Biliary tract

OXIII/691

PROBABILITY OF DEVELOPING GALLSTONES AND RELATED RISK FACTORS IN 400 CIRRHOTICS. D. Conte, D. Barisani, M. Fraquelli, P. Casarin1, P. Bodini2, M. Borzoi3, G.P. Aimo4, C. Mandelli. Instituto di Scienze Mediche e 3Ospedale Fatebenefratelli-Milano, and Ospedali di 1Sicile, 2Cremona, and 4Sud-Italy.

Background: Several studies have described an increased prevalence of gallstones (GS) in cirrhotic; furthermore, an annual incidence of 2.8% (3) and a global cumulative incidence of 5.2% in 100 patients who have recently (2) been reported.

Aim: To estimate the probability of GS development and related risk factors in 400 cirrhotics with different etiology, GS free at enrollment.

Patients: 400 patients (257 men and 143 women; mean age 58 ± 10 yr, and 51 ± 10 yr, respectively) with cirrhosis related to alcohol abuse (n=169), HBV or HCV infection (n=138), HBV or HCV chronic infection (n=77) and alcohol abuse (n=50) and miscellaneous (n=43). At enrollment 231 patients were in Child's A class, 130 in B and 39 in C. Follow up consisted of clinical examination, serum tests for liver function, and ultrason sound liver scan (US) every six months. Diagnosis of GS was based on consistent US findings or emergency cholecystectomy for GS.

Methods: The cumulative probability of developing GS was estimated by the Kaplan-Meier method. Possible risk factors for GS were studied by logistic regression analysis which included the following variables: sex, age, body mass index (BMI), alcohol intake, Child's class, etiology of cirrhosis, HBsAg, albumin, bilirubin, AST, ALT, yGT, alkaline phosphatase, cholesterol, triglycerides.

Results: The mean duration of follow up was 48 mo. (range 6-323), 94 patients (23.5%) developed GS. The cumulative probability of developing GS according to years of follow up is given in the table.

Follow up (yr) 0 2 4 6 8
Patients at risk (n) 400 366 256 156 86
Patients with GS (n) 6 2 4 6 8
Probability (%) - 5 5 15 24 38

The risk of developing GS increased significantly with BMI (p<0.01, odds ratio 1.05) and was significantly higher in women than men (p<0.01, odds ratio 2.16) and in Child's class B/C than A (p<0.01 in both cases with odds ratio of 2.84 and 5.39 respectively).

Conclusions: Terms of both prevalence (3) and incidence, cholelithiasis represents a major problem in cirrhosis and an accurate follow up of cirrhotic patients, GS free at enrollment, is mandatory.


OXIII/645

INTRACELLULAR SECOND MESSENGERS RESTORE THE IMPAIRED CONTRACTILITY OF HUMAN GALLBLadders WITH CHOLESTEROL STONES. P.Yu, P. Biancani and J.Behar. Department of Medicine, Rhode Island Hospital, Brown University Medical School, Providence, R.I., U.S.A.

Previous studies have shown that human gallbladders with cholesterol stones (CS) have a weak contraction in response to the octapeptide of cholecystokinin (CCK-8). In contrast, gallbladders with pigment stones (PS) appear to have a more normal contractility. The CCK stimulation of gallbladder muscle contraction is mediated by two pathways: (1) the action of low concentrations of CCK (10-12 M) is mediated by inositol triphosphate (IP3), which releases calcium from biliary glycerolipid DAG-protein kinase C (PKC) pathway; (2) high concentrations of CCK (10-8 M) also act through IP3 and intracellular calcium which then activates the calmodulin (CaM) and light myosin chain kinase pathway. The aim of this studies was to examine the functional integrity of the intracellular pathways that mediate CCK induced contraction in muscle cells from specimens with CS. Sixteen gallbladders with cholesterol and fourteen with pigment stones were included in these studies. All patients had the gallbladders removed electively and none had a history of acute cholecystitis. Studies were performed in enzymatically digested intact or permeabilized smooth muscle cells. Muscle cells were permeabilized by exposure to saponin (75 µg/kg for 4 minutes). Dose-response studies with CCK-8 (10-10 - 10-8 M) showed that the contraction of muscle cells from gallbladders with PS was greater than that of muscle cells from specimens with CS (p<0.05). Dose-response studies with IP-3 (10-10 - 10-5 M), DAG (10-13 - 10-5 M) and CaM (10-11 to 10-5), activated with 1.32 µM of calcium, increased the contractility of muscle cells from cholesterol stones compared to the contraction induced by CCK (p<0.05). In contrast, there was no difference between the contraction induced by the second messenger and by CCK in muscle cells from gallbladders with PS. There was also no difference between the contraction of muscle cells from specimens with CS and from specimens with PS in response to IP-3, DAG and CaM. The CCK induced contraction appears to be mediated by Gi3 protein since it was almost completely blocked by a specific antibody against the a3 subunit. GTPS (10-4 - 10-4 M), the non-hydrolysed GTP, also increased the contractility of muscle cells from gallbladders with CS to levels similar to those from specimens with PS. It is concluded that the contractility of muscle cells from specimens with CS is restored by activating the postsynaptic CCK pathways and the muscle defect may be due to abnormal receptor function and/or abnormal interaction between receptors and G proteins.

OXIII/687

ACUTE & CHRONIC EFFECTS OF ORAL CHOLESTYRAMINE ON GALLBLADDER & ANTRAL MOTILITY IN OBES & LEAN INDIVIDUALS. P. Fortiussella, A.D. Cialle, V. Palmieri, G. Palasciano, Institute of Clinica Medica I, University Medical School, Bari, Italy.

Gallbladder (GB) stasis is frequent in obesity which are at increased risk for gallstone crystal formation. We showed that cholestyramine (CH) enhances cholecystokinin release and postprandial GB evacuation in healthy subjects and ameliorates GB hypomotility in gallstone patients (Gastroentero 1992, 102;673 and 102;A239).

We, thus, studied CH-induced GB emptying in obes and controls, also measuring gastric motor function which can be affected by CCK levels.

METHODS: Basal/residual GB volumes & antrum areas were assessed by scintigraphy every 5-10 min during 2 hr in 12 obes (2M,10F, age 37±13 yrs, BMI 39±12 kg/m2) and 7 lean volunteers (4M,3F, age 39±13 yrs, BMI 24±1). On different days subjects ingested either 200 mL of test meal alone (2 egg yolks) or in combination with CH 4g. Tests were repeated after 1 mo in 9 obes on CH 4g (3 times weekly).

RESULTS: (mean±SEM):

<table>
<thead>
<tr>
<th>GB Volume</th>
<th>Basal/L</th>
<th>Sequence A</th>
<th>Sequence B</th>
<th>Sequence C</th>
</tr>
</thead>
<tbody>
<tr>
<td>mL</td>
<td>18.3±1.8</td>
<td>18.0±1.2</td>
<td>18.7±1.2</td>
<td>18.6±1.2</td>
</tr>
<tr>
<td>Basal/ML</td>
<td>18.5±1.8</td>
<td>18.0±1.2</td>
<td>18.7±1.2</td>
<td>18.6±1.2</td>
</tr>
<tr>
<td>Residual/L</td>
<td>4.4±0.6</td>
<td>4.3±0.4</td>
<td>4.3±0.4</td>
<td>4.3±0.4</td>
</tr>
<tr>
<td>Residual/ML</td>
<td>4.4±0.6</td>
<td>4.3±0.4</td>
<td>4.3±0.4</td>
<td>4.3±0.4</td>
</tr>
<tr>
<td>AUC(10-5,M)</td>
<td>459±30</td>
<td>609±82</td>
<td>615±82</td>
<td>615±82</td>
</tr>
<tr>
<td>t/2,min</td>
<td>22±2</td>
<td>22±2</td>
<td>22±2</td>
<td>22±2</td>
</tr>
<tr>
<td>Tmax,min</td>
<td>22±2</td>
<td>22±2</td>
<td>22±2</td>
<td>22±2</td>
</tr>
</tbody>
</table>

CONCLUSION: CH-induced GB emptying was maintained. Gastric emptying increased transiently in cholecystokinin (AUC 5064±50) and cholecystokinin+CH (AUC 495±28) (p<0.05 vs prior meal+CH).

OXIII/663

CLOSING AND FUNCTIONAL EXPRESSION OF A CCK-R RECEPTOR FROM GUINEA PIG GALLBLadder. A. de March, JF Pirespa, SA Wank, Digestive Diseases Branch, HODGE, National Institutes of Health, Bethesda, MD 20892.

The contractile response to cholecystokinin (CCK) on isolated smooth muscle cells may be mediated by two distinct CCK receptor subtypes, one CCK preferring and the other gastrin preferring. We have recently isolated a cDNA from guinea pig gallbladder with sequence homology to cDNA receptor subtype. Its receptor expression pharmacology is consistent with the physiological data for a CCK preferring receptor subtype.

Screening a guinea pig gallbladder cDNA library with a [32P labelled, rat CCK receptor cDNA coding sequence probe in order to identify a second CCK receptor subtype with gastrin preferring pharmacology. The two longest cDNA clones identified (2.9 kb insert) contains a single long reading frame encoding a 172 amino acid polypeptide with 90% homology with the rat CCK receptor and a calculated molecular weight of 48,2 KDa. Hydropathy analysis indicates several regions of hydrophobic residues corresponding to putative transmembrane domains expected for members of the G-protein-coupled superfamil of receptors. The sequence allows for three potential N-linked glycosylation sites in the extracellular amino terminus. Expression analysis of the receptor cDNA subcloned into the mammalian expression vector pCDL-4RB-alpha and transiently expressed in COS-7 cells shows specific and saturable binding of [125I]-Bolton-Hunter labelled CCK-8 (125I-BH-CCK), CCK-8 and gastrin 17-1 inhibited binding with nearly equal potency (IC50 = 1.0nm) and was nearly complete in the presence of CCK receptor antagonist L 364,718. These results are similar to results seen for native CCK receptor in brain and gastric glands and is consistent with CCK receptor subtype. Conclusion: These studies demonstrate that in addition to a CCK-R, receptor subtype 2 exists which expresses a second CCK receptor with a typical CCK receptor subtype pharmacology.
INHIBITION OF CARCINOL-LIBERATED GALLBLADDER CONTRACTION AND PP RELEASE BY TELENEPEpine in Man. G. Dahmen, B.L. Keple*, P. Malfertheiner, G. Adler. Dept. of Internal Medicine, University of Ulm, and Medical Institute Klinikum Essen*, University of Munich, Germany.

Nonselective muscarinic receptor blockade with atropine inhibited caerulein-induced (GB) contraction and pancreatic polypeptide (PP) release in man. Studies using pirenzepine yielded conflicting results on the involvement of muscarinic receptor subtypes. We therefore investigated caerulein-induced GB-contraction and PP release during selective ML-receptor blockade by telenepine (T).

Methods: Eleven healthy male medical students volunteered for two series of experiments (4–7 days apart). T (1 mL/h) or placebo were infused i.v. over 2 h (randomized, double-blind). GB volume was assessed by ultrasonography before and during i.v.-infusion of two consecutive doses of caerulein (10 ng/kg i.v. at 45 min, then 50 ng/kg i.v. at 45 min). Calcium concentrations were measured at 15 time intervals by highly specific radioimmunoassays.

Results: Basal GB volume was 25% smaller and was not altered by T infusion (35 ml) before caerulein (C)-stimulation. Infusion of C caused GB volume reduction to 36% of initial volume at 75 min (end of low-dose C) and to 72% at 120 min (end of high-dose C). T inhibited integrated GB contraction (areas above the curves) from 3665±1038 to 1095±1001 (p<0.001). With regard to the different doses of C, T inhibited low-dose response by 78% (p<0.001) and high-dose response by 25% (n.s.). Basal and stimulated (due to C infusion) CCK plasma levels were not different on both days. PP plasma levels were increased by C infusion from 79±10 pg/ml to 104±25 pg/ml (n.s.) at 75 min and to 193±33 pg/ml (p<0.01) at 120 min. T completely suppressed stimulation of PP release, and PP plasma levels even fell below basal (from 82±25 pg/mI to 57±24 pg/ml at 120 min, p<0.01).

Conclusions: Selective ML-receptor blockade inhibits caerulein-induced GB contraction in man, especially during stimulation with a "physiologic" dose. These data support the hypothesis that ML-muscarinic receptors are involved in the anticholinergic inhibition of CCK-induced GB contraction in man. We furthermore have confirmed that an intact cholinergic innervation via ML-receptors is crucial for the stimulation of PP release.

Oxi/3/693

PROMOTION OF GALLBLADDER EMPTYING AND CHOLECYSTOKININ RELEASE BY RAPID INTRAVENOUS ADMINISTRATION OF AMINO ACIDS. G. Zoli, A. Ballinger, J. Healy, LID O'Donnell, ML Clark, M.JG Farthing. Depts. Gastroenterology & Radiology, St Bartholomew's Hospital, London, UK & I Medical Pathology, University of Bologna, Italy.

Patients receiving total intravenous nutrition have inert gallbladders and as a consequence develop gallbladder sludge and gallstones; these can be prevented by enhancing gallbladder emptying. We have explored the efficacy of rapid infusions of intravenous amino acids (IVAA) in stimulating cholecystokinin (CCK) release and gallbladder contraction, and determined the minimum infusion regimen capable of producing gallbladder emptying.

Eight healthy fasted subjects on four mornings received in random order four different infusion regimens of an IVAA mixture (Synthamin 14, B5I amino acids). Gallbladder volumes were determined by ultrasonography before and at 5 min intervals for 60 min after commencing the infusion. Blood was obtained by an indwelling i.v. cannula before and at 10, 20, 30, 45 and 60 min after the start of the infusion. Plasma CCK was measured by radioimmunoassay. Mean (SEM) fraction excretion with the various IVAA regimens were: 61:1.2%±12:5% with 250ml in 30 min, 76:8±8:5 with 250 ml in 10 min, 63:6±8:4 with 125ml in 5 min, and 24:4±8:0 with 50ml in 5 min. The latter regimen produced significantly less emptying than the other two regimens (p<0.01). The produced significant rise in plasma CCK concentrations. Peak and integrated CCK concentrations were not different with infusions of 250ml in 30 min (mean 5:9±1:1 pmol/l, 211:3±3:2 pmol/l/60 min, respectively) 250ml in 10 min (8:2±0:5, 235:6±6:13), 125ml in 5 min (7:0±0:7, 192:9±2:08), but were significantly lower with the infusion of 50 ml in 5 min (2:1±0:2, 82:4±6:3; p<0.001).

Intermittent rapid infusion of IVAA in a load as low as 125ml in 5 min promotes CCK release and gallbladder emptying and should prove useful therapeutically, not only during intravenous nutrition, but also in other situations associated with gallbladder inactivity, such as critically ill patients in intensive care units and during the post-operative.

Oxi/3/653


When used in the treatment of acromegaly, the somastostatin analogue octreotide (OT), induces gallbladder stones (GBS) in 40-70% of patients. We postulate that the unusual explanation for these iatrogenic stones is that OT impairs GB motility, but we showed recently that OT also induces isoeugenol changes in biliary acid (BA) and cholesterol (CH) composition (Dub 1992 33:1S26, 33:2S57). Nothing, however, is known about OT's effects on biliary phospholipids (PLs) - of importance since increases in arachidonic acid (20:4)-rich PLs (AAPLs) predict the development of GB stones.

Methods: Therefore, in 5 acromegalic patients studied before and during 3 mo of OT treatment (100 µg tds by SQI), we obtained samples of fresh GB bile by US-guided percutaneous fine-needle puncture, measured the molecular species of phosphatidylcholine (PC - the principal biliary PL) by HPLC, and related the results to the relative proportions (meq/%) of total BA, PL, and CH (measured enzymatically) and to the resultant CH saturation indices (CSI). Results: The relative proportions of the major PC molecular species were: PL 16:0-18:1, were similar before and during OT. However, PC 16:0-20:4 (the predominant AAPL) increased from 10% to 23% during OT (p<0.05), while PC 18:0-20:4 rose from 4.3% to 28% during OT (p<0.01). These changes in AAPLs were associated with an increase in the CSI, from 0.89±0.08 to 1.18±0.4 (p<0.05).

Summary: In 5 acromegalic patients, OT increases the proportion of AAPLs in gallbladder bile, with an associated rise in the biliary CSI. These conclusions are similar to those found in patients with CH GBs, and suggest that biliary AAPLs may also be important in the pathogenesis of OT-induced GBs.