CONTRIBUTIONS TO THE INVESTIGATION OF DRUG INTERACTIONS BETWEEN ANTITUBERCULOSIS MEDICATION AND OMEPRAZOLE: THE THERAPEUTICAL EFFECT / TOXICOLOGICAL RISK RATIO

ABSTRACT OF THE PhD THESIS

Scientific supervisor,

Prof. Univ. PhD. Elena BUTNARU

PhD student,

Magda COSTIN

IAŞI 2013
By virtue of the decision of the Rector of ”Grigore T. Popa” University of medicine and Pharmacy Iaşi no of , the following PhD thesis commision was approved:

The PhD Commission is formed of:

**President:** Univ. Prof. PhD, Monica Hăncianu Dean, ”Grigore T. Popa” University of Medicine and Pharmacy of Iaşi

**Scientific supervisor:** Univ. Prof. PhD Elena Butnaru”Grigore T. Popa” University of Medicine and Pharmacy of Iaşi

Scientific reviewers:

**Prof. Univ. Dr. Daniela Baconi**

”Carol Davila” University of Medicine and Pharmacy of Bucureşti

**Prof. Univ. Dr. Cristina Dehelean**

”Victor Babeş” University of Medicine and Pharmacy of Timișoara

**Prof. Univ. PhD. Traian Mihăescu**

”Grigore T. Popa” University of Medicine and Pharmacy of Iaşi
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**The PhD thesis includes:**

- 153 pages (Present state of the domain - 33 pages, original contribution – 120 pages)
- 44 figures
- 72 tables
- 200 bibliographical citations
- Approval for the study
- Agreement
- Scientific papers: 1 ISI; 1 B+

The present abstract resumes the same numbering as in the thesis for contents, tables, figures and bibliographical citations.

**Keywords:** isoniazid, omeprazole, antitubercular medication, adverse reactions, isoniazid – omeprazole interactions.
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<tr>
<td>Consent</td>
<td></td>
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</table>
INTRODUCTION. MAIN OBJECTIVES

Medicinal interactions resulting from the association of the various groups of drugs represent a topic of special interest for both pharmacologists and toxicologists. Medicinal associations are useful for a simultaneous treatment of several diseases or symptoms, especially those producing interactions which reduce the pharmaco-dynamic action and intensify the adverse effects, on one side, or those which intensify the pharmacodynamic action and diminish the adverse effects, on the other.

Medicinal interactions represent a major cause of adverse reactions, following polydrug therapies, their incidence being directly proportional with the number of associated drugs.

Interactions may be manifested either prior to drug administration, as a consequence of certain physical or physical-chemical phenomena-defined as in vitro interactions representing incompatibilities or of their in vivo administration, as a result of some pharmacokinetic, pharmacodynamic or pharmacotoxicological interactions.

Especially interesting are the pharmacokinetic interactions, occurring as a result of drugs’ hepatic biotransformation. As generally known, the liver represents a key factor in the biotransformation of exogenous substances, on the basis of the principle of a higher solubility, assuring a higher elimination rate off the organism. An important role is played, in hepatic biotransformation, by hepatic microzomes and by cytochrome P450. The competition manifested between drugs for the isoforms of cytochrome P450 is a determining factor of drug interactions, increasing the risk of the under or overdosage phenomena, known as responsible for therapeutical inefficiency and exacerbated adverse reactions.
Abstract

The effects of medicinal interactions may be either synergistic or antagonistic. Pharmacodynamic interactions are of synergistic type, when the drugs act in the same direction, and of antagonistic type, when they act in reverse direction.

The literature of the field specifies that medicinal interactions may provoke loss of the therapeutical effect or a higher drug toxicity; also, they may increase efficiency, by inducing a more intense effect, and reduce toxicity.

Association of drugs may influence metabolization through phenomena of enzymatic induction or enzymatic inhibition.

Association of the two classes of drugs – antitubercular medication (isoniazid 300 mg) and proton pump inhibitors (omeprazole 20 mg) causes enzymatic inhibition, a phenomenon frequently met in the medical practice at the Clinical Hospital of Pneumophthisiology of Iași. The consequences of enzymatic inhibition may be of pharmaco-therapeutical type, when the therapeutic effect is increased, or of pharmacotoxicological type, which results in a higher incidence and seriousness of the adverse effects.

Investigation of the two simultaneously administered groups of drugs started from the reports of clinicians, obliged to continue the treatment with antitubercular medication over a longer period of time than the standard one, recommended by World Health Organization. Worth mentioning in this respect is the fact that, suffering from gastro-duodenal diseases, all patients had received, besides antitubercular medication, proton pump inhibitors (omeprazole 20 mg).

To the best of our knowledge, the literature of the field provides no studies proving that association of the two classes of
Abstract

drugs - namely antitubercular medication and proton pump inhibitors – might influence isoniazid absorption by the presence of omeprazole.

Objectives of the investigation

The research put forward in the present PhD thesis – based on a minute documentation and thorough consultation of the existing literature in the domain – is aimed at elucidating the following objectives:

- Quantitative determination of isoniazid through UV-VIS spectrophotometry
- Elaboration and validation of a HPLC-UV method for a simultaneous determination of isoniazid and omeprazole presence in biological fluids (human serum)
- Application of the validated HPLC-UV method for a simultaneous determination of the serum concentrations of isoniazid and omeprazole in the patients participating to the study
- Statistical evaluation of the biochemical and hematological parameters of the investigated subjects vs a reference group
- Statistical analysis of the adverse reactions induced by antitubercular medication administered in the Clinical Hospital of Pneumophthisiology of Iaşi along a 72 month period (2007- 2012)
- Experimental analysis of the isoniazid – omeprazole interaction.
Abstract

The thesis is structured into two main parts, as follows: ”Present-state of knowledge” (chapters 1–3) and ”Original contribution” (chapters 4 – 9).

A. PRESENT-STATE OF KNOWLEDGE

Analysis of the present-state of knowledge in the here investigated domain includes general considerations on antitubercular medication, classification of chemical-therapeutical antitubercular drugs, mechanisms of action, information on isoniazid, such as: toxico-kinetic, toxico-dynamic peculiarities, adverse reactions, symptomatology of acute and chronic intoxications, treatment. Also included are some general considerations on the proton pump inhibiting medication, with reference to the symptomatology of active ulcer, etiology of ulcerous disease, information on omeprazole - toxico-kinetic, toxico-dynamic peculiarities, adverse reactions, symptomatology of acute and chronic intoxications. The analysis routes applied to isoniazid and omeprazole include chromatographic, spectral and electrochemical methods.

B. ORIGINAL CONTRIBUTION

5. DEVELOPMENT AND VALIDATION OF A METHOD OF SIMULTANEOUS DETERMINATION OF ISONIAZID AND OMEPRAZOLE THROUGH HPLC-UV

5.1. Introduction

The present study was devoted to the separation and identification of isoniazid (HIN) and omeprazole through HPLC
Abstract

analysis with UV detection on the same chromatogram, as well as to the development of a selective method, with reference to other antitubercular drugs (pyrazinamide, ethambutol, rifampicine, streptomycin) usually recommended in tuberculostatic treatments.

Objectives

The main objective of the study was to elaborate and validate a HPLC-UV analysis method for a simultaneous determination of isoniazid and omeprazole from biological fluids (human serum), on following ICH recommendations and specialized literature.

DEVELOPMENT AND VALIDATION OF THE METHOD

5.2. Materials and method

Reactives

- isoniazid CRS (Sigma Aldrich, Germany);
- 2-pyridylamine (internal standard) CRS (Sigma Aldrich, Germany);
- omeprazole CRS (Molekula BioChimica, Germany);
- lansoprazole (internal standard) CRS (Molekula BioChimica, Germany);
- triethylamine R, formic acid R, acetonitrile R, ammonium (Merck, Germany);
- methanol (Sigma Aldrich, Germany);

Equipments
Abstract

All determinations were made on a **Thermo- Fischer Scientific Surveyor HPLC Plus System** equipped with autosampler and UV-VIS detector with photodiode network. The stationary phase was the Octasyl C 8 (Purospher RP8) chromatographic column, and the mobile phase: 10 mM triethylamine, pH 10.5 (10% solution of orthophosphoric acid) and acetonitrile 67:33 (v/v). Along the whole investigation, an isocratic solution with a flow maintained at 1 mL/min was applied, at a temperature of 25°C. The following wavelengths were selected:

- 260 nm for isoniazid and 2-pyridylamine (internal standard);
- 300 nm for omeprazole and lansoprazole (internal standard).

The injection volume was of 5 µL. Development and validation of the HPLC method was based on the same software - Chrom Quest – for data processing. The following validation parameters were analyzed: specificity/selectivity, linearity of the method, precision, accuracy/mean recovery, detection limit and quantification limit.

5.3. Results. Validation of the method

5.3.1. Selectivity / Specificity

Identification of the peaks corresponding to isoniazid and 2-pyridylamine (internal standard), omeprazole and lansoprazole (internal standard) involved preparation of solutions of various concentrations, for each substance in part, the following determinations being performed:

- isoniazid 1 000 ng/mL and 5 000 ng/mL;
Abstract

- 2-pyridylamine 10 000 ng/mL;
- omeprazole 1 000 ng/mL and 5 000 ng/mL;
- lansoprazole 5 000 ng/mL.

The retention times of the main compounds were followed for both concentrations, and the maximum values of the molecular absorption spectra of each compound were recorded. The following retention time were recorded: for isoniazid - about 2.35 min, for 2-pyridylamine - around 3.55 min (figure 5.1.a), for omeprazole - about 4.05 min, and for lansoprazole - about 6.71 min, respectively (figure 5.1.b). Also, analysis of a blanc solution at 260 nm and 300 nm evidenced no secondary signals interfering with the here discussed substances. The method is specific for a simultaneous determination of isoniazid and omeprazole from serum samples.

![Chromatogram of isoniazid and 2-pyridylamine at 260 nm]

**Fig. 5.1.a Chromatogram of isoniazid and 2-pyridylamine at 260 nm**
Fig. 5.1.b Chromatogram of omeprazole and lansoprazole at 300 nm

5.3.2. Linearity

To study the linearity of the method, 9 sets of solutions were prepared for each of the two reference substances – isoniazid and omeprazole – over the 50 – 5 000 ng/mL concentration interval, and 3 successive determinations were performed. The quantification method employed was that of the internal standard, using 2-pyridylamine in a concentration of 10 000 ng/mL, and lansoprazole in a concentration of 5 000 ng/mL, on also establishing the linearity domain (50 – 5 000 ng/mL). The calibration curve obtained shows a direct proportionality relation between the zone of the analytical signal and sample’s concentration over the studied interval, which demonstrates the
Abstract

linearity of the method (figure 5.5). The equation of the calibration line is:

**Peak area = 42.621 x concentration (ng/mL) + 1993.6**

![Calibration line of HPLC-UV isoniazid determination](image)

In the case of omeprazole, the obtained calibration curve evidences a direct proportionality relation between the area of the analytical signal and sample’s concentration over the studied interval, which demonstrates the linearity of the method (figure 5.6.). The equation of the calibration line is:

**Peak area = 51.48 x concentration (ng/mL) + 1,170.4**
Abstract

Fig. 5.6. Calibration line of HPLC-UV omeprazole determination

5.3.3. Detection limit

- detection limit for isoniazid LD = 63 ng/mL
- detection limit for omeprazole LD = 11.66 ng/mL

5.3.4. Quantification limit

- quantification limit for isoniazid LQ = 213 ng/mL
- quantification limit for omeprazole LQ = 38.89 ng/mL

5.3.5. Interval (working domain)

All determinations were performed over the 50-5000 ng/mL concentration domain.

5.3.6. Precision

The obtained chromatograms permitted measurement of peak areas and calculation of the mean value, standard deviation
Abstract

and relative standard deviation. The experimental values obtained when establishing the precision of the HPLC-UV determination method are listed in table 5.XIII.

Table 5.XIII. Precision of the HPLC-UV determination method of isoniazid and omeprazole

<table>
<thead>
<tr>
<th>No.</th>
<th>Day</th>
<th>Isoniazid</th>
<th>Omeprazole</th>
<th>2-pyridyl amine</th>
<th>Lansoprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>149652</td>
<td>206399</td>
<td>281797</td>
<td>293190</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>148958</td>
<td>206986</td>
<td>282658</td>
<td>295653</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>149632</td>
<td>207865</td>
<td>284256</td>
<td>294566</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>148658</td>
<td>207566</td>
<td>286167</td>
<td>282858</td>
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<tr>
<td>5</td>
<td></td>
<td>147896</td>
<td>205965</td>
<td>286588</td>
<td>285658</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>148236</td>
<td>206253</td>
<td>284566</td>
<td>286545</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>149563</td>
<td>206856</td>
<td>275559</td>
<td>281797</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>147856</td>
<td>207456</td>
<td>275986</td>
<td>282658</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>147236</td>
<td>207996</td>
<td>278956</td>
<td>284256</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>147655</td>
<td>207409</td>
<td>286687</td>
<td>286687</td>
</tr>
<tr>
<td>Mean value</td>
<td>148534.2</td>
<td>207075.1</td>
<td>282733.4</td>
<td>286847</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>889.6003</td>
<td>698.473</td>
<td>4207.465</td>
<td>287337.7</td>
</tr>
</tbody>
</table>
Abstract

| RSD (%) | 0.5989 | 0.3373 | 1.4881 | 1.2625 |

As, according to the obtained experimental data, the value of standard deviation (RSD) was below the imposed limit of 2%, one may assert that the HPLC-UV method of simultaneous determination of isoniazid and omeprazole is a precise one.

5.3.7. Accuracy/Exactness

To establish the exactness of the method of isoniazid and omeprazole determination, the working variant with a minimum of 9 determinations, covering the specific concentration domain, was preferred. The obtained values are listed in table 5.XIV.

In the study on the precision of the HPLC-UV method of simultaneous isoniazid and omeprazole determination, mean recovery takes a value of 100% over 95.8-104.4% interval for isoniazid and of 93.7%, respectively, over the 90.6-97.7% interval for omeprazole, - values supporting method’s precision.

Table 5.XIV. Precision of the HPLC-UV method of isoniazid and omeprazole determination
### Abstract

<table>
<thead>
<tr>
<th>No</th>
<th>Isoniazid concentration</th>
<th>Omeprazole concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Theoretical</td>
<td>Practical</td>
</tr>
<tr>
<td>1</td>
<td>300</td>
<td>313.3</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>299.2</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>287</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>500.5</td>
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<tr>
<td>5</td>
<td></td>
<td>494.8</td>
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<td>6</td>
<td></td>
<td>504.7</td>
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<td>7</td>
<td>700</td>
<td>709.6</td>
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<td>8</td>
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<td>697.2</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>693.3</td>
</tr>
<tr>
<td></td>
<td>Mean recovery</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>95.8</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>104.4</td>
</tr>
</tbody>
</table>

### 5.4. Conclusions

The HPLC-UV method elaborated for simultaneous dosing of isoniazid and omeprazole from human serum is characterized by selectivity, sensitivity (low detection and
Abstract

quantification limits), linearity over the studied domain (50 - 5000 ng/mL), precision and exactness (accuracy).

The HPLC-UV method was applied for isoniazid and omeprazole determination in the human serum of consumptive patients.

6. APPLICABILITY OF THE HPLC-UV METHOD FOR A SIMULTANEOUS DETERMINATION OF SERUM CONCENTRATIONS OF ISONIAZID AND OMEPRAZOLE IN PATIENTS WITH TUBERCULOSIS

6.1. Objectives

- Evaluation of serum concentrations of isoniazid and omeprazole, determined by HPLC-UV methods, after administration of antitubercular medication (isoniazid and proton pump inhibitors - omeprazole).

- Statistical evaluation of the serum concentrations of isoniazid and isoniazid with omeprazole, determined by HPLC-UV methods, in the first, third, fifth and seventh day of treatment.

6.2. Materials and method

**Reactives and biological samples**

- isoniazid (reference substance), 2-pyridylamine (internal standard) – Sigma Aldrich-Germany;

- omeprazole (reference substance), lansoprazole (internal standard) – Molekula BioChimica – Germany;
Abstract

- triethylamine (R), acetonitrile (R), formic acid (R), aqueous solution of ammonium (25%) - Merck KgaA – Germany;
- methanol (R) – Sigma Aldrich – Germany;
- human serum collected from TBC patients treated with HIN, and from patients treated with HIN and omeprazole.

6.3. Results

Table 6.III. centralizes the personal data of the patients from the two groups as a function of sex, age and social origin.

Table 6.III. Structure of groups by sex, origin and groups of age

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Sex</th>
<th>Social origin</th>
<th>Maximum age</th>
<th>Minimum age</th>
<th>Average age</th>
<th>Groups of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>F</td>
<td>Urban</td>
<td>Rural</td>
<td>Under 20</td>
<td>21 - 30</td>
</tr>
<tr>
<td>Lot 1</td>
<td>30</td>
<td>21</td>
<td>9</td>
<td>6</td>
<td>24</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>Lot 2</td>
<td>30</td>
<td>24</td>
<td>6</td>
<td>8</td>
<td>22</td>
<td>24</td>
<td>43,70</td>
</tr>
</tbody>
</table>

Seric concentrations of isoniazid, determined by HPLC-UV, are listed in table 6.V. as a function of the time of their collecting (first, third, fifth and seventh day), and minimum, maximum and mean values for group 1.

Table 6.V. Minimum, maximum and mean concentrations of isoniazid in serum

<table>
<thead>
<tr>
<th>Day</th>
<th>D1</th>
<th>D3</th>
<th>D5</th>
<th>D7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>
In the patients forming group 2, the serum concentrations of isoniazide and omeprazole were determined by HPLC-UV along the 7 days of the treatment, namely in the first, third, fifth and seventh day. Also, the values of the minimum, maximum and mean concentrations of isoniazid and omeprazole were determined (table 6.VII.).

<table>
<thead>
<tr>
<th>Patients</th>
<th>Report areas</th>
<th>Isoniazid concentration</th>
<th>Report areas</th>
<th>Isoniazid concentration</th>
<th>Report areas</th>
<th>Isoniazid concentration</th>
<th>Report areas</th>
<th>Isoniazid concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>0.11</td>
<td>0.28</td>
<td>0.36</td>
<td>0.96</td>
<td>0.40</td>
<td>1.06</td>
<td>0.50</td>
<td>1.36</td>
</tr>
<tr>
<td>Mean</td>
<td>0.51</td>
<td>1.36</td>
<td>0.70</td>
<td>1.89</td>
<td>0.82</td>
<td>2.23</td>
<td>1.02</td>
<td>2.76</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.86</td>
<td>2.32</td>
<td>0.96</td>
<td>2.61</td>
<td>1.14</td>
<td>3.11</td>
<td>1.41</td>
<td>3.85</td>
</tr>
</tbody>
</table>

Table 6.VII. Minimum, maximum and mean concentrations of isoniazid and omeprazole in serum
**Abstract**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Determined isoniazid concentration</th>
<th>Determined omeprazole concentration</th>
<th>Determined isoniazid concentration</th>
<th>Determined omeprazole concentration</th>
<th>Determined isoniazid concentration</th>
<th>Determined omeprazole concentration</th>
<th>Determined isoniazid concentration</th>
<th>Determined omeprazole concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>0.32</td>
<td>0.01</td>
<td>1.08</td>
<td>0.62</td>
<td>0.94</td>
<td>0.92</td>
<td>0.89</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean</td>
<td>2.33</td>
<td>0.37</td>
<td>2.54</td>
<td>0.39</td>
<td>2.40</td>
<td>0.32</td>
<td>1.96</td>
<td>0.36</td>
</tr>
<tr>
<td>Maximum</td>
<td>3.67</td>
<td>2.77</td>
<td>4.22</td>
<td>1.26</td>
<td>4.13</td>
<td>0.84</td>
<td>2.89</td>
<td>1.07</td>
</tr>
</tbody>
</table>

**6.4. Discussion**

Data listed in table 6.V. show that, after oral administration of isoniazid, the mean value of serum concentration determined by HPLC-UV was slightly increasing in the first day (1.36 mg/L), continuing until the seventh day of the treatment (2.76 mg/L).

Table 6.VII. synthesizes the minimum, maximum and mean seric concentrations of isoniazid and omeprazole, determined by HPLC-UV, in the patients from group 2. The observation made was that the mean values of seric concentrations of isoniazid are higher in the presence of omeprazole, in the first (2.33 mg/L), third (2.54 mg/L), and fifth day of treatment (2.40 mg/L). The mean values of seric concentration of omeprazole were: 0.37 mg/L in the first day, 0.39 mg/L in the third, 0.32 mg/L in the fifth day and 0.36 mg/L, respectively, in the seventh day.
Abstract

Such low values of omeprazole serum concentrations indicate a delayed absorption of the proton pump inhibitor. In spite of the fact that both drugs here under analysis – isoniazid and omeprazole - produce enzymatic inhibition, isoniazid acts as an inhibitor of omeprazole metabolization, which permits the conclusion that isoniazid has a biphasic – i.e., inhibition and induction – effect upon omeprazole.

6.5. Conclusions

- The serum concentrations of isoniazid were determined in the patients of group 1 (21 men and 9 women) treated in the Spitalul Clinical Hospital of Pneumophthisiology of Iasi by the validated HPLC-UV method.

- The values of serum concentrations of isoniazid, recorded in group 1, varied between 0.8 mg/L, in the first day, and 3.85 mg/L, respectively, in the seventh day of treatment.

- The serum concentrations of isoniazid and omeprazole were determined simultaneously, by the validated HPLC-UV method, in the patients forming group 2 (24 men and 6 women) hospitalized in the same medical unit.

- The values of serum concentrations of isoniazid in the patients forming group 2 varied between 0.32 mg/L in the first day and 4.22 mg/L, respectively, in the third day of treatment.

- In the first treatment day, the serum concentrations of omeprazole varied between 0.01 mg/L and 2.77 mg/L.

- In group 1, the mean values of serum concentrations of isoniazid increased from the first up to the seventh day of the treatment in group 1 while, in group 2, the mean values of serum
concentrations of isoniazid remained high along the whole duration of the study, a slight decrease being observed from the third (2.54 mg/L) up to the seventh day of treatment (1.96 mg/L).

7. STATISTICAL STUDY OF BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN TUBERCULAR PATIENTS TREATED IN THE CLINICAL HOSPITAL OF PNEUMOPTHISIOLOGY OF IAŞI IN 2011-2012

7.1. Introduction

Objectives

- Monitorization of the biochemical (TGP, TGO, glucose, creatinin, urea) and hematological (WBC, RBC, HGB, HCT, PLT) parameters in group 1, formed of patients treated with HIN.

- Evaluation of the minimum, maximum and mean values of the biochemical and hematological parameters in days 1, 3, 5, 7 of treatment, in patients from group 1.

- Monitorization of biochemical (TGP, TGO, glucose, creatinin, urea) and hematological (WBC, RBC, HGB, HCT, PLT) parameters in the patients of group 2, treated with omeprazole.

- Evaluation of the minimum, maximum and mean values of the biochemical and hematological parameters in days 1, 3, 5, 7 of treatment, in patients from group 2.

- The study was performed comparatively with a reference group.
7.2. Materials and method

Reactives and biological samples

- COBAS INTEGRA case with 500 tests for TGO, TGP, glucose, urea determination - Roche Diagnostics Ltd, Switzerland;

- COBAS INTEGRA case with 700 tests for creatinin determination - Roche Diagnostics Ltd, Switzerland;

- stromatolyser – 4 DL, Sysmex – Japan;

- stromatolyser – DS, Sysmex – Japan;

- sulfolyser, Sysmex – Japan;

- blood samples taken over from both patients and healthy subjects.

Equipments

- COBAS INTEGRA 400 plus automated analyzer for biochemistry - Roche Diagnostics Ltd, Switzerland

- Sysmex XS -1000i automated analyzer for hematology - Sysmex – Japan

7.3. Results

The study was developed on 60 patients, hospitalized in the Clinical Hospital of Pneumopthisiology of Iasi between 2011-2112, and on 30 healthy healthy volunteers, whose written consent was obtained prior to the investigation.

Table 7. IX.Mean values of the biochemical and hematological
parameters in the 1st, 3th, 5th and 7th day of treatment, comparatively with the reference group (L2 and LM)

<table>
<thead>
<tr>
<th></th>
<th>Biochemical parameters</th>
<th>Hematological parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean values</td>
<td>TGP</td>
<td>TGO</td>
</tr>
<tr>
<td>Mean L1/Z1</td>
<td>19.75</td>
<td>20.66</td>
</tr>
<tr>
<td>Mean L1/Z3</td>
<td>21.85</td>
<td>27.48</td>
</tr>
<tr>
<td>Mean L1/Z5</td>
<td>27.09</td>
<td>29.73</td>
</tr>
<tr>
<td>Mean L1/Z7</td>
<td>32.76</td>
<td>35.47</td>
</tr>
<tr>
<td>Mean L1 along 7 days</td>
<td>25.36</td>
<td>28.33</td>
</tr>
<tr>
<td>Maximum L1 along 7 days</td>
<td>32.76</td>
<td>35.47</td>
</tr>
<tr>
<td>Minimum L1 along 7 days</td>
<td>19.75</td>
<td>20.66</td>
</tr>
<tr>
<td>Mean value of the reference</td>
<td>17.81</td>
<td>20.13</td>
</tr>
</tbody>
</table>

Table 7.IX. centralizes the mean values obtained in the determination of the biochemical and hematological parameters in the first, third, fifth and seventh day of treatment for group 1. Also centralized were the minimum, maximum and mean values

27
**Abstract**

recorded along seven days of the treatment, comparatively with the normal mean values of the reference.

Table 7.XIV. centralizes the mean values of the biochemical and hematological parameters in the first, third, fifth and seventh day of treatment with omeprazole (group 2), and the minimum, maximum and mean values recorded along seven days of the treatment, comparatively with the normal reference values.

Table 7.XIV. Mean values of the biochemical and hematological parameters in the 1st, 3rd, 5th and 7th day of treatment, comparatively with the reference (L2 and LM)

<table>
<thead>
<tr>
<th></th>
<th>Biochemical parameters</th>
<th>Hematological parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean values</td>
<td>TGP</td>
<td>TGO</td>
</tr>
<tr>
<td>Mean L2/Z1</td>
<td>17.35</td>
<td>14.49</td>
</tr>
<tr>
<td>Mean L2/Z3</td>
<td>19.79</td>
<td>22.41</td>
</tr>
<tr>
<td>Mean L2/Z5</td>
<td>22.41</td>
<td>25.50</td>
</tr>
<tr>
<td>Mean L2/Z7</td>
<td>25.84</td>
<td>28.45</td>
</tr>
<tr>
<td>Mean L2 along 7 days</td>
<td>21.34</td>
<td>23.96</td>
</tr>
<tr>
<td>Maximum L2 along 7 days</td>
<td>50.30</td>
<td>63.92</td>
</tr>
<tr>
<td>Minimum L2 along 7 days</td>
<td>8.20</td>
<td>11.27</td>
</tr>
<tr>
<td>Mean reference value</td>
<td>17.81</td>
<td>20.13</td>
</tr>
</tbody>
</table>
Abstract

<table>
<thead>
<tr>
<th>Normal minimum-maximum values</th>
<th>max</th>
<th>max</th>
<th>60 - 110</th>
<th>0.60 – 1.30</th>
<th>10.00 – 50.00</th>
<th>4.00 – 10.00</th>
<th>4.25 – 5.50</th>
<th>12.00 – 17.00</th>
<th>37.00 – 47.00</th>
<th>150 – 450</th>
</tr>
</thead>
</table>

7.4. Discussion

Analysis of the biochemical parameters presented in table 7.IX shows a slightly higher mean value of TGP (25.36 U.I.) and TGO (28.33 U.I.), comparatively with the mean value of the reference TGP (17.81 U.I.) and TGO (20.13 U.I.), remaining nevertheless within normal limits, if compared with the maximum reference values. In the case of hematological parameters, the HGB value obtained was 11.62 g/dL, vs the mean value of the reference HGB (14.06 g/dL), as well as a lower HCT (37.26 %), comparatively with the mean reference HCT value (41.74 %).

Data in table 7.XIV. indicate an increase of TGP (21.34 U.I.) and TGO (23.96 U.I.) comparatively with the mean value of the reference TGP (17.81 U.I.) and TGO (20.13 U.I.), however this tendency remains insignificant. In the case of hematological parameters, a decreasing tendency was noticed for hemoglobin HGB (12.18 g/dL) comparatively with the mean value of the reference HGB (14.06 g/dL), as well as a lower hematocryte HCT (36.35 %) comparatively with the mean reference HCT (41.74 %). In the case of thrombocytes (PLT), a higher mean value of PLT (367.37 x10³ / mmc blood) was registered, comparatively with the mean value of the reference PLT (269.30 x10³ / mmc).

7.5. Conclusions

In the patients of group 1, treated with HIN, lower mean values of HGB and HCT and higher mean PLT values were obtained, comparatively with the reference, as well as
Abstract

insignificantly higher mean TGP and TGO values, comparatively with the mean reference ones

In the patients of group 2, treated with HIN and omeprazol, no significant mean values of the hematological and biochemical parameters were recorded, comparatively with the reference.

8. INCIDENCE OF ADVERSE REACTIONS TO ANTI-TUBERCULOSIS MEDICATION IN THE PATIENTS TREATED IN THE CLINICAL HOSPITAL OF PNEUMOPHTHISIOLOGY OF IAŞI (2007-2012)

8.1. Introduction

Objectives: The adverse reactions recorded in the observation sheets of the patients and in the files of spontaneous registration have been analyzed statistically from the viewpoint of their incidence on year of study, number of drugs (one ar several) having caused them, groups of sex, age, degree of seriousness.

8.2. Materials and method

8.2.1. Statistical study of the adverse reactions registered in the patients of the Clinical Hospital of Pneumophthisiology of Iaşi (2007-2012)

8.3. Results

8.3.1. Weight of adverse reactions (AR) to a single and to several anti-tubercular drugs

Statistical analysis of data evidenced that most of the AR (74.26%) had been induced by a single drug (pyrazinamide 500 mg, rifampicine 300 mg, isoniazid 300 mg), while 25.74% of them had been caused by several drugs involved in the treatment scheme.

8.3.2. Weight of the adverse reactions vs the active substance

Adverse reactions have been also induced by isoniazid 300 mg (17.9%), manifested as pain in the right hypochondrium, asthenia, nausea, vomiting, sclero-tegumentary icterus, extended lupus eritematous with drug etiology and a significant increase of hepatic transaminases (TGP = 173 U.I., TGO = 149 U.I., TGP = 282 U.I., TGO = 297 U.I.).

8.3.3. Weight of the adverse reactions as a function of disease seriousness

The most severe form of AR manifestation was hepatotoxicity, which gradually increased to 8.25% in the year 2008, to 8.93% in 2009, 12.86% in 2010 and 12.68%, respectively, in 2011. Nausea, vomiting, abdominal pain have been recorded along the whole period of the study, the highest value (7.56%) being recorded in 2010. Another severe form of AR manifestation was the maculatas pruriginous eruption, which recorded values of 9.08% in 2010 and of 6.06%, respectively, in 2012 (table 8.V.).

Table 8.V. Weight of adverse reactions vs years of study
Abstract

<table>
<thead>
<tr>
<th>ADVERSE REACTIONS</th>
<th>2007 (%)</th>
<th>2008 (%)</th>
<th>2009 (%)</th>
<th>2010 (%)</th>
<th>2011 (%)</th>
<th>2012 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatotoxicity</td>
<td>5.25</td>
<td>8.05</td>
<td>8.93</td>
<td>12.86</td>
<td>12.68</td>
<td>7.56</td>
</tr>
<tr>
<td>Nausea, vomiting, abdominal pain</td>
<td>5.25</td>
<td>4.75</td>
<td>4.16</td>
<td>7.56</td>
<td>3.61</td>
<td>5.3</td>
</tr>
<tr>
<td>Sclero-tegumentary icterus</td>
<td>3.48</td>
<td>absent</td>
<td>absent</td>
<td>2.26</td>
<td>1.59</td>
<td>absent</td>
</tr>
<tr>
<td>Hyperuricemia</td>
<td>3.51</td>
<td>7.61</td>
<td>7.73</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>Maculary pruriginous eruption</td>
<td>3.46</td>
<td>2.83</td>
<td>3.56</td>
<td>9.08</td>
<td>3.15</td>
<td>6.06</td>
</tr>
<tr>
<td>Fever, intense shivering</td>
<td>absent</td>
<td>0.95</td>
<td>1.8</td>
<td>1.5</td>
<td>absent</td>
<td>3.01</td>
</tr>
<tr>
<td>Patients</td>
<td>19</td>
<td>35</td>
<td>28</td>
<td>22</td>
<td>21</td>
<td>22</td>
</tr>
</tbody>
</table>

8.3.4. Weight of adverse reactions on groups of age

The most affected groups of age have been the 45-54 year (37.1%) ones, in 2009, and the 55-64 year (25.7%) ones, respectively, in 2008.

In recent years, the incidence of pulmonary tuberculosis increased in children and, consequently, the incidence of adverse reactions, manifested by hepatotoxicity phenomena induced by isoniazid, through a slight increase of seric transaminases.

8.3.5. Weight of adverse reactions on sexes

As to AR incidence on sexes, it has been observed that males are much more affected (88.8%) than females (11.2%), the higher incidence of tuberculosis in men being possibly associated
with advanced age, more numerous comorbidities, less chances of carrying out the treatment, etc.

8.4. Discussion

The results of the present study confirm the fact that primary antituberculous medication is responsible for various adverse reactions, representing a serious problem for the patients treated against pulmonary/extrapulmonary tuberculosis.

The major adverse effects (observed in 147 patients) were: hepatotoxicity, nausea, vomiting, abdominal pain and hyperuricemia.

The medical staff of the Clinical Hospital of Pneumophthisiology of Iaşi is constantly interested in pharmacovigilance activities, firstly by analyzing the cases of adverse reactions induced by antituberculous medication, the AR files being also forwarded, by the dispensary of the hospital, to the National Drug Agency (ANM), as well as by presenting such cases to the young trainee physicians and specialists.

Management of active tuberculosis includes initiation and carrying out of antituberculosis therapies, and intervention in situations in which adverse reactions might be manifested.

8.5. Conclusions

Analysis of the data provided by the Department of Statistics and by the closed-circuit dispensary of the hospital, collected between 2007-2012, on the number of patients treated against pulmonary/extrapulmonary tuberculosis and on the incidence of possible adverse reactions led to the following conclusions:
Abstract

- Most of the observed AR had been caused by a single drug (74.26%), vs the ratio recorded for several drugs (25.74%).

- Most of the AR had been produced by isoniazid 300 mg (17.90%), in the year 2009.

- AR analysis according to seriousness showed that the most frequent symptom had been hepatotoxicity: 8.05% in 2008, increasing to 8.93% in 2009, 12.86% in 2010 and to 12.68%, respectively, in 2011. The pruriginous macularty eruption was present in 9.08% of the patients in the year 2010, in 6.06% of cases in 2012 and in 3.56%, respectively, in 2009.

- AR weight on groups of age shows that most of them (37.1%) were registered in the 45-55 year group of age in the year 2009, while the 0-14 year group recorded the highest number of AR considered for the study (22.73%) in the year 2012.

- AR weight on sexes indicates a high ratio (88.8%) among men in 2009, comparatively with women (38.1%) in 2011.

9. EXPERIMENTAL EVALUATION OF THE ISONIAZID – OMEPRAZOLE INTERACTION

9.1. Introduction

The working hypothesis of the present investigation considers that, in medical practice, the patients affected with tuberculous disease associated with either gastric ulcer or hyperacid gastritis are frequently subjected to a concomitant administration of omeprazole. The duration of a concomitant administration of omeprazole in association with isoniazid differs
Abstract

according to the type of digestive pathology, duration of the treatment with isoniazid and other associated pathologies. The present study aimed at an experimental evaluation of the isoniazid–omeprazole interaction.

Objectives:

- macroscopic and microscopic evaluation of ulcer healing
- macroscopic and microscopic evaluation of hepatic toxicity
- analysis of the alimentary behaviour and of bodily weight.

9.2. Materials and method

The experiments, developed on the model of rat ulcer induced with indomethacin, aimed at evaluating acidity and the alimentary behaviour of the sample animals.

9.2.1. Medicinal substances

Indomethacin (Sigma Aldrich, Germany)

Omeprazole (Molekula BioChimica, Germany)

Isoniazid (Sigma Aldrich, Germany)

Tragacanth gum (Sigma Aldrich, Germany)

9.2.2. Laboratory animals used in the study

The tests were made on white, male, adult Swiss rats with a bodily weight of 20-25 g, obtained from the "Cantacuzino"
Abstract

Institute of Researches of București, on the basis of a conformity certificate.

All experimental procedures necessary for the present study had been performed in the Laboratory of Experimental Pharmacodynamics of the discipline: Pharmacodynamics and Clinical Pharmacy, on a minimum number of animals, special efforts being taken for producing as low suffering as possible, on strictly observing the international bioethical regulations referring to studies on laboratory animals, as well as the rules of the ”Grigore T. Popa” UMF of Iași.

9.2.3. Evaluation of ulcer healing

9.2.3.1. Macroscopic evaluation

The gastric mucous membrane was visualized through transparency with a x10 magnifying glass.

9.2.3.2. Microscopic evaluation

For histopathological examination, the fragments of ulcerated stomach were fixed into a saline solution of formol 10%, for 24 hours, followed by gradual dehydration in ethanol, introduction treatment with xylene, fixing in paraffin blocks and sectioning. The obtained sections were placed on glass lamellae and coloured with hematoxylin-eosine and van Gieson, then read on the microscope and interpreted.

9.2.4. Evaluation of hepatic toxicity

9.2.4.1. Macroscopic evaluation of the liver
Abstract

- The liver collected through a microsurgical technique was immersed into a saline solution with physiological pH and subjected to macroscopic analysis with a x10 magnifying glass.

9.2.4.2. Microscopic evaluation of the liver

- For histopathological examinations, the fragments of liver taken over from the same lobe were fixed in a saline solution of formalin 10% for 24 hours, followed by gradual dehydration in ethanol, introduction treatment with xylen, fixing in paraffin blocks and sectioning. The obtained sections were placed on glass lamellae and coloured with hematoxylin-eosine and van Gieson, then read on the microscope and interpreted.

9.2.5. Evaluation of alimentary behaviour and weight

Along the whole experiment, the alimentary behaviour, water and food consumption, and bodily weight were evaluated, on having in view that digestive or hepatic suffering may induce several modifications. The data are calculated as mean values, being correlated with either weight or pathology.

9.2.6. Methods of analysis and interpretation of experimental data

For data interpretation, the mean and standard deviation should be calculated. The inhibition degree of ulcers was calculated with formula:

\[ \text{inhibition (\%) } = 100 - \frac{T}{M} \times 100 \]

where:

- M- inhibition degree of the control group
Abstract

T- value of the inhibition degree of the treated group

Statistical analysis is based on the ANOVA variance method and the Newman-Keuls test.

Statistical analysis for all tests of statistical significance considered that, for p values < 0.05, a statistically significant difference exists between the groups under comparison.

9.3. Results

9.3.1. Macroscopic and microscopic evaluation of the healing extent of ulcers

The test was performed on groups formed of 15 white Swiss mice (with the exception of the group treated with indomethacin, including 20 animals) with a weight of 20-30 g each, randomly selected and placed in special 40x60 cm Plexiglas cages, in a room with controlled temperature (21°C ± 2°C) and a 12h/12h light-dark cycle, with food and water ad libitum. Access to food was stopped for 24 hrs prior to inducing the ulcers. 8 groups of animals have been prepared, distributed and treated as follows:

Group I – 2% p.o. tragacanth gum mucilage

Group II – omeprazole 3 mg/kg/body p.o.

Group III – isoniazid 15 mg/kg/body p.o.

Group IV – indomethacin 18 mg/kg/body p.o. unique dose

Group V – indomethacin 18 mg/kg/body p.o. unique dose + omeprazole 3 mg/kg/body p.o.
Abstract

Group VI – indomethacin 18 mg/kg/body p.o. unique dose + isoniazid 15 mg/kg/body p.o.

Group VII – indomethacin 18 mg/kg/body p.o. unique dose + omeprazole 3 mg/kg/body p.o. + isoniazid 15mg/kg/body p.o. – concomitant administration

Group VIII indomethacin 18 mg/kg/body p.o unique dose + omeprazole 3 mg/kg/body p.o + isoniazid 15 mg/kg/body p.o. at an 1 hr interval between administrations.

The experiment was performed along 7 days, 5 animals from each group being sacrificed in days 3, 5 and 7, the organs of interest being taken over and prepared for macro and microscopic examinations.

The obtained data were subjected to statistical analysis.

The following aspects were registered in the sacrificed animals in the 3rd day of treatment: diffuse atrophy, zones with normal aspect; in the 5th day of treatment, the mucous membrane of the stomach evidences: uneven mucosa, ulcer, zones with normal aspect; in the 7th day of treatment, respectively: diffuse atrophy of the mucous membrane, periglandular inflammation.

In the case of group VII, including patients with indomethacin-induced ulcer, treated concomitantly with omeprazole and isoniazid, a slow, plateau healing tendency may be observed up to the 5th day of treatment, followed by a rapid evolution, so that in day 7, the inhibition ratio recorded is of 78.2%, and the total ulcer index is 3. Global evaluation indicates a 43.76% healing ratio and the presence of 1-2 lesions of reduced seriousness.
Abstract

Day 3 of treatment put into evidence the following aspects in the sacrificed animals: atrophy, zones with normal aspect, zones with diffuse inflammation, reduced ulceration in some areas; in day 5 of treatment, the mucous membrane of the stomach displays: zones with normal aspect, inflammatory zones, while day 7 permitted the following observations: atrophy, periglandular inflammation, extended hyperplastic zone.

Fig. 9.5. Hyperplasic gastritis (Col. HE x 200)

In group VIII, with indomethacin-induced ulcer treated with omeprazole and isoniazid, with an 1 hour interval between administrations, a rapid, progressively gradual healing tendency may be noticed, with an inhibition ratio of 100% in day 5, the global percent value recorded being of 79.53%. Also in day 7, macroscopic examination showed a very low lesion degree and an increased total ulcer index. Histological analysis discovered no visible lesions, even if hyperplasia characteristic to the treatment with omeprazole could be observed (figure 9.5).
Abstract

The obtained data were analyzed statistically. Group IV, representing the untreated ulcer, was compared with each of the groups having received treatment, as shown in table 9.IX.

Table 9.IX. Statistical parameters obtained by the Newman-Keuls test

<table>
<thead>
<tr>
<th>No</th>
<th>Compared groups</th>
<th>Statistical parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group IV + V</td>
<td>F: 2.077 F(95%) = 7.710 n = 5 ns</td>
</tr>
<tr>
<td>2</td>
<td>Group IV + VI</td>
<td>F = 1.0511 F(95%) = 7.7100 n = 5 ns</td>
</tr>
<tr>
<td>3</td>
<td>Group IV + VII</td>
<td>F = 7.9500 F(95%) = 7.7100 n = 5 , p&lt;0.05*</td>
</tr>
<tr>
<td>4</td>
<td>Group IV + VIII</td>
<td>F = 9.9248 F(95%) = 7.7100 n = 5 , p &lt; 0.01**</td>
</tr>
<tr>
<td>5</td>
<td>Group VI + VII</td>
<td>F = 2.356 F(95%) = 7.7100 n = 5 ns</td>
</tr>
</tbody>
</table>

The statistical parameters of analysis provided statistically significant data (p < 0.05) for the group with ulcer treated with omeprazole and isoniazid, concomitant and highly statistically significant (p < 0.01) for the group with ulcer treated with omeprazole and isoniazid, with 1 an hour-interval between administrations.

9.3.2. Macroscopic and microscopic evaluation of hepatic toxicity

In all groups under study, macroscopic investigations showed rare cases of liver with increased volume, yet the deviation from the normal liver weight was not significant.

Microscopic examination evidenced the following aspects: Group V, **day 3 of treatment**: granulo-vacuolar degenerescence, Kupfferian reaction, superhepatic and capillary stasis. Group V,
Abstract

day 5 of treatment: Kupfferian hypertrophy, granulo-vacuolar degenerescence, leucocitary margination. Group V, day 7: zonal, isolated granulo-vacuolar degenerescence.

Group VI, day 3 of treatment: steatosis, granulo-vacuolar degenerescence, Kupfferian hypertrophy. Group VI, day 5 of treatment: mucosa hypertrophy, abcess, granulo-vacuolar degenerescence stasis. Group VI, day 7 of treatment: steatosis, stasis, granulo-vacuolar degenerescence.

Fig. 9.8. Necrosis-affected liver (Col. HE x 100)
Abstract

Fig. 9.10. Hepatic abscesses (Col. HE x 200)

**Group VIII, day 3 of treatment:** stasis, granulo-vacuolar degenerescence. **Group VIII, day 5 of treatment:** stasis, granulo-vacuolar degenerescence. **Group VIII, day 7:** stasis, hepatocitary degenerescence, granulo-vacuolar degenerescence, necrosis (figure 9.11.).
Abstract

Fig. 9.11. Necrosis-affected liver (Col. HE x 200)

9.3.3. Evaluation of the alimentary behaviour and of weight

The experimental data on the evaluation of the alimentary behaviour and weight evidenced no major differences.

9.4. Conclusions

The laboratory study performed on experimental animals, devoted to the isoniazid–omeprazole interaction, evidenced the following aspects:

- Group VI, with ulcer induced with indomethacin and treated with isoniazid, showed a higher degree of lesion and an increased total ulcer index, which suggests the presence of severe lesions. The lesion inhibition ratio (46.5%) suggests a slow healing.

- The indomethacin-induced ulcer treated with omeprazole and isoniazid, with an 1 hour interval between administrations (group VIII), evidenced a gradual progressive healing tendency, with an inhibition ratio of 100% in day 5, and a high global ratio (79.5%). Hyperplasia, characteristic to the treatment with omeprazole, was also present.

- The statistical parameters of analysis provide statistically significant data (p < 0.05) for the group treated with omeprazole and isoniazid concomitantly, with group VII and highly statistically significant (p < 0.01) for the ulcer treated with omeprazole and isoniazid, with an 1 hour interval between administrations, in group VIII.
Abstract

- Microscopic analysis of liver put into evidence stasis, granulo-vacuolar degenerescence, hepatocytary degenerescence, necrosis, in all days of the treatment: 3, 5, 7.

- The mean values of the parameters analyzed showed no differences of alimentary behaviour or weight deviations.

10. GENERAL CONCLUSIONS

The investigations described in the present PhD thesis permitted the following general conclusions:

1. Quantitative determination of isoniazid through UV-VIS spectroscopy showed that dosing of isoniazid with the vanillin from the biological sample (horse serum) does not lead to convincing results, as other substances present in horse serum may also intervene, the determined concentrations being much higher than the theoretical values. However, in the case of isoniazid dosing with a PABA 1% solution in alcohol, the concentrations determined have been close to the theoretical ones.

2. For a simultaneous determination of isoniazid and omeprazole from human serum through HPLC-UV, an original method has been established and validated by the author of the present thesis. The determinations were made on a Thermo-Fischer Scientific Surveyor HPLC Plus System chromatograph with UV-VIS detector.

Chromatographic separation was performed on an Octasilyl C 8 column (Purospher RP8) (of the 250 mm x 4.6 mm i.d., type 5 µg), the mobile phase being formed of triethylamine 10 mM and acetonitrile 67:33 (v/v); UV-VIS detection was performed at two wave lengths: 260 nm for isoniazid and 2-pyridylamine (internal standard) and 300 nm for omeprazole and lansoprazole (internal standard). The validation parameters
taken into study were: selectivity/specificity, linearity, detection limit, quantification limit, linearity of the method, precision, exactness. The domain of linearity ranged between 50 – 5 000 ng/mL.

3. The validated HPLC-UV method was applied for the determination of serum concentrations of isoniazid and isoniazid with omeprazole from the biological fluid (human serum) in the patients with pulmonary/extrapulmonary tuberculosis and gastro-duodenal diseases treated in the Clinical Pneumophthisiology Hospital of Iaşi. Along the 7 days of treatment, the determined concentrations of isoniazid for group 1 of patients varied between 0.28 mg/mL and 3.85 mg/mL. Determinations of seric concentrations for the patients of group 2 evidenced a variation of isoniazid concentrations between 0.32 mg/mL and 4.22 mg/mL. In the case of omeprazole, the determined seric concentrations varied between 0.32 mg/mL and 0.39 mg/mL. The obtained data evidence low seric concentrations of omeprazole in the presence of isoniazid, which may induce a delayed absorption of the proton pump inhibitor and, in the case of a treatment with omeprazole concomitantly with the tuberculostatic treatment, the schemes of a long-term treatment should be well-established.

4. Statistical study on the dynamics of the biochemical: TGP, TGO, glucose, urea, creatinin, and hematological: WBC, RBC, HGB, HCT, PLT parameters, respectively, studied comparatively with a reference group, showed slight modifications of the hepatic enzymes TGP and TGO from the first up to the 7th treatment days for both groups of patients, as well as lower hemoglobin and hematocryte values, again in both groups. Another modified hematological parameter was the increased number of thrombocytes in groups 1 and 2.
5. Statistical analysis of the incidence of the adverse reactions to antituberculosis medication outlined the importance of registering and reporting of the adverse reactions to the National Drug Agency (ANM). The study, performed along a 72 month period (January 2007 – December 2012), recorded a number of 147 files of adverse reactions, distributed on years. Statistical analysis demonstrated that most of the adverse reactions had been produced by a single drug: 74.26%, comparatively with the adverse reactions induced by several drugs: 25.74%. As to the contribution of the active substance, isoniazid 300 mg registered in the year 2010 a ratio of 17.9%. The most affected group of age was the 45 – 54 year one (37.1%) in 2009, and the 55 – 64 year one, respectively, (25.7%) in 2008. The year 2012 registered the most numerous adverse reactions in the 0-14 year group of age (22.73%) for the whole period taken into study. According to sex distribution, men registered the highest values - 88.8% - in the year 2009 while women recorded the highest values (38.15%) in 2011. Analysis of the adverse reactions according to their seriousness evidenced that the most frequently manifested form was hepatotoxicity, which varied between 5.25% in the year 2007 and 12.86%, respectively, in 2010. Digestive phenomena have been also registered in 2010 (7.56%) and cutaneous manifestations in 2010 (9.08%).

6. The experimental study devoted to the isoniazid–omeprazole interaction was performed on white male adult Swiss rats. During the 7 days of the study, the animals were orally treated with suspensions from the substances administered either alone or associated with indomethacin, expected to induce ulcer, followed by sacrifices in days 3, 5 and 7. Especially interesting was the fact that administration of isoniazid did not provoke gastric ulcerative problems while, when administered in patients with ulcer, the degree of spontaneous healing has significantly decreased, with a value of 32.76%, along with slowing down of the action of omeprazole. This aspect is especially important in
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medical practice, as a significant reduction of the degree of spontaneous healing may increase the gravity of the ulcerous disease during treatments with antituberculous medication. At hepatic level, microscopic modifications have been evidenced from the first up to the seventh day of treatment, as follows: granulo-vacuolary degrenescence, sinusoidal stasis, suprahepatic circulation stasis, necrosis. No modifications of the alimentary behaviour or weight deviations have been observed in the experimental groups.

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Abstract


Abstract


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