Isotope-Selective Infrared Spectroscopy Reveals Pathological Changes in the Liver

**Jana VRANOVÁ** (CZ) jana.vranova@if3.cuni.cz

**Jozef ROSINA** (CZ) rosina@fbmi.cvut.cz

**Jiří HORAK** (CZ) horak@fnkv.cz

**Miluše HENDRICHova** (CZ) milusehendrich@centrum.cz

**Karolina KRATKA** (CZ) karolinakratka@seznam.cz

**BIOGRAPHICAL NOTES**

Ing. Jana Vránová, PhD. is an assistant professor at the Department of Medical Biophysics and Medical Informatics, 3rd Faculty of Medicine, Charles University in Prague. She graduated in 1984 at the Electrotechnical Faculty, Czech Technical University in Prague, specialisation: the technical cybernetics. Her professional orientation is the analysis of medical issues. She is an author of many hospital information systems (modules) for both, internal and surgical disciplines, such as: oncology, internal clinic, ophthalmologic clinic, surgery, urology, neurosurgery, pediatric surgery.

doc. M.D. Jozef Rosina, PhD. is an associate professor at the 3rd Faculty of Medicine, Charles University in Prague. On the present he is undertaking a function of the Dean of the Faculty of Biomedical Engineering, Czech Technical University in Prague and at the same time he is the Head of the Department of Medical Biophysics and Medical Informatics, 3rd Faculty of Medicine, Charles University in Prague. He graduated at the 2nd Moscow Faculty of Medicine in 1981. He is a member of many prestigious councils and commissions.

**KEY WORDS**

$^{13}$C-methacetin, dose, cumulative dose, infrared spectroscopy, transmittance, absorbance, cirrhosis

**ABSTRACT**

An overview of new diagnostic device IRIS (Wagner Analysen Technik, Germany) is provided. This device uses the $^{13}$C stable isotope together with non-dispersive infrared selective spectroscopy for non-invasive quantification of hepatic function parameters, including kinetics (type of liver damage) and capacity (percentage of recovery yield of the liver). The short physical principle of infrared spectroscopy and acoustooptical detector of IRIS was presented. In the final section we verified the diagnostic quality of this new diagnostic device (using ANOVA) on cohort of 76 patients divided into 5 groups according to diagnosis and the various level of liver damage.

**INTRODUCTION**

Investigation of liver function stretches the entire history of modern hepatology.
Once widespread chromoexcretory tests such as bromsulfophthalein test or so-called galactose elimination test was gradually abandoned and replaced by metabolic tests using radioactive isotopes, which also had their significant limitations. Isotope $^{13}\text{C}$-labeled substances have the last word in the quantification of liver function. They occur in nature, do not emit ionizing radiation and are therefore completely safe. The principle of testing is simple - proband consumes appropriate material labeled by $^{13}\text{C}$ carbon, for example methacetin. It is metabolized in the liver to carbon dioxide and the amount of $^{13}\text{C}_2\text{O}$ in exhaled air is a measure of the intensity of hepatic metabolism. Test results correlate very well with Child-Pugh classification of liver cirrhosis and in addition are applicable for monitoring changes in all liver functions. For the determination of $^{13}\text{C}_2\text{O}$, in exhaled air is used infrared spectroscopy isotope. We themselves use for several years in our department apparatus IRIS (InfraRed Nondispersive Isotope-Selective Spectroscopy) from Wagner Analysen Technik, Germany [1]. IRIS is supplied with software which is able to distinguish between normal liver, stimulated hepatic function (metabolism of the liver is very fast, stimulated by permanent degradation of toxic substances (alcohol, hepatotoxic drugs, etc.), liver cirrhosis (metabolism of the liver is highly damaged). The values of measured quantities, i.e. $\%$ $^{13}\text{C}$ dose/hr and $\%$ $^{13}\text{C}$ cumulated dose in the expired air sample for these three cases, together with reference ranges that define the zones of normal hepatic function, are shown in Fig. 1 and 2.

**MAIN PRINCIPLES**

$^{13}\text{C}$-Methacetin

$^{13}\text{C}$ commonly occurs in nature, and under normal conditions it represents 1.1% of the total carbon content in the human body. $^{13}\text{C}$-methacetin is metabolized as well as widespread carbon $^{12}\text{C}$, it is non-toxic, non-radioactive, and represents a stable isotope, therefore suitable for use in diagnostics. $^{13}\text{C}$-methacetin is metabolized in the hepatic mixed-function oxidase system using subgroups of the P450 cytochrome (CYP2E1 and CYP1A2) by demethylation/decarboxylation to acetaminophen and $\text{CO}_2$. The formed carbon dioxide is quickly absorbed into the blood, circulates to the lungs and is exhaled. Use of $^{13}\text{C}$-marked methacetin allows quantification of hepatic function parameters using the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio in exhaled breath; these parameters include kinetics ($\%$ $^{13}\text{C}$ dose/hr) that defines the type of liver damage, and capacity ($\%$ $^{13}\text{C}$ cumulated dose) that determines the percentage of recovery yield. Additionally, since cytochrome CYP2E1 is active in alcohol metabolism, and is also damaged and stimulated by alcohol, the $^{13}\text{C}$-methacetin breath test is particularly valuable in assessing hepatic diseases caused by alcohol [1].

The $^{13}\text{C}$-methacetin breath test is also used to determine the stage of NASH (non-alcoholic steatohepatitis, characterized by hepatic involvement, morphologically similar to hepatic involvement in alcohol abuse) or ASH (alcoholic steatohepatitis - alcoholic liver damage), and assessing liver fibrosis, steatosis, cirrhosis; the test provides information...
on the reserve capacity of the liver and is also a very suitable instrument for accurate timing of liver transplantation [2].

**INFRARED SPECTROSCOPY**

Infrared spectroscopy is an analytical technique designed for identification and structural characterization of organic compounds and inorganic substances. It is based on an interaction of the measured sample with infrared radiation (electromagnetic waves with wavelengths from 800 nm to 1 mm). The energy of infrared photons (1 kJ/mol - 60 kJ/mol) is not sufficient to excite electrons in molecular orbits; however, it is sufficient to change the vibrational or rotational state of molecules. When the change of vibrational and rotational states is associated with a change of dipole moments, absorption of radiation occurs, which is characteristic for a given bond within the molecule. Infrared spectroscopy observes the amount of infrared radiation absorbed as a function of the wavelength. The amount of absorbed radiation is expressed either as percent of transmittance (%T) or in units of absorbance (A). Transmittance is defined as the ratio of intensities of passed radiation (I) to the original beam (I₀) [3]:

\[
T = \frac{I}{I_0}
\]

Absorbance determines how much radiation was absorbed by the measured sample. Using transmittance, it is defined as follows:

\[
A = \log\left(\frac{1}{T}\right) = -\log(T) = c.l.ε
\]

where c = molar concentration; l = cell length; ε = molar absorption coefficient, tabulated.

This relationship is also called the Lambert - Beer law; according to this law, absorbance is proportional to the concentration of the absorbing substance [4].

Analytical output of infrared spectroscopy takes the form of infrared spectra, thus a graphical depiction of functional dependence of absorbed energy, expressed precisely by transmittance or absorbance, relative to wavelength. The infrared spectrum is characteristic for individual substances to such an extent that there are virtually no two compounds with an entirely identical infrared spectrum. An example of a typical infrared spectrum is shown in Fig. 3.

![Fig. 3 IR spectrum of the analyzed sample. Transmittance (up), absorbance (down)](image)

Together with non-dispersive infrared spectroscopy, IRIS also uses a broadband light source and an acousto-optical detector (of the Luft - Lehrer type), which is sensitive only to those wavelengths at which the examined gases are IR-absorbing. With two such detectors, which are individually sensitive to the absorption spectra of ¹²CO₂ and ¹³CO₂, the concentrations of these two gas components to be related to each others for ¹³C/¹²C-isotope ratio determination are measured [2]. The IR-absorption spectra of the asymmetrical stretch oscillations modes of both the ¹³CO₂- and ¹²CO₂-molecules are separated almost completely.

**Luft – Lehrer Detector**

The basic scheme of the detector is shown in Fig. 4 [5]. The detector consists of a sealed chamber where molecules of the absorbing substance, which may be present in the sample gas, are placed. The flexible membrane plate of the capacitor partitions the rear section of the detector where the solid plate capacitor is located, from the front, absorbing part, where the window with high optical transmittance is located, and through which the chopped infrared radiation enters the chamber. A detector sig-
nal arises from the change in the capacitor voltage generated by the displacement of the flexible or movable capacitor plate. The gas in the absorption compartment is heated by the IR energy entering through the optical window. With the temperature rise, there is a corresponding pressure increase and the gas expands against the membrane. Chopped IR beams produce membrane displacement oscillations at the chopping frequency. The intensity of the IR beam entering the detector is inversely proportional to the concentration of the absorbing species in the sample cell.

SUBJECTS AND METHODS

Patients come in a fasting state for the procedure; they breathe into the first breath bag before consuming the substrate drink (provides baseline 13C-methacetin). Immediately, they consume the test drink (75 mg of 13C-methacetin dissolved in 200 ml of unsweetened tea or water). Breath samples were collected at 10, 20, 30, 40, 50, 60, 80, 100 and 120 minutes following ingestion of the 13C-methacetin substrate according to the protocol of Liver Function 2 (IRIS User’s Guide). Samples were collected by having patients take a deep breath and slowly exhale into additional breath bags.

IRIS is capable to distinguish between a normal, stimulated, or cirrhotic liver. The main goal of our study was to determine whether there were statistically significant differences between the measured values of the dose and cumulated dose of 13C-methacetin measured using IRIS among 5 different patient groups which had been divided according to diagnosis for all time intervals. Additionally, the goal was to determine which sample times show the greatest difference, in other words, which sample time or times provide the "highest diagnostic power". The measurement was done on 76 patients [6], [7]. Group 1 consisted of patients with no serious disease - the control group (N=11); group 2 consisted of patients with cirrhosis (N=10); group 3 consisted of patients with compensated ischemic heart failure (N=30); group 4 consisted of patients with concurrent nephrological and cardiovascular disease (N=10); and group 5 consisted of patients with decompensated heart failure (N=15). Group 4 and 5 were included to determine whether severe liver damage is present in severe heart damage or renal insufficiency.

Analysis of variance (ANOVA) with subsequent application of the Bonferroni’s test for multiple comparisons was used to evaluate the data. STATISTICA,
Fig. 5 Graph of mean values and confidence intervals \(^{13}C\) dose/hr [%]. Yellow line-Controls, Pink line-Cirrhosis, Blue line-Compensated Heart Failure, Red line - Concurrent Nephrological and Cardiovascular Disease, Green line-Decompensated Heart Failure

Tab. 2 ANOVA test results. Time intervals with the highest discrimination ability are highlighted.

<table>
<thead>
<tr>
<th>Test Time [min.]</th>
<th>Dose/hr [%]</th>
<th>Cumulated dose [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>10</td>
<td>13,374</td>
<td>0,000000</td>
</tr>
<tr>
<td>20</td>
<td>15,606</td>
<td>0,000000</td>
</tr>
<tr>
<td>30</td>
<td>18,377</td>
<td>0,000000</td>
</tr>
<tr>
<td>40</td>
<td>16,380</td>
<td>0,000000</td>
</tr>
<tr>
<td>50</td>
<td>14,020</td>
<td>0,000000</td>
</tr>
<tr>
<td>60</td>
<td>13,684</td>
<td>0,000000</td>
</tr>
<tr>
<td>80</td>
<td>13,240</td>
<td>0,000000</td>
</tr>
<tr>
<td>100</td>
<td>9,112</td>
<td>0,000005</td>
</tr>
<tr>
<td>120</td>
<td>7,219</td>
<td>0,000062</td>
</tr>
</tbody>
</table>

version 9 from StatSoft Inc., was used for statistical analysis.

RESULTS

Table 1 shows mean values of the measured dose and the cumulated dose of \(^{13}C\)-methacetin in all time intervals for all 5 patient groups. Tab.2 summarizes ANOVA results-only F-test values and values of obtained significance level p.

Table 2 shows that significance levels are very low for all times; p=0,00 for almost all sample times. The discrimination power of IRIS can be thus evaluated according to the F-test value; the higher the F value, the lower the significance level, and the higher the discrimination power. It can be concluded from the values that the highest discrimination power for the \(^{13}C\) dose occurs at 30 and 40min., while the highest discrimination power for the cumulated dose occurs at 50, 60 and 80min. Fig. 5 and 6 present graphs of mean values and confidence intervals for the measured dose value and the cumulated dose of \(^{13}C\) for all 5 patient groups.

CONCLUSIONS

The results show clearly that slightly stimulated hepatic function is present in patients with concurrent nephrological and cardiovascular disease (this is probably due to long-term use of large amounts
of drugs many of which may induce the liver enzymes). In patients with decompensated heart failure, we observed the presence of highly impaired hepatic function, approaching liver cirrhosis. Results of the study are in absolute agreement with those expected and indicate that IRIS is a diagnostic apparatus of superior quality, which has a high ability to effectively distinguish between various degrees of liver damage.

REFERENCES

[1] Breath testing in clinical Diagnose [online] [cit 2010-06-18], http://www.wagner-bremen.de/


