Nondestructive Analysis of Cholesterol and Collagen in Atherosclerotic Plaques using NIR-FT-Spectroscopy

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Introduction:
Detailed examination of human arteries, especially of coronary arteries, in respect to the narrowing of the lumen, the consistency, and the special features of the arterial wall can well be performed using the most recent catheter techniques. Now, however, the chemical composition of atherosclerotic plaques could not be determined without surgical intervention. Plaques formed by atherosclerotic lesions are heterogeneous. A sudden rupture of the plaque followed by a local obstruction thrombosis is the most prominent cause of an acute coronary event. The risk of plaque disruption indeed depends more on its composition and vulnerability than on the degree of stenosis. In a practical sense, knowledge of the chemical composition of these plaques could influence therapeutical strategies significantly. To do this, determination of the main compounds of the arterial wall (cholesterol, collagen) without danger for the endothelial cells.

Aim:
The aim of our investigation was to examine whether near infrared spectroscopy is a useful tool for the quantitative determination of the main components of the aortic wall (cholesterol, collagen) without danger for the endothelial cells.

Questions:
- Can the total cholesterol and collagen content of the arterial intima be estimated with acceptable precision in vitro by NIRS? Despite the matrix inhomogeneity of the plaques and their great anatomic variability?
- How deep can NIRS radiation penetrate into arterial tissue and which dose is sufficiently to measure the cholesterol and collagen content in coronary plaques without danger for the endothelial cells?
- Is the penetration sufficient to obtain information on lipid accumulation?
- Can acceptable results be obtained using a fiber optic strand with a coronary catheter-like diameter (d=1-1.5 mm) with right-angled light exit?

Material and Methods:
- FT-spectrophotometer DFS 20 (Bruker Avantik GmbH, Karlsruhe)
- a) InGaAs-Detector, Cell: beam splitter, wavelength 1000 – 2500 cm⁻¹, spectral resolution 8 cm⁻¹, time of measurement 30 sec (20 scans)
b) Fiber optic strand: optical window d=4 mm, length 1.5 m
- Cholesterol: RP-HPLC according Vercaemst et al.
- Artificial model mixtures: composition see table 1
- Human aortic specimens obtained at autopsy, 10 different aortas
- Apparatus: Spectrospin 800 (Spectrospin GmbH, Berlin)
- Chemical reference method for determination of free and esterified cholesterol: RP-HPLC according Vercaemst et al.
- Vitality indicators of endothelial cell cultures: mitochondrial potential

Conclusions:
- Using near infrared diffuse spectroscopy (λ = 1500 – 2500 nm) and fiber optic strand (diameter d=4 mm) the cholesterol content of model mixtures of the main compounds of aortic wall can be determined with high precision (r=0.907, y=0.922+0.806)
- The NIRS value of cholesterol in human aortic samples was not possible.
- Using a thin coronary catheter-like fiber optic strand (d=1 mm) the cholesterol determination in the intima is also acceptable (r=0.837, y=0.795+1.801)
- The energy dose of 71 mW/cm² (4 mm fiber optic strand) seems to be not hazardous for endothelial cells with measuring times of 30 seconds. Also higher doses (up to 50-100 nm) do not injure human endothelial cells in culture.

Using a thin fiberoptic strand (d=400µm) with a right-angled light exit the cholesterol and collagen determination in model mixtures is possible with acceptable results: cholesterol: r=0.837, y=0.922+0.806
- Collagen: r=0.705, y=0.795+1.801

Unfortunately, the spectra of human aortic samples showed a low signal/noise ratio and a poor reproducibility (Figure 5 shows the spectra of a non-atherosclerosis, an atherosclerosis and a human aortic sample). That is why, quantitative determination of cholesterol in human aortic samples was not possible.

Results:

- Figure 1: Correlation between the determination of total cholesterol in human aortic specimens by HPLC and NIRS with fiberoptic strand d=4 mm
- Figure 2: Correlation between the determination of total collagen in human aortic specimens by HPLC and NIRS with fiberoptic strand d=1 mm
- Figure 3: Determination of total cholesterol and correlation between the true value in the model mixtures and the determination with NIRS
- Figure 4: Determination of collagen and correlation between the true value in the model mixtures and the determination with NIRS
- Figure 5: NIRS spectra (first derivative) of model mixtures and human aortic samples