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INDOCYANINE GREEN CLEARANCE IN THE UNDERNOURISHED ADULTS

A. TULPULE AND K. KRISHNASWAMY

Food & Drug Toxicology Research Centre
National Institute of Nutrition
Jamai-osmania, Hyderabad

1. Indocyanine green clearance was studied in eight undernourished subjects.
2. The data showed that in mild to moderately, undernourished adult males, the plasma concentration of ICC were low, the t 0.5 tended to prolong, as a result of increased volume of distribution.
3. However, the ICC clearance was not found to be affected indicating that the clearance of drugs with a high hepatic extraction ratio is unlikely to be altered in these subjects.

Introduction

One of the important factors that is likely to alter drug distribution and kinetics is the nutritional status of an individual (Krishnaswamy, 1978). We have used indocyanine green (ICC) as a prototype to study the clearance of drugs with a high hepatic extraction ratio in the undernourished. ICC, when administered in the low doses used in this study, is rapidly removed from the circulation by the liver with hepatic extraction ratio's in man varying from 50% to 80% (Caeser, Shaldon, Chiandussi et al, 1961; Cherrick, Stein, Leavy et at, 1960: Weigand, Ketterer, Rapaport, 1960). ICC is extensively bound to alpha-l-lipoprotein (Baker, 19%) to be distributed in plasma without evidence of extravascular distribution (Cherrick, Stein, Leavy et al, 1960). Following uptake into the liver, ICC is actively transported to bile without metabolic transformation (Caeser, Shaldon, Chiandussi, 1961) and once excreted into the small intestine it remains within the gastrointestinal tract and does not undergo enterohepatic circulation (Wheeler, Craton, Meltzer, 1958).

Ohkubo et al (1978) reported that the plasma clearance rate of ICC is increased following a 72 hour caloric restriction in man (Ohkubo, Musha, Okuda, 1978). Jahoor and Jackson in(1982)reported that the ICC uptake by liver is impaired in rats fed with protein deficient diet. However, there is no data on the clearance of ICG in the undernourished humans.

Methods

SUBJECTS:

Sixteen adult male subjects, aged 25 to 40 years, participated in the study. Of them 8 were normal and healthy and B were undernourished. Subjects having an

$$\text{anthropometric index} \quad \frac{\text{Weight (Kg)}}{\text{Height (cm)}^{*}} \times 100$$

below 0.18 were considered undernourished (Jaya Rao, Mukherjee and Rao, 1972). They were free from recognisable diseases and none had oedema. None of the subjects were alcoholics or chronic smokers. Care was taken to record that the occasional smoking habits of the undernourished were similar to the habits of the normal subjects. The subjects investigated did not take any drug during the week prior to or on the day of the investigation. The purpose of the study was explained

to all subjects and their consent obtained. The study was cleared by the Ethics Committee of our institute. The liver function status of all subjects was assessed by determining SGOT, SGPT (Bergmeyer and Bernt, 1974) and gamma GT (Rosalki and Tarlow, 1974) levels. Serum albumin (Gustafsson, 1976) and haemoglobin (Davie and Lewis, 1975) values were also determined.

STUDY DESIGN:

As food and physical stress are known to alter KG clearance (Marigold, Gilmore and Thompson, 1981, Swartz, sidell and Cucinell, 1974), the study was conducted after an overnight fast and after rest with the subject in reclining position. Five ml. of antecubital venous blood was withdrawn to be used as serum blank and then 0.5 mg/kg body weight of ICC was injected intravenously as a bolus. Venous blood samples were withdrawn from the opposite antecubital vein at 1,3,6, 9,12 and 15 minutes after the injection.

ANAL YTICAL METHODS:

ICG analysis -was performed by measuring optical density of serum samples directly at 805 nm (Owen, 1973); OD readings were converted to concentrations of KG with the use of a standard curve determined by adding known amounts of ICC to normal human serum. The minimum amount detectable was 1 /µg/ml of serum by this method.

PHARMACOKINETIC ANALYSIS:

The terminal half-lives were calculated by the method of least squares and the area under time and concentration curve AUC $_{0\sim\infty}$ by the Trapezodial rule. The clearance was calculated by dividing the dose given by AUC $_{0}$ ..Volume of distribution was calculated by dividing ckarance by the elimination rate constant.

Statistical analysis was evaluated by Students 't' test.

Results

Clinical details of subjects are given in Table 1. Apart from having a significantly lower anthropometric index, the undernourished subjects also had significantly lower mid arm circumference and skin fold thickness. Their serum albumin levels were also lower than in the normals. However, the haemoglobin concentrations were comparable in both the groups. SGOT, SGPT and gamma GT levels were within the normal range in both the normals and the undernourished Pharmacokinetic data of KG following an1 V bolus of 0.5 mg/kg body weight are given in table 2.

The serum concentration of ICC at time zero tended to be lower in the undernourished. The volume of distribution was significantly higher in these subjects AS a consequence the half life of the drug tended to be prolonged in the undernourished. However, the ckarance and the AUC were not different in the two groups.

Discussion

The mechanisms whereby KG is bound and transported across the hepatocyte for excretion into the bile is dependent upon receptor and carrier proteins which are thought to belong to the family of glutathione-s-transferase (Kaplowitz, 1980). They comprise upto 10% of total protein content of the liver. The net content of these proteins is dependent on their relative rates of synthesis and degradation and must relate to the overall state of protein metabolism within the hepatocyte and be responsive to the dietary -state (Ohkubo, Musha and Okuda, 1978; Stein, Mishkin, Fkischner et al, 1976).

Table 1. Relevant ceinical data of subjects who participated in ICC kinetic study

Group	AI	MAC (cm)	SFT (mm)	Serum Albumin	Haemoglobin (g/dl)	SGOT (IU)	SGPT (IU)	γ GT (IU)
Normal	0.18± 0.004	24.1 ± 1.25	120f 1.74	4.0± 0.09	13.9± 0.81	22.7 ± 1.14	21.0 ± 1.31	24.3± 1.34
Under-nowkhed	0.16± 0.004	21.0+± 0.68	6.8+ ± 0.68	3.0± 0.16	12.8± 0.84	23.6± 2 u	18.2± 3.02'	26.1± 2.36

Figures are mean ± SEM (n=8 each group)

• P<0. 001; • * P<0.01; + P<0.02; • * PLO.05

AI = Anthropometric Index

MAC = Mid arm circumference

SFT = Skin fold thickness

Table 2. ICC Kinetic Parameters

Group	co (/ug/ml)	Clearance (ml/min/kg)	Vd (L % Body weight)	'0.5 (min.)	Auc o~∝ (ug-ml/min)
Normal	13.5± 2.21	11.0± .72	4.3+ 1.51	2.6 ± 0.39	52.5 + 7.57
Undernourished	7.8*•± .49	15.8±3.56	8.3*± .51	4.2±0.59	49.9± 285

Figum are Mean c SEM of 8 subjects in each group
* P<0.05, • i PLO.1

In clinical situations it has been observed that in general wherever there is an identifiabk derangement in hepatic function, there is a failure of the liver to remove ICG from the drculation (Leevy, Smith, Longuevflk et al,,1967). In both man and rat subtle metabolic alterations, such as energy restriction, are shown to alter the clearance of ICG. (Ohkubo, Musha and Okuda, 1978). However, our data indicate that the extraction capacity of ICC by the liver appears to be normal in the chronic undernourished subjects. Hence the ckarance of ICC is normal in undernourished. This situation seems akin to that reported by Johoor and Jackson where they have reported the phasic response on low protein diet, with initial impairment in ICC clearance andapparent recovery of clearance on the 12th day. Thk may reflect a changing pattern of protein synthesis and degradation in the liver with time, during the process of adaptation to a low protein intake (Garlick, Millward, James et al,1975).

It remains to be seen whether ICC clearance is altered in' severe cases of malnutrition (Nutritional oedema in adults and severe PEM in children). However, our data show that in mild to moderately undernourished adult males ICC clearance is not altered indicating that the clearance of drugs with high hepatic extraction ratio is unlikely to be altered in these subjects.

References

BAKER, K.J. (1966): Binding of sulphobromophthalein (BSP) sodium abd indocyanine green (ICC) by plasma alpha-l- lipoproteins. Proc. Soc. exp. biol. med. 122: 957-963.

BERGMEYER, H.U. and BERNT, E. (1974): Methods of Enzymatic analysis. ed. H.U. Bergmeyer, Vol. 2, p. 727 and 755-757, Academic Press. Inc. New York.

CAESER, J., SHALDON, S., CHIANDUSSI, L.,GUEVERA, L., and SHERLOCK, S. (1961): The use of indocyanine green in the measurement of hepatic blood flow and as a test of hepatic function. Clin. Sci. 21: 43-57.

CHERRICK, G.R, STEIN, S.W., LEAVY, C.M. and DAVIDSON, C.S. (1960) Indocyanine green: Observations on its physical properties,Plasma decay and hepatic extraction J. C/in. Invest 39 : 592-600.

DAVIE, J.V. and LEWIS, S.M. (1975): Practical Haematology Churchill Livingston.

GARLICK, P.J., MILLWARD, D.J., JAMES, W.P.T. and WATERLOW,J.C.(l975):The effect of protein deprivation and starvation on the rate of protein synthesis in tissues of the rat. Biochem. Biophys. Acta. 414: 71-84

GUSTAFSSON, J.E.C. (1976): Improved specificity of serum albumin determination and estimation of acute phase reactants' by use of the bromocresol seen reaction. C/in Chem. 22: 616-622.

JAHOOR, F. and JACKSON, A. (1982): Hepatic function in rats with dietary - induced fatty liver, as measured by the uptake of indocyanine green. Br. J. Nutr. 47: 391-398.

JAYA RAO, K.S., MUKHERJEE, N.R.andRA0,K.V. (1972): A survey of diabetes mellitus in a rural population in India. Diabetes. 21: 1192-1196

KAPOLWITZ, N. (1980): Physiological significances of glutathione-s-transferase. Am. J. physiol. 239: 439444.

KRISHNASWAMY, K. (1978): Drug metabolism and pharmacokinetics in malnutrition. *Clin.* Pharmacokinet. 3: 216-240

LEVY, CM., SMITH, F., LONGUEVILLE, J., PAUMGARTNER G., and HOWARD, M.M. (1967): Indocyanine Green Clearance as a test for hepatic function. J. Am. med. Ass. 200: 236-240

MARIGOLD, J.H., GILMORE, LT., and THOMPSON, R.P.H. (1981): Effects of meal on plasma clearance of c^{14} glucocholic acid & ICC in man. Clin. Sci. 61: 325-330

OHKUBO, HIDEKI, HIROTAKA, MUSHA. and KUNIO

OKUDA (1978): Effects of caloric restriction on the kinetics of indocyanine green in patients with liver diseases and in the rat. Am. J. Dig. Dis. 23: 1017-1024.

OWEN, V.M.]. (1973): Laboratory note. Estimation of indocyanine green concentrations. Clin. Biochem. 6: 132-135.

ROSALKI, S.D. and TARLOW, D. (1974): Optimizeddetermination of γ-Glutamyl transferase by reaction rate analysis. Clin. Chem. 20: 1121-1124.

STEIN, L.B., MISHKIN, S., FLEISCHNER, G.,GATMAITAN, Z. and IRWIN, M.A. (1976): Effect of fasting on hepatic ligandin, Z protein and Organic Anion transfer from plasma in rats. *Am. J.* Physiol. 237: 1371-1376.

SWARTZ, R.D., SIDELL, F.R. and CUCINELL,S.A. (1974): Effects of physical stress on the disposition of drugs eliminated by the liver in man.J. Pharmac. exp. Ther. 188: 1-7.

WEICAND, D.B., KETTERER, S.G. and RAPAPORT,E. (1960). The use of indocyanine green for the evaluation of hepatic function and blood flow in man. Am. J. Digest Dis. 5: 427-436.

WHEELER, H.O., CRATON, W.I., and Meltzer, 1.1. (1958): Hepatic uptake and biliary excretion of indocyanine green in the dog. Proc. Sco. *exp.* biol. med.99: 11-16.

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Dr. Kamala Krishnaswamy
Food & *Drug Toxicology* Research Centre,
National Institute of Nutrition,
Janiai Osmania, Hyderabad 500 007.