THE EFFECT OF GLUTATHIONE DEPLETOR AND INDUCERS IN THE BILIARY EXCRETION OF CADMIUM AND ZINC IN RATS.

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ABSTRACT

The effect of hepatic glutathione (GSH) depletion and enzymes Induction on hepatic glutathione S-transferase (GST's) activity, biliary excretion of GSH cadmium, zinc was studied in rats.

The GSH depletor Methyl Iodide did not Influence hepatic GST activity but depending on the substrate used , Phenobarbital benzopyeren increased it by 50-60% , 20-30% respectively GSH depletor decreased (60%) , benzopyrene did not effect

Phenobarbital increase(90%) the transport of GSH into bile.The biliary excretion of cadmium and zinc was reduced by methyl iodide depletor (52-65%) and enhanced by Phenobarbital (99- 110)% . treatment with benzopyrene did not affect the excretion of zinc into bile ,but decreased that of cadmium, these result do not provide evidence for the role of hepatic GST but support the importance of biliary GSH excretion in the hepatobiliary transport of cadmium & zinc .

It is assumed that Phenobarbital enhance the biliary excretion of metals by increasing the transport of GSH as a chemical complexation (cadmium-glutathione, zinc-glutathion) while the benzopyrene was less effect .

INTRODUCTION

Biliary excretion is an transportant route for the elimination of metals[1].
Organometals such as cadmium and zinc [2] were found in bile, at least partly as glutathione (GSH) complexes.

Depletion of hepatic GSH markedly by decreased their excretion into bile. [3, 4,5],
Cadmium and zinc[6] have high effinities for GSH , therefore complexation of GSH with these metals can take place as a chemical reaction.

The present study was designed to present evidence for the relative importance of the biliary excretion of GSH and GST's (glutathione –s-transferase) in the excretion of cadmium and zinc into the bile , for this purpose, we have investigated the effect of GSH depletor such as methyl iodide and enzyme Inducer benzopyrene, Phenobarbital , on the hepatic GST's activity and GSH level, transport of GSH into bile and biliary excretion of cadmium and zinc

MATERIALS AND METHODS

Chemicals:

Benzopyrene, Phenobarbital (as inducers compounds), cadmium chloride, zinc sulfate (as trace elements), methyl iodide(as depletory)

The animals:

6male rats for each group(three groups) (Spreng-dewely or Rattus norvegicus) weighting (150-200 gm)were used they were housed in animal room and maintained on standard diet (pellets free diet) access to tap water about 16 hr before bile collection the food was with drawn .

Treated one group with methyl iodide (depletory) ,The second group treated with benzopyrene , Phenobarbital (inducers),The third group as control. The experiment was started at 10 hr a.m.

Treatment:

GSH depletor injected intraperitonally in the following dose: methyl iodide 70 mg/kg (14 mg/rat) Inducers were administered Intra peritonally in a volume of 4ml/kg (0.8 ml/rat) for six days using the following daily.

Doses: benzopyrene 25 mg/kg ,(5 mg /rat)Phenobarbital 70 mg/kg (14mg/rat) inseparate experiment.

Bile pulled:

Biliary excretion of metals, The bile duct of rats were connulated with by anaesthesia with needle attached to poly ethylene tubing the body temperature of the animals was kept at 37Cº by means of a heating lamp .For measurement of the biliary excretion of GSH, bile was collected in 3 hr into 0.4 ml 5% meta phosphoric acid to inorder prevent oxidation of the GSH [7].

Bile was collected in 1 hr periods for 3hrs after the injection of $CdCl_4$ (2mg/kg) and zinc sulfate(2.5 ml/kg) into the femoral vein. Bile flow was measured volumetrically.

Determination of GSH & GST activities. The rats were killed by decapitation, The livers was rinsed & homogenized in six volumes of ice-cold 0.25 m sucrose -0.01 m potassium phosphate buffer PH 7.4 & centrifuged at 80,000 for 2 hrs ,GST activities of the supernatant were determined by the specrophotometric method [8]. Protein in the liver cytosol was measured by the method of [9]. GSH being the major non protein sulphydryl compound intra bile (4). And liver (8) measurement of protein sulphdryl content was taken as estimate of GSH content .

treatment	Liver weight g/kg wt	GSH concentration in liver µmol/g	DCNB , DNCB GST actives (n mol /min/mg protein) in liver	
controls	20±o.33 a	6.59±0.32 a	1020±60 a	40.0±3.1 a
Methyl iodide	20.6±0.44 a	1.08±0.08 b	1030±54 a	42.0±3.2 a
Benzopyrene	36.2±0.09 c	8.54±0.42 c	1310±34 c	50.0±5.1 c
phenobarbitol	36.1±0.07 c	8.96±0.91 c	1390±60 d	65.8±4.9 d

Statistics:

The means of the treatment and control group were compared by the LSD with P<0.05 as the level of significance difference.

Table which induct effect of glutathione (GSH) depletor and enzyme inducers on the liver weight, hepatic GSH concentration and (GST) activities.

Rats were injected with methyl iodide (70mg/kg IP) 45 min .Prior to sacrifice. Inducers were administered for six days at the following does benzopyrene 25mg/kg, Phenobarbital 70mg/kg in saline (5 mg/rat,14 mg/rat).

Values are means ±SE at six rats

DNCB=1-chloro 3,4 di nitrobenzene

DCNB=1-dichloro nitrobenzene.

Significantly different (P<0.05) from the values of untreated rats

RESULTS AND DISCUSSION

The table was indicate to the significant different (P<0.05)between treated animals and untreated animals (control) show the significant different(P<0.05) with methyl iodide group depletory markedly reduced hepatic GSH concentration level compared with second and third groups(1.08± 0.08), (8.54±0.42, 8.69±0.91), (6.59±0.32) µmol/g respectively. due to the relationship between the biliary excretion of metals with the excretion of GSH into bile was less closed, therefore complexation of GSH with this metals can take place as a chemical reaction[3].

Methyl iodide not influencing on liver weight and GST activities such as notice in the table above(20.6±0.44) (20±o.33) ,(1030±54) (1020± 60) (42.0±3.2)(40.0±3.1) compared with second and third group due to the effect of metals as compound complexation with chronic damage effect to these metals on the liver cells and bile duct while the third group that which treated with benzopyrene and Phenobarbital show significant different (p<0.05) markedly increase the liver weight and hepatic GST's activities (36.2±0.09, 36.1±0.07)(20±o.33)(1310±34, 1390±60) (1020± 60), (40.0±3.1), (40.0±3.1) compared with control group respectively .because the stimulatory effect of benzopyrene on the excretion of cadmium and zinc was long lasting and weaker then that produced by Phenobarbital and appeared only in the early phase of metals of excretion. In addition benzopyrene which did not influence significantly the biliary excretion of cadmium [4,5].

These finding indicate that the effect of inducers on the biliary excretion of cadmium and zinc the transport of GSH on bile It has been reported that an eightfold elevation of hepatic concentration of metallothione reduced the biliary excretion of cadmium and zinc reduced to 1% and 40% respectively [1] therefore it might be supposed that in the case of cadmium and zinc the change in their biliary excretion rates a combination of their effect on biliary GSH transport and hepatic metallothione in level[1].

Our result do not support the role of GST in the transport of cadmium and zinc from the blood to bile [9]. Failed to increase the biliary excretion which metals (cadmium and zinc) This finding suggest that GST's, either as enzyme or as binding protein do not limit the rate of the biliary excretion of cadmium and zinc and that increase in the biliary excretion of these metals following treatment with Phenobarbital can be attributed to their stimulatory effect on the transport of the GSH from liver to bile rather than to their capability to induces GST's in liver. In conclusion, our results support the importance of biliary GSH excretion and call in question the role of hepatic GST's in the hepatobiliary transport of cadmium and zinc enzymes inducers which increase the excretion of GSH into bile can enhance the biliary excretion of these metals to a smaller or greater extent[10]. it was shown that some none of the parameter studied was significantly influenced by the intra peritoneal treatment of rats

تأثيرالكلوتاثيون في استنزاف وتحريض الطرح الصفراوي للكادميوم والزنك في الجرذان

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الخلاصة

تمت هذه الدراسة لمعرفة تأثير فعالية الكلوتاثيون(GSH) وفعالية الأنزيمات الناقلة للكلوتاثيون (GST's) في تزايد الطرح الصفراوي لبعض العناصر النزرة (الكادميوم والزنك) اجريت هذه الدراسة باستخدام بعض المواد الكيميائية المحقونة للجرذان تمثلت بمثيل اليود كمادة مستنزفة والفينوباربيتال والبنزوبايرين كمواد محرضة للطرح الصفراوي وأثبتت عدم تأثير مادة اليود على فعالية الأنزيمات الناقلة للكلوتاثيون وبالتالي على الطرح الصفراوي للكادميوم والزنك بينما وجدت زيادة فعالية الكلوتاثيون GSH عند الحقن بالفينوباربيتال والبنزوبايرين بنسبة 50-60% و20-30% على التوالي، بينما حدث اختزال في نقل الكلوتاثيون بنسبة 60% عند حقن الجرذان بمثيل اليود مقارنة مع البنزوبايرين ،اضافة الى اختزال الطرح الصفراوي للكادميوم والزنك عند الحقن بمثيل اليود للجرذان بنسبة 52-60% وعند معاملة الجرذان بالفينوباربيتال ازداد الطرح الصفراوي للكادميوم والزنك بنسبة 99-110% أما عند حقن البنزوبايرين لم يلاحظ

أي تأثير للأخير على طرح الزنك في الصفراء لكنه قلل من طرح الكادميوم.، ومن خلال هذه النتائج لم يتضح أي دور للـ (GST) الكبدي لكن أعطت اسناداً واضحاً لفعالية الكلوتاثيون في الطرح الصفراوي للكادميوم والزنك. نستنتج من ذلك حصول زيادة الطرح الصفراوي للكادميوم والزنك بشكل معقدات كيميائية (كادميوم-كلوتاثيون وزنك-كلوتاثيون) عند حقن الجرذان بالفينوباربيتال وبنسبة أقل عند الحقن بالبنزوبايرين.

REFERENCES

1-Klassen,C.D.(1987):Effect of metallothionein on hepatic disposition of metals. Amer .J. physiol . 200.,P.30-32

2-Alexander,J:J.Aaseth and T.Refsvik.(1984):Excretion of zinc in rat bile :A role of glutathione. Acta pharmacol. and toxicol.;59,79-90.

3-Cherian,M.G. (1990):Biliary excretion of cadmium in rat: effects of chelating agents and change in intracellular thiol content on biliary transport and tissue distribution of cadmium.J. Toxicol .Environ Health,60 379-391.

4-Cherian,M.G.and J.J. Vostal.(1992):Biliary excretion of cadmium in rat: Dose-dependent biliary excretion and the form of cadmium in bile .J, Toxicol. Enviro .Health,4,800-815 .

5-Eberle ,D.,R.Clarke and N. Kaplowiz.(1991): Rapid oxidation in vitro of endogenous glutathione in bile of rats ,J. boil Chem .,256 ,2115-**2118**

6-Clarksent.T.W.and Magos ,L.(1985):Effect of Phenobarbitone on the biliary excretion of cadmium and zinc in rats and mice .Nature ,102-106

7-Habig,H.M. Inou ,I.M .Arias and I. Listowsky.(1993) : glutathione s-transferase : A family of multifunctional proteins in the rat .In :function of glutathione :biochemical ,physiological ,toxicological and clinical aspects . Eds.: Alarsson ,A. Holmgren . B Mann ervik and S.Orrenius . Raven press ,New York , ,pp.89-97.

8-Lowry,O.H.,N.J.Reson brought,A.L.Farr and R.J.Randall .(1995):Protein measurement with the folin phenol reagent.J.biol.chem.105,300-303.

9-Perrin ,D.D and A.E. Walt.(1999) :complex formation of zinc and cadmium with glutathione . BioChem .BioPhys Acta ,pp.99-102 .

10-Gergos ,Z.M ,C.D .Klassen and J.B. Watkins. (1992) : Effect of microsomal enzyme inducers on the soluble enzyme of hepatic phase II. Biotransformation Toxicol . Appl . Pharmacl ,66 ,400-407 .