S glutathione and ^{3}H glutathione (intraperitoneal injection) in healthy mice

The diffusion of the glutathione tritiated at the CH_2 group of glycine is compared to the diffusion of the glutathione marked with ^{35}S on the cysteine (Tables 1 and 2).

With exception (see Section 4), glutathione does not directly penetrate cells. Once administered, glutathione is first hydrolysed to γ-glutamylcysteine and glycine in the plasma. It is then synthesised in the intracellular compartment when its metabolites are captured [4]. At different time intervals, the blood rates for the ^{3}H glycine increase whereas those of the ^{35}S γ-glutamylcysteine decrease. This observation shows the efficient rupture of the molecule of glutathione at the level of the peptide bond between the cysteine and the glycine. This is particularly evident 2 h after administration, because under our conditions, at the time intervals of 15 min and 1 h, the blood rates were not statistically different between the two markings. In the heart, the

Table 1
Administration of ^{35}S glutathione intraperitoneally in healthy mice. Percent of radioactivity for each tissue relative to the administered radioactivity

Time (min)	Blood	Heart	Liver	Kidney	Uterus	Rectum
15	4.61 ± 1.50	2.27 ± 0.94	7.00 ± 2.36	14.29 ± 4.03	10.06 ± 3.68	5.87 ± 4.00
60	2.50 ± 0.32	1.68 ± 0.32	15.54 ± 1.57	19.61 ± 1.00	4.20 ± 1.61	4.15 ± 0.53
120	1.75 ± 0.09	1.61 ± 0.16	12.77 ± 0.40	13.42 ± 0.75	3.23 ± 0.41	3.91 ± 0.42
240	1.59 ± 0.24	1.46 ± 0.28	11.79 ± 1.51	9.09 ± 1.35	4.03 ± 0.44	3.76 ± 0.12

Table 2
Administration of ^{3}H glutathione intraperitoneally in healthy mice. Percent of radioactivity for each tissue relative to the administered radioactivity

Time (min)	Blood	Heart	Liver	Kidney	Uterus	Rectum
15	3.74 ± 0.72	1.90 ± 0.18	6.08 ± 1.61	11.47 ± 0.49	7.62 ± 2.44	19.63 ± 4.33 *
60	3.18 ± 0.41	0.71 ± 0.05 *	4.17 ± 0.33 *	7.21 ± 1.10 *	5.11 ± 0.85	31.53 ± 11.3 *
120	3.68 ± 0.62 *	0.99 ± 0.02 *	4.91 ± 0.87 *	9.65 ± 3.12	5.31 ± 1.04 *	26.13 ± 6.12 *
240	4.24 ± 0.73 *	0.68 ± 0.17 *	4.63 ± 0.72 *	7.94 ± 0.58	4.79 ± 0.53	23.30 ± 18.26

* Significant statistical difference $p < 5\%$ with respect to the administration of ^{35}S glutathione.

Table 3
Rectal administration of glutathione in non-irradiated mice. Percent of radioactivity for each tissue relative to the administered radioactivity

Time (min)	Blood	Heart	Liver	Kidney	Uterus	Rectum
15	0.99 ± 0.42	0.75 ± 0.40	2.03 ± 0.21	3.47 ± 2.06	0.92 ± 0.48	4.84 ± 3.51
60	2.20 ± 2.53	1.07 ± 1.04	14.97 ± 5.88	8.64 ± 9.10	2.08 ± 2.28	2.76 ± 2.73
120	0.91 ± 0.72	0.68 ± 0.52	14.83 ± 4.84	9.43 ± 1.21	1.39 ± 1.21	6.31 ± 1.34
240	3.12 ± 0.75	1.79 ± 0.44	4.49 ± 2.84	5.59 ± 1.21	3.40 ± 1.21	3.30 ± 3.33

Table 4
Rectal administration of glutathione in irradiated mice. Percent of radioactivity for each tissue relative to the administered radioactivity

Time (min)	Blood	Heart	Liver	Kidney	Uterus	Rectum
15	0.57 ± 0.15	1.00 ± 0.63	1.67 ± 0.43	1.07 ± 0.35	0.83 ± 0.22	10.20 ± 2.48
60	1.36 ± 0.02	1.27 ± 0.60	4.91 ± 1.17 *	3.26 ± 0.14	1.77 ± 0.32	6.26 ± 0.53
120	2.12 ± 0.65	2.48 ± 0.88 *	20.11 ± 6.90	15.60 ± 4.39	4.73 ± 0.90 *	6.06 ± 1.74
240	2.02 ± 0.31 *	1.69 ± 0.46	14.01 ± 2.94 *	6.02 ± 2.21	2.99 ± 1.67	8.61 ± 3.53

* Significant statistical difference $p < 0.042$ with respect to non-irradiated mice.

organ/blood ratio of glutathione is always lower than 1 whatever the time or the marking. The rates of ^{3}H glycine are significantly weaker than the rates of ^{35}S γ-glutamylcysteine at 1, 2 and 4 h. In the liver, the concentration of ^{35}S γ-glutamylcysteine is about seven times more than in blood 1 h after administration and until the end of the measurements. For this same time, the marking by the ^{3}H glycine in the liver is significantly a lot weaker, besides, the measured values are nearly identical to those determined for blood. In the kidneys, the marking with ^{35}S is globally eight times higher than in blood. The marking with ^{3}H is lower; its concentration is only twice that of the blood. In the uterus, impregnations by ^{3}H glycine or ^{35}S γ-glutamylcysteine are not statistically different, except at 2 h. In the rectum, the marking by the tritiated glycine is significantly greater than that produced by ^{35}S and for all determinations. It ranges from five to ten times greater than in blood.

3.2. Diffusion of the glutathione ^{35}S in non-irradiated healthy mice and irradiated mice (rectal injection)

In this second experience, the diffusion of ^{35}S glutathione is observed in healthy animals and in animals undergoing some radiolesions at the pelvic region. These lesions concern anatomically the uterus and the rectum. The precocious effects of radiation involve an inhibition of the division of cells, 72 h after the irradiation. The cytotoxicity of the rays depends on the speed of the renewal of cells in a tissue, so the digestive mucous membrane is particularly radiosensible, and the myometrium is more resistant to the ionisations (Tables 3 and 4).

Between the batch of healthy mice and the one that has been irradiated, some statistically significant differences occur. The uterus and the heart are labelled twice in the irradiated mice, 2 h after administration. The liver is three times less marked, 1 h after administration, but three times more 4 h after administration, during which the blood demonstrates a percentage of radioactivity lower than the one in the healthy mouse. Concerning the rectum or the kidneys, no difference of impregnation is measurable between the two batches. Otherwise, the marking of the organs in the healthy non-irradiated mice after rectal administration (suppository) shows a weaker impregnation of the organs in relation to the intraperitoneally administration.

4. Discussion

Physiologically, plasmatic glutathione is essentially of hepatic and renal origin. The cells of the liver and kidneys, and to a lesser extent, the cells of the muscles [5,6], release glutathione in plasma. Plasmatic glutathione will be able to be used by cells possessing a γ-glutamyl transpeptidase. This enzyme couples the γ-glutamyl cysteine, resulting from the extracellular degradation of glutathione, to an acceptor amino acid, allowing its transportation into the cells [7]. The liver delivers glutathione under a reduced form (GSH) in the plasma. There, it combines by a disulfur bridge to plasmatic constituents or delivers the disulfur form (GSSG) [23]. Some cellular types have the possibility to capture intact glutathione. These are notably the cells of the proximal tubules of the nephrons and the enterocytes of the small intestine. This is due to a cotransport system exchanging two sodium ions with a molecule of glutathione [8].

4.1. Comparison of the diffusion of ^{35}S glutathione and ^{3}H glutathione in healthy mice

The rates of renewal of glutathione in the liver and kidneys are fast; the half-lives of the hepatic and renal glutathione are of the order of 1–5 h [9]. The hepatic clearance of the absorption of glutathione is estimated to be 50 ml/h per gram of tissue in physiological conditions [10]. So, an intraperitoneal administration of glutathione amplifies the concentration of glutathione in the sub-hepatic veins in the rat by 68%, but the inhibition of the γ-glutamyltransferase opposes this efflux of glutathione [11]. The liver restores glutathione after plasmatic hydrolysis [12]. In the kidneys, the absorption of glutathione can be achieved without hydrolysis, so the markings measured in our study correspond, in part, to intact glutathione. However, as the impregnation of the kidneys by ^{3}H glycine is three times less than by ^{35}S, one can estimate that about only a third of intact glutathione is absorbed. In mice, the intraperitoneal administration of GSH or GSSG permits an increase in renal concentration in 20 min. This occurs even in the presence of an inhibitor of γ-glutamyl transpeptidase [13]. Otherwise, it has been shown that the cells of the proximal tubules of the nephrons are able to oxidise GSH and to hydrolyse glutathione, however, the absorption of intact glutathione is the first means to maintain the intracellular concentration in the kidneys [8,14]. In the heart and blood, the half-life of glutathione is very long, of the order of 68–118 h [9]. This could explain the weak impregnations observed during the 4 h studied. It has been demonstrated, in the heart that an increase of intracellular glutathione can be obtained in an *in vitro* model of isolated rat myocytes, by the addition of GSSG in the middle of culture; but the addition of GSH does not demonstrate this. In this model, the inhibition of the glutathione reductase suppresses the cardiac absorption of glutathione [15]. The absorption of ^{3}H glycine by the heart is very weak, about three times less than the values measured for blood. This emphasises the fact that the renewal of the cardiac glutathione does not result from a re-synthesis from the plasmatic metabolites of glutathione. In blood, the rate of ^{3}H glycine is rather steady. It is significantly more important than the rate of ^{35}S γ-glutamylcysteine up to 2 h after administration. The half-life of the plasmatic glycine is longer than that of γ-glutamylcysteine or glutathione. The rate of ^{35}S γ-glutamylcysteine constantly decreases from the beginning of the measurements. This is because the plasmatic concentration of glutathione is quickly regulated to reach the physiological concentration by capture by the cells and by renal and biliary elimination [16]. The uterus absorbs ^{35}S γ-glutamylcysteine and ^{3}H glycine to the same extent; there does not appear, in this organ, to be a significant difference of marking between ^{35}S and ^{3}H. Several glutathione S-transferases, as well as some glutathione peroxydases, were characterised in the uterus [17,18]. This equipment permits a large use of glutathione in the uterus. The ^{35}S γ-glutamylcysteine is better absorbed, or at least as well, than ^{3}H glycine in all organs studied but the rectum. For all times of measurement, it contains five to ten more ^{3}H glycine than that determined in blood. In the rectum, the rate of renewal of the glutathione is relatively slower than in the liver and kidneys [9]. Glycine is one of the most abundant amino acids found in the rectum [19]. This can explain the strong absorption measured in our study. The rectal mucous membrane possesses only 5% of glutathione S-aryltransferase activity relative to the liver and glutathione peroxidase activity is a lot weaker than that found in the liver. On the other hand, the glutathione disulfur reductase activity is twice that in the liver [20]. The enzymatic equipment of the rectum allows therefore, reduction of the already in place GSSG, rather than to turn them into conjugated forms [4] (see Fig. 1 for more details). This contributes to preserve the glutathione molecules in place while recycling them, thanks to the glutathione reductase, rather than to lose molecules of conjugated glutathione. The conjugated glutathione is indeed converted in mercapturic acid, and then eliminated by renal and biliary excretion.

4.2. Comparison of the rectal or intraperitoneal diffusion of ^{35}S glutathione in the healthy mice

The diffusion of ^{35}S glutathione in the organs after rectal administration is generally weaker in relation to

an intraperitoneal administration. The important marking of the liver during the first 2 h after administration indicates that glutathione administered rectally is strongly removed by the liver. Glutathione once re-synthesised in the liver will later reach the other organs after hepatic excretion. In these conditions, especially during the first 15 min after administration, the studied organs are less labelled.

4.3. Comparison of the diffusion of ^{35}S glutathione rectally in the healthy mice and in the irradiated mice at the pelvic region

The physiological answer facing an irradiation is the setting of repair mechanisms. Among them, the 'weak molecular weight system' is in part represented by glutathione. Irradiations produce a reduction in glutathione S-transferase activity without modification of the power of the glutathione peroxydase, nor glutathione reductase [21]. The intervention of these two enzymes permits the maintenance of the concentration of GSH without the necessity of resynthesis [22], nor of capture of extracellular glutathione. The irradiation does not modify the absorption of ^{35}S glutathione in the rectum during a short term (< 72 h). There does not appear to be a statistically significant difference of absorption between the healthy rectum and the irradiated one. In the kidneys, no difference of absorption is shown between the irradiated and the healthy animals during the 4 h of observation. In both groups, the absorption is maximal 2 h after administration. In the irradiated uterus, the absorption of the ^{35}S γ-glutamylcysteine is significantly higher 2 h after administration in relation to the healthy uterus. The uterus contains some glutathione S-transferases [17,18]. These enzymes convert glutathione to a conjugated form that is excreted in the extracellular fluids, which leads to an irreversible loss of GSH in cells. The maintenance of the concentration of glutathione requires, in this case, a re-synthesis and therefore absorption of γ-glutamylcysteine. The reduction of the half-life of the glutathione in the irradiated organs lacking glutathione reductase requires a re-synthesis that can occur in the liver. The liver being the main circulating glutathione supplier; it is not therefore surprising to note a significant increase of absorption of ^{35}S γ-glutamylcysteine at 2 and 4 h in the liver of the irradiated animals. At 4 h, this increase of the absorption produces a significant reduction of blood concentration of ^{35}S γ-glutamylcysteine. The amplification of the marking in the heart, 2 h after administration, in the irradiated animals, can be best explained by a transient increase of concentration of the plasmatic GSSG. Under this form, glutathione is captured by the myocytes.

5. Conclusions

The kinetic of absorption of γ-glutamylcysteine and glycine after the plasmatic hydrolysis of administered glutathione is different from one organ to another. The absorption varies according to the devices of capture of each organ and depends on the metabolism of the glutathione in each organ. It also varies according to the method of administration, intraperitoneal or rectal. In the animal locally irradiated at the site of the pelvic area, the homeostasis of glutathione is modified in most organs even far from the irradiation site. In these animals, the contribution of glutathione rectally increases its availability in tissues, especially at the uterus. The absorption of the metabolites, which permits the synthesis of glutathione, does not constitute the major source of the renewal of GSH in the rectum. However, the exogenous glutathione contribution, while increasing its biodispensibility, permits to maintain, at the rectum, a comparable availability in the healthy and in the irradiated animal. This contribution could be put to profit in radiotherapy for gynaecological cancers. On the one hand, to protect the healthy rectum situated in the cone of irradiation and on the other hand to protect only the healthy parts of the uterus. It is indeed well known that the hypoxia that reigns within the tumours opposes the generation of peroxy free radicals. Thus, the radioprotector power of the glutathione only exercises itself in the normoxics parts of the uterus and thus does not protect the tumour.

Acknowledgements

The authors thank Professor Jean Bernard Dubois, Director of the Centre Régional de Lutte contre le Cancer of Val d'Aurelle, Montpellier, and Dr René Delard, Physicist of the Radiotherapy Service, for their contribution to this survey. The authors thank the National League against Cancer, Departmental Committee of the Hérault, for the financing of these works.

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