Thin Layer Chromatographic and IR Spectral Evidence for the Presence of Phosphonolipids in Human Sperm

Michael C. Moschidis

A. Dedoussi E. E., Pharmaceutical Company, Schimatari, Viotias, Greece

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Phosphono Analogue of Phosphatidyl Choline, Phosphonolipids, Phospholipids

The total phosphonolipids from human sperm have been isolated by means of preparative TLC and among these the phosphono analogue of phosphatidyl choline has been identified by chromatographic and spectral techniques.

Introduction

Evidence for the presence of phosphonolipids in humans is scant. Herein is provided evidence for the presence of phosphonolipids in human sperm. The phosphonolipids have been isolated by thin layer chromatography (TLC) and have been identified by TLC, IR spectroscopy, and nitrogen-phosphorus determinations.

Experimental

Materials

The solvents used were of pro-analysis or analytical reagent grade and were distilled before use. Reagents were purchased from Merck (Darmstadt, FRG) and VIORYL (Kifisia, Athens, Greece). Silica Gel G was purchased from Merck (Darmstadt, FRG) and silicic acid for column chromatography from Sigma (St. Louis, Mo., USA).

Human sperm was obtained from a sperm bank in Athens, Greece from a 30 year-old healthy male.

Methods

The human sperm was obtained from the same male subject on the same day and weighed 6.702 g. It was homogenized in methanol–chloroform (2:1, v/v) with a Sorvall homogenizer.

Preparative TLC was performed on glass plates coated with silica gel G to a thickness of 0.75 mm. The chromatograms were developed in methanol–water (2:1, v/v) (system A) [1] and the run normally took 80 min for full development. The solvent system chloroform–methanol–water (65:25:4), v/v/v) (system B) was also used for identification purposes and for quantitative isolation of the phospholipid classes.

The spots and bands were made visible with iodine, ammonium molybdate, ninhydrin and α-naphthol-sulfuric acid spray reagents and the Stillway and Harmon procedure [2].

IR spectra were recorded on a double-beam Perkin-Elmer 197 grating IR spectrophotometer as thin films from dry chloroform.

Total phosphorus and phosphono-phosphorus were determined by the procedure of Kapoulas [3] and total nitrogen and lipid nitrogen by the procedure of Kjeldahl and Lea *et al.* [4]. A glass column of length 40 cm and I.D. 2.4 cm was used for the column chromatography of the isolated phosphonolipids.

Procedure

The lipids from the homogenized sperm were extracted according to the procedure set out by Kates [5], and the solvents were evaporated under vacuum using a bath of 35 °C. The total lipids were then extracted with acetone and the total phospholipids so obtained were dried in a vacuum desiccator over phosphorus pentoxide for 24 h.

The total phospholipids were dissolved in 10.0 ml of chloroform–methanol (1:1) and subjected to preparative TLC with solvent system A. The band of R_F 0.81–0.98 was scraped off and the phosphonolipids were obtained from the silica gel with chloroform.

The phospholipids were similarly obtained from the silica gel with chloroform–methanol (2:1).

The total phosphonolipids were fractionated on a silicic acid column. 8 g of silicic acid were used and the column was loaded to a height of 4.6 cm and a total volume of 20 ml. The flow rate maintained in the elution was 1.7–1.9 ml/min.

Results

Human sperm, 6.702 g, furnished, after extraction, 0.167 g of phospholipids, of which 3.2% per cent were phosphonolipids.

Chromatography of the phosphonolipid-free phospholipids (TLC in solvent system B) provided evidence for the presence of the following phospholipids: spingomyelin (R_F = 0.18), phosphatidyl ino-

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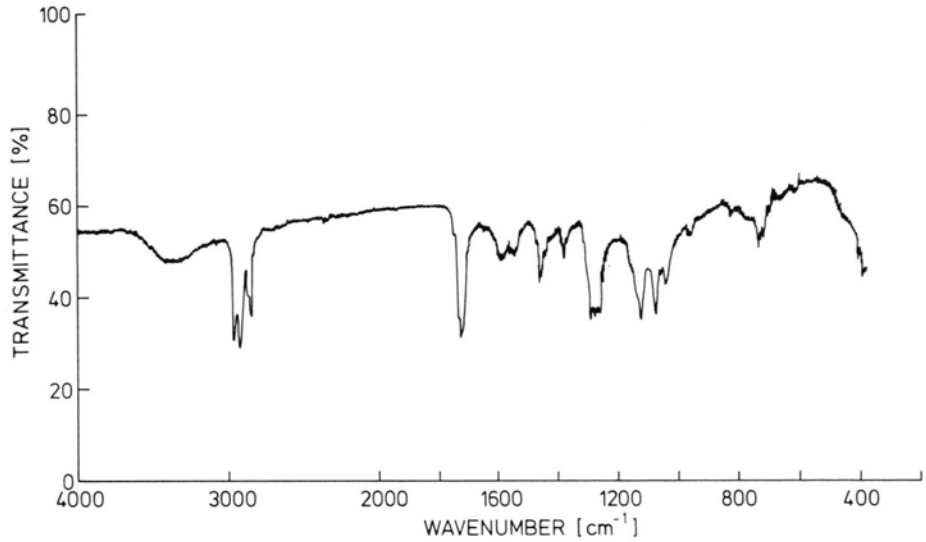

Fig. 1. IR spectrum of total human sperm phosphonolipids taken on sodium chloride optics as a thin film from dry chloroform.

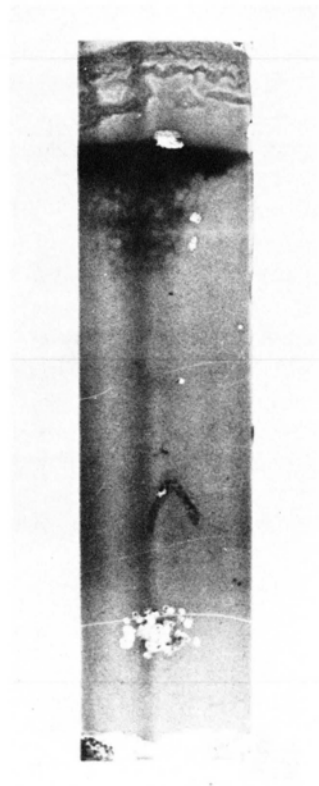

Fig. 2. Thin layer chromatographic profile of one of the constituents of the total phosphonolipid fraction, possessing an R_F value of 0.41, and identified as phosphonolecithin. Chromatography using silica gel G and the solvent system chloroform–methanol–water (65:25:4, v/v/v). Visualization was effected with ammonium molybdate spray.

sitol (R_F = 0.25), phosphatidyl choline (R_F = 0.33), phosphatidyl-N,N-dimethyl ethanolamine (R_F = 0.55), phosphatidyl ethanolamine (R_F = 0.66), cardiolipin (R_F = 0.84) and one additional spot with R_F = 0.09 attributed to lyso-lecithin, and one spot with R_F = 0.49 identified as phosphatidyl glycerol.

The IR spectrum of the total human sperm phosphonolipids is shown in Fig. 1.

The isolated phosphonolipids released no phospholipid phosphorus and was shown to contain phosphono-phosphorus when subjected to respective phosphorus determinations [3]. Also the phosphonolipid extract was free from amino-acids and sugars, as no indication was obtained for their presence on the chromatograms when treated with the respective sprays.

The following spots were obtained when the phosphono extract was chromatographed in solvent system B: R_F (a) 0.17; (b) 0.41 and (c) 0.56.

Of these only spot (b) amounted to any appreciable quantity, was subsequently scraped off the silica gel and extracted with chloroform. The substance when rechromatographed in solvent system B, exhib-

ited an R_F value of 0.41. Analysis for nitrogen gave 2.10% and for phosphorus 4.37% with a total abundance of 17.80% in the original phosphonolipid extract. The substance was identified as the phosphono-analog of phosphatidyl choline.

In Fig. 2 is shown the thin-layer chromatographic profile of the isolated phosphono-lecithin from human sperm.

Discussion

By the combined use of thin-layer chromatographic and IR spectroscopy has been possible to isolate the human sperm phosphonolipids and identify phosphonolecithin as one of the constituents of the human sperm phosphonolipids.

The complete identification of all the inherent phosphonolipids has not been possible, because of the limited quantities of human sperm available and the low abundance of phosphonolipids in the total phospholipid fraction.

The evidence for the presence of phosphonolipids in human sperm, may prove to be of value from a biochemical point of view, in the elucidation of certain aspects of human metabolism.

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