Journal of Steroid Biochemistry. 1975. Vol. 6. pp. 1371–1372. Pergamon Press. Printed in Great Britain

SHORT COMMUNICATION

MASS FRAGMENTOGRAPHIC DETERMINATION OF CONJUGATED NEUTRAL 17-KETOSTEROIDS IN PERIPHERAL AND PORTAL VENOUS BLOOD: EFFECT OF AMPICILLIN ADMINISTRATION

FINIAN MARTIN,* HERMAN ADLERCREUTZ,* BJÖRN LINDSTRÖM,† HANS DENCKER,‡ ULF RIMÉR‡ and NILS-OTTO SJÖBERG‡

* Department of Clinical Chemistry, University of Helsinki; † IV Department of Surgery, University Central Hospital, Helsinki, Finland; and ‡ Department of Surgery, University of Lund, Lund, Sweden

(*Received* 16 *December* 1974)

SUMMARY

Dehydroepiandrosterone, androsterone and epiandrosterone sulphate and androsterone glucuronide were determined by mass fragmentography in pools of peripheral and portal venous plasma from postmenopausal women. Samples were analyzed both before and after oral administration of ampicillin for 4 days (2 g per day). The peripheral ketosteroid monosulphate concentrations were within the normal range for postmenopausal women reported in the literature. Androsterone glucuronide was present in concentrations of 42 μg/l plasma. The peripheral and portal venous metabolite levels did not differ from each other either before or after ampicillin administration. With the exception of decreasing dehydroepiandrosterone sulphate levels ampicillin did not affect either portal or peripheral metabolite concentrations. The higher pre-ampicillin dehydroepiandrosterone sulphate concentrations may reflect a stress situation as the mean cortisol levels were also higher, but not significantly so in these samples.

As ampicillin administration has been shown to cause reduced urinary excretion of both estrogen [1, 2] and neutral steroid metabolites [3, 4] during pregnancy, most probably by interfering with the enterohepatic circulation of these steroids at the level of the intestinal tract [2, 4] it was of interest to test the effect of administering this drug on endogenous portal venous steroid concentrations. Okishio and Nair[5] have demonstrated that it is possible to assess the effects of antibiotic therapy on the enterohepatic circulation of bile acids in rats by analyzing portal venous blood. Thus, sulphate and glucuronide conjugated 17-ketosteroid levels were measured in peripheral and portal venous blood from postmenopausal women, before and after ampicillin administration, by mass fragmentography. Vihko[6] and K. Sjövall *et al.*[7–9] have previously quantitated plasma sulphate conjugated neutral steroids in pre- and postmenopausal women and during pregnancy using a similar gas chromatographic method.

The subjects were five postmenopausal women, aged 47–55 years. All had been operated upon for carcinoma of the colon. The patients were examined with portography for metastases of the liver and only patients with no demonstrable liver secondaries were included. At the time of the study the patients were in good general condition; they were ambulant and on a normal hospital diet, bowel function was normal. Catheterization of the portal vein and sampling of blood were performed as previously described [10].

Peripheral and portal venous blood were collected before and after the administration of ampicillin, 2 g per day orally for 4 days. Because of the limited volume of portal venous blood available it was necessary to pool the samples for steroid determination. Plasma dehydroepiandrosterone, androsterone and epiandrosterone monosulphate and androsterone glucuronide were determined by a mass fragmentographic procedure which has been described in detail recently [11]. All determinations were carried out in triplicate. In this series of experiments procedural losses were corrected for on the basis of the recovery of [^{3}H]-testosterone sulphate and [^{3}H]-testosterone glucuronide added to the plasma, the recovery values were 65·4 ± 8·8% (mean ± S.D.) and 67·7 ± 4·5% (mean ± S.D.), respectively. Plasma cortisol was determined using an enzymatic technique [12].

The results of the conjugated 17-ketosteroid determinations are shown in Table 1. The concentrations of the 17-ketosteroid monosulphates in the peripheral plasma pool before ampicillin administration are similar to the mean values reported in the literature for post-menopausal women [6, 13]. Androsterone glucuronide was the principal ketosteroid component of the glucuronide fraction. Etiocholanolone and dehydroepiandrosterone were detected in the glucuronide fraction but in amounts too small to permit accurate quantitation under the conditions used.

There were no differences between the concentrations of the conjugated ketosteroids in the peripheral and portal venous pools either before or after ampicillin treatment (Table 1). In addition, after ampicillin administration the concentrations of the ketosteroids in both the portal and peripheral pools were the same as in the control samples, with the exception of the dehydroepiandrosterone sulphate levels which were 20–30% lower (Table 1). The significance of this change in dehydroepiandrosterone sulphate levels is difficult to evaluate. The cortisol levels in the portal and peripheral plasma from the five individuals were measured before and after ampicillin administration. The mean pre-ampicillin levels for the group [peripheral: 0·35 ± 0·16 (μmol/l ± S.D.); portal: 0·38 ± 0·12] were higher than the post-ampicillin levels (peripheral: 0·24 ± 0·05; portal: 0·28 ± 0·09) but not significantly so. Thus the higher cortisol and dehydroepiandrosterone sulphate levels in the control samples may reflect a post-operative stress situation.

Androsterone, etiocholanolone and dehydroepiandrosterone are secreted in human female bile as glucuronides and monosulphates in amounts from 10–680 μg/l [14]. In the gastrointestinal tract these conjugates are probably cleaved and some fraction of the liberated steroids reab-

Table 1. 17-Ketosteroid sulphate and androsterone glucuronide levels in pools of peripheral and portal venous plasma from postmenopausal women before and after the administration of ampicillin (2 g/day) for 4 days. The values are expressed as μg/l. The mean and range (in brackets) of three analyses of each plasma pool are shown

	MONOSULPHATES			GLUCURONIDE
	Dehydroepiandrosterone	Androsterone	Epiandrosterone	Androsterone
Peripheral venous plasma				
- before ampicillin	434 (352-538)	144 (108-164)	44 (32-58)	42 (36,46)[x]
- after ampicillin	338 (322,352)[x]	147 (142,152)[x]	38 (34,41)[x]	40 (28-54)
Portal venous plasma				
- before ampicillin	500 (484-512)	130 (114-150)	44 (38-50)	38 (32-45)
- after ampicillin	366 (326-392)	170 (160-186)	44 (38-54)	36 (32-42)

[x] In these cases one sample was lost and the results of the other two determinations are given.

sorbed and reconjugated in the intestinal mucosal wall [15, 16] although little is known of their enterohepatic circulation [17].

Qualitative and quantitative similarity between the peripheral and portal venous pools probably rules out any significant formation of characteristic intestinal mucosal metabolites of these compounds. In addition, the concentration of endogenously reabsorbed material in this area of the circulation was not measurable by this approach. Using the present ampicillin administration and blood sampling regimen no overall change in metabolite concentration was seen in the portal venous pool. Again, the lack of a portal/peripheral venous concentration difference probably eliminates the possibility of detecting any changes caused by ampicillin in the enterohepatic circulation of these compounds in this way.

Endogenous steroids have not previously been determined in portal venous blood. It may be concluded that in postmenopausal women the peripheral and portal venous blood 17-ketosteroid conjugate concentrations are not significantly different.

Acknowledgements—The excellent technical assistance of Miss Leena Roiha and Mrs. Anja Manner is gratefully acknowledged. F.M. is in receipt of a post-doctoral fellowship from WHO.

REFERENCES

1. Willman K. and Pulkkinen M. O.: *Am. J. Obstet. Gynec.* **109** (1971) 893–896.
2. Tikkanen M. J., Pulkkinen M. O. and Adlercreutz H.: *J. steroid Biochem.* **4** (1973) 439–440.
3. Trybuchowski H.: *Clin. chim. Acta* **45** (1973) 9–18.
4. Martin F., Peltonen J., Laatikainen T., Tikkanen M. and Pulkkinen M.: *Clin. chim. Acta* **55** (1974) 71–80.
5. Okishio T. and Nair P. P.: *Biochemistry* **5** (1966) 3662–3668.
6. Vihko R.: *Acta endocr., Copenh.* Suppl. **109** (1966) pp. 1–67.
7. Sjövall K.: *Ann. clin. Res.* **2** (1970) 393–408.
8. Jänne O., Vihko R., Sjövall J. and Sjövall K.: *Clin. chim. Acta* **23** (1969) 405–412.
9. Sjövall J. and Sjövall K.: *Ann. clin. Res.* **2** (1970) 321–337.
10. Dencker H., Göthlin J., Meeuwisse G. and Tranberg K.-G.: *Scand. J. Gastroent.* **7** (1972) 451-453.
11. Adlercreutz H., Martin F., Wahlroos Ö. and Soini E.: In Proceedings 4th International Congress on Hormonal Steroids Mexico Sept. 1974. *J. steroid Biochem.* (1975) **6** (1975) 247–260.
12. Härkönen M. and Adlercreutz H.: *J. steroid Biochem.* **5** (1974) 527–528.
13. Heyns W.: In *Gas Chromatography of Hormonal Steroids* (Edited by R. Scholler and M. F. Jayle). Dunod, Paris and Gordon and Breach, New York (1968) pp. 403–404.
14. Laatikainen T.: *Ann. clin. Res.* **2** (1970) 338–349.
15. Fries N., Knapstein P., Wendlberger F. and Oertel G. W.: *Acta endocr., Copenh.* **56** (1967) 705–712.
16. Knapstein P., Wendlberger F. and Oertel G. W.: *Z. physiol. Chem.* **348** (1967) 89–92.
17. Taylor W.: *Vitam. Horm.* **29** (1971) 201–285.