

Glucuronidation of bile acids in human liver, intestine and kidney

An in vitro study on hyodeoxycholic acid

M. Parquet, M. Pessah, E. Sacquet⁺, C. Salvat, A. Raizman and R. Infante

INSERM Unité de Recherches d'Hépatologie U.9, Hôpital Saint-Antoine, 184 rue du Fg Saint-Antoine, 75571 Paris Cedex 12 and ⁺Laboratoire d'Ecologie Microbienne, CNRZ-INRA, 78350 Jouy en Josas, France

Received 5 June 1985

The activities of UDP-glucuronyl transferase(s) in homogenates and microsomal preparations of human liver, kidney and intestine were tested with hyodeoxycholic acid (HDC). The various kinetic parameters of the UDC-glucuronidation were determined from time course experiments. In both liver and kidney preparations, HDC underwent a very active metabolic transformation: liver $K_m = 78 \mu\text{M}$, $V_{\max} = 3.3 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \text{ protein}$; kidney $K_m = 186 \mu\text{M}$, $V_{\max} = 9.9 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \text{ protein}$. To our knowledge this is the first observation of both an extensive and comparable bile acid glucuronidation occurring in renal and hepatic tissues.

Liver Kidney Intestine Bile acid Hyodeoxycholic acid UDP-glucuronyl transferase

1. INTRODUCTION

In man and other animals, UDP-glucuronyl transferase(s) (UDP-GT) (EC 2.4.1.17) has been shown to be a very active enzyme in detoxification mechanisms [1]. It catalyzes the transfer of the activated form of glucuronic acid to a large variety of substrates, such as phenolic and naphtholic derivatives, morphine, menthol, testosterone, etc. [2] or bilirubin [3]. Different human organs commonly possess UDP-GT activities, glucuronidation is observed in the liver [4], kidney [5] and intestine [6]. In 1974, Back et al. [7] were the first to detect the presence of bile salt glucuronides in the urine of man, thereafter the formation of bile acid glucuronides was described by several authors

studying various pathologies (cholestasis, cirrhosis and intestinal malabsorption).

Subsequently, Matern et al. [8,9] demonstrated UDP-GT activities on bile acids, in microsomal preparations of liver, kidney and intestine. In spite of these in vitro demonstrations of enzymatic activities, in vivo glucuronidation of bile acids remains a minor metabolic pathway. However, hyodeoxycholic acid (HDC), a 6 hydroxylated bile acid has been shown to be extensively glucuronidated and excreted in the urine of patients [10] and in healthy subjects [11] after oral administration. A search for the organs responsible for this extensive glucuronidation was therefore undertaken.

2. MATERIALS AND METHODS

2.1. Human samples

Liver biopsies, or the whole organ, were obtained from human donors in an irreversible coma

Abbreviations: HDC, hyodeoxycholic acid; UDP-GT, uridine diphosphate glucuronyl transferase; GLC, gas liquid chromatography; TLC, thin layer chromatography

(accident, suicide). Kidneys or fragments of intestine were respectively obtained during nephrectomies (cancer) or ileal resections for Crohn's disease or adenocarcinoma. The liver and kidney specimens were immediately sliced into thin pieces, frozen in liquid N₂ and then stored at -70°C. Intestinal mucosa was prepared as described by Weiser [12]. Histological studies of the tissues used were made to ensure that they were normal. 20% homogenates (w/v) were prepared with sucrose (0.3 M) using a glass homogenizer with a teflon pestle and microsomal preparations were obtained after centrifugation of the post mitochondrial supernatants at 105 000 × *g* for 60 min. The final pellets were resuspended in 0.3 M sucrose containing 15 mM Tris-HCl (pH 7.5).

2.2. Analytical procedure

Labelled [24-¹⁴C]HDC was prepared by extraction from the faeces of Fischer rats, which had ingested [24-¹⁴C]chenodeoxycholic acid [10], and then purified by separation on a silica gel column.

The UDP-GT activity was measured by incubation, at 37°C, of the hepatic, renal and ileal homogenates or microsomal proteins (1 mg) in a reaction mixture containing UDP-glucuronic acid (3 mM), MgCl₂ (5 mM), delipidated bovine albumin (7.5 μM), [24-¹⁴C]HDC (40 μM, spec. act. 1.18 μCi/μmol), with or without Brij 58 (0.5 mg/mg protein added). The reaction was stopped at different times by adding 1 ml cold methanol. The proportion of HDC metabolite was determined by TLC of the medium on silica gel using butanol-acetic acid-water 10:1:1 (v/v) as solvent (system I) and then by examination of the plates with a Berthold radioscanner.

The HDC metabolite was identified by TLC of the bile acid in system I or TLC of the methylated derivative using chloroform-acetone-methanol 70:25:5 (v/v) (system II) as the solvent. Glucuronide was hydrolyzed using a β-glucuronidase isolated from *Escherichia coli* under the following conditions: a microsomal incubation (as described above) was lyophilised and diluted in 1 ml phosphate buffer (4 mM, pH 6.8) containing 100 Sigma units of β-glucuronidase. Hydrolysis was continued for 20 h at 37°C. The medium was then acidified to pH 1 with 6 M HCl, extracted with ether and evaporated to dryness. Extracts

were methylated with diazomethane, fractionated on TLC (system II) and analysed with a Berthold radioscanner. For GLC analyses, extracts were methylated and trifluoroacetylated with trifluoroacetic anhydride and injected into the glass column (*l* = 1.5 m, i.d. = 3 mm) of a 7400 Packard chromatograph. Injections were made using a solid injector at a temperature of 210°C, the column was packed with a 2% OV 210 fixed on Gas Chrom Q 100–200 mesh as stationary phase.

2.3. Chemicals

[24-¹⁴C]Chenodeoxycholic acid (50 mCi/mmol) was purchased from NEN Chemicals (Boston, USA) and the unlabelled compound from Serva (Heidelberg, FRG). UDP-glucuronic acid, Brij 58, sucrose, β-glucuronidase (*E. coli*) and free lipid albumin were obtained from Sigma (St. Louis, MO) and saccharolactone from Calbiochem-Behring (San Diego, CA). Diazald was obtained from Ega-Chemie (Steinheim, FRG) and trifluoroacetic anhydride from Merck (Darmstadt, FRG).

3. RESULTS AND DISCUSSION

3.1. Hepatic glucuronidation

Both liver homogenates and microsomal preparations actively transformed HDC into its corresponding glucuronide. The proportion of synthesized glucuronide after 60 min incubation, varied from 25% in homogenates to 81% in microsomal preparations (fig.1). The activities of both preparations were increased when the detergent Brij 58 was added (fig.1). These observations may be related to the location of UDP-GT in the endoplasmic reticulum of hepatocytes [1] and to the known activation of membrane bound enzyme by compounds which alter the phospholipid environment [13]. Another parameter which could have caused variations or underestimations of the HDC glucuronidation is the reverse reaction by hepatic β-glucuronidase. Experiments were therefore carried out using saccharolactone, an inhibitor of this enzyme. No effects on the rate of HDC-glucuronidation or on the proportion of HDC glucuronide formed were observed, even when the concentration of inhibitor reached 10-times that of HDC.

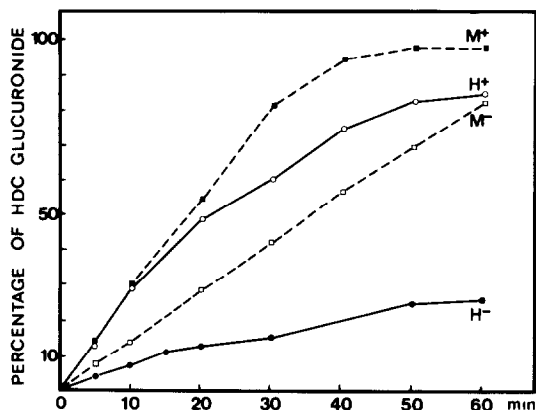


Fig.1. Time courses of HDC glucuronidation in preparations of hepatic human tissues. 1 mg of either homogenate (H) or microsomal proteins (M) were incubated with $[24\text{-}^{14}\text{C}]\text{HDC}$ ($40\text{ }\mu\text{M}$) and UDP-glucuronic acid (3 mM). UDP-glucuronyl-transferase activity was measured with (+) or without (-) the detergent Brij 58.

3.2. Comparison between the different organs

Time courses of HDC glucuronidation in kidney homogenates and microsomal preparations are presented in fig.2.

The UDP-GT activity observed in this organ was similar to that of the liver tissues. The same differences were observed between homogenates and

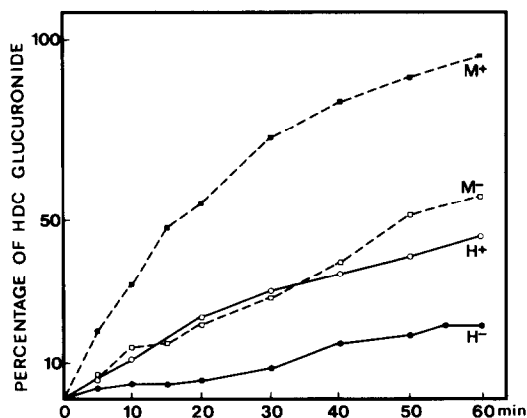


Fig.2. Time courses of HDC glucuronidation in preparations of renal human tissues. Experimental conditions were identical to those used in liver preparations (see text). M and H represent microsomal and homogenate preparations, + and - indicate the presence or absence of detergent Brij 58.

microsomal preparations, and the UDP-GT activity was increased by detergent treatment (Brij 58). These results are in agreement with a microsomal location of renal HDC-UDP-GT activity. Lineweaver-Burk plot (fig.3) for hepatic and renal UDP-GT activities gave 2 straight lines from which the apparent K_m and V_{max} were calculated: liver $K_m = 78\text{ }\mu\text{M}$, $V_{max} = 3.3\text{ nmol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ protein and kidney $K_m = 186\text{ }\mu\text{M}$, $V_{max} = 9.9\text{ nmol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ protein. Such a level of renal UDP-GT activity for bile acids has never been observed in normal human tissues. This fact could account for the important urinary excretion of HDC glucuronide seen in patients with T-tubes [10] and in healthy subjects [11], after HDC oral administration.

The glucuronidation of HDC in the ileum occurred at a lower rate than in other organs (fig.4) and was not increased by the addition of Brij 58.

3.3. Comparison with chenodeoxycholic acid

Time courses experiments comparing the glucuronidation of HDC and chenodeoxycholic acid were conducted under identical experimental conditions. Wide variations were observed between the substrates (fig.5). In both the liver and kidney preparations, HDC undergoes a more active glucuronidation than chenodeoxycholic acid.

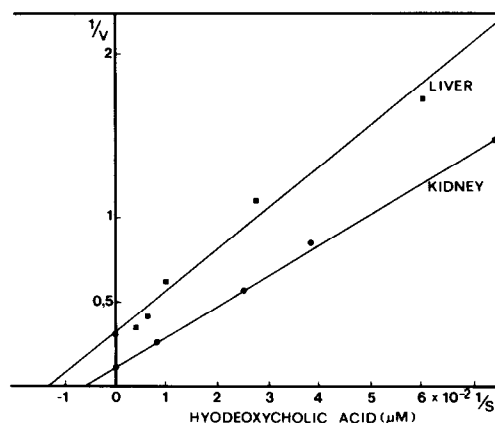


Fig.3. Lineweaver-Burk plots of HDC glucuronidation in the liver and kidney. Microsomal proteins (1 mg) of different tissues were incubated with Brij 58 (0.5 mg) at various known concentrations of $[24\text{-}^{14}\text{C}]\text{HDC}$. Incubations were stopped after 15 min and the proportion of HDC glucuronide determined (see section 2.2).

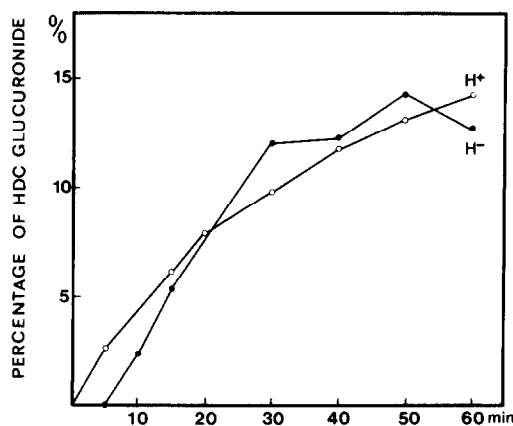


Fig. 4. Time courses of HDC glucuronidation in ileal preparations of human intestine. Incubations were carried out (as described in section 2.2) on homogenates of mucosa cells with (+) or without (-) the detergent Brij 58.

It is interesting to note that a slight modification in the chemical structure of these bile acids (i.e. HDC is dihydroxylated in 3α and 6α positions and chenodeoxycholic acid in 3α , 7α) should produce such variations in UDP-GT activity.

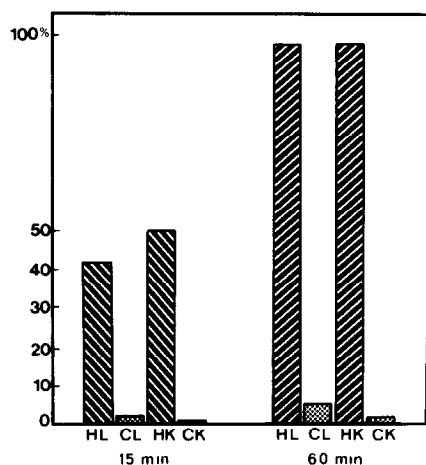


Fig. 5. Comparison of HDC and chenodeoxycholic acid glucuronidation in the liver and kidney. HL, HDC in liver; CL, chenodeoxycholic acid in liver; HK, HDC in kidney; CK, chenodeoxycholic acid in kidney. 1 mg microsomal preparations were incubated with the detergent Brij 58. The percentages of the 2 bile acid glucuronides were measured at 2 different times.

3.4. Identification of HDC glucuronide

The metabolic transformations of HDC by hepatic and renal tissues, have produced only one compound whose R_f (0.40) on silica gel plates with system I as solvent, clearly discriminated it from HDC ($R_f = 0.93$). This metabolite possessed the same R_f as the glucuronide isolated from urine of healthy subjects after oral administration of HDC [11]. In addition, β -glucuronidase treatment of the incubation medium (containing 70–94% of HDC metabolite) produced a single radioactive compound. Its characterization was achieved by comparing migration (plates, system II) of a HDC methyl ester (standard) with the methylated derivative and by GLC analysis of the trifluoroacetylated methyl ester derivatives. Equivalent structural determinations were obtained from ileal homogenates although the proportion of glucuronide (15%) was lower.

It is possible to conclude that HDC undergoes no biotransformation, in these in vitro experiments, other than glucuronidation. In particular, no modification of the initial steroid structure of HDC (hydroxylation, oxidation, etc.) was detected, in the microsomal preparations.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs M.E. Bouma, Dr Michel, Dr Fraioli and Dr Hurel for providing the biopsy specimens.

REFERENCES

- [1] Dutton, G.J. (1980) CRC Press, Boca Raton, FL.
- [2] Boutin, J.A., Antoine, B., Batt, A.M. and Siest, G. (1984) *Chem.-Biol. Interact.* 52, 173–184.
- [3] Black, M., Billing, B.H. and Heirwegh, K.P.M. (1970) *Clin. Chim. Acta* 29, 27–35.
- [4] Bock, K.W., Lilienblum, W. and Von Bahr, C. (1984) *Drug Metab. Disposition* 12, 93–97.
- [5] Hobkirk, R., Green, R.N., Nilsen, M. and Jennings, B.A. (1974) *Can. J. Biochem.* 52, 9–14.
- [6] Hoensch, H.P., Steinhardt, H.J., Weiss, G., Haug, D., Maier, A. and Malchow, H. (1984) *Gastroenterology* 86, 1519–1530.
- [7] Back, P., Spaczynski, K. and Gerok, W. (1974) *Hoppe-Seyler's Z. Physiol. Chem.* 355, 749–752.
- [8] Matern, H., Matern, S., Schelzig, C. and Gerok, W. (1980) *FEBS Lett.* 118, 251–254.

- [9] Matern, S., Matern, H., Farthmann, E.H. and Gerok, W. (1984) *J. Clin. Invest.* 74, 402–410.
- [10] Sacquet, E., Parquet, M., Riottot, M., Raizman, A., Jarrige, P., Huguet, C. and Infante, R. (1983) *J. Lipid Res.* 24, 604–613.
- [11] Parquet, M., Raizman, A., Berthaux, N. and Infante, R. (1984) in: *Advances in Glucuronide Conjugation* (Matern, S. et al. eds) pp.411–412, Press, England).
- [12] Weiser, M.M. (1973) *J. Biol. Chem.* 248, 2536–2541.
- [13] Zakim, D. and Vessey, D.A. (1982) in: *Membranes and Transport* (Martonosi, A.N. ed.) vol.1, pp.269–273, Plenum, New York.