Isolation and Purification of Human Biliary Vesicles With Potent Cholesterol Nucleation-Promoting Activity

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Abstract

1. Cholesterol nucleation is a critical step in the formation of cholesterol gallstones. This nucleation takes place after aggregation and fusion of cholesterol-rich biliary vesicles, a process probably modulated by biliary proteins. The present study was conducted to identify specific proteins associated with native cholesterol-rich biliary vesicles and to explore their effect on the cholesterol-nucleation time of supersaturated artificial bile.

2. Hepatic bile was obtained from six patients with cholesterol gallstone disease. Biliary vesicles were isolated by ultracentrifugation and were purified by gel filtration chromatography. A small amount of protein (less than 1% by weight) remained associated with the purified cholesterol-rich biliary vesicles. The electrophoretic profile of these proteins was remarkably similar in all six patients, showing the presence of at least six polypeptides (of molecular mass from 52 to 200 kDa), five of them having carbohydrate residues (except the 52 kDa one). The effect of reconstituted biliary vesicle solutions, containing their specific vesicular proteins, on cholesterol-nucleation time was studied by mixing the vesicle solution with artificial supersaturated bile. A potent cholesterol-pronucleating activity, reflected in a 20–70% reduction in nucleation time, was present in the biliary vesicle solutions compared with control solutions having a similar lipid composition. The pronucleating activity disappeared on heating and was not detected in the micellar fraction containing the major proportion of biliary proteins.

3. These results indicate that cholesterol-rich biliary vesicles containing a unique and defined glycoprotein profile can be isolated and purified from human hepatic bile. The potent cholesterol-pronucleating activity of the biliary vesicles from patients with gallstones was unrelated to their lipid composition or cholesterol content. This activity was probably associated with their glycoprotein component, supporting the view that vesicular proteins may have a central role in the pathogenesis of cholesterol gallstone disease.