(54) Title: PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF CHRONIC HEPATOSIS

(57) Abstract

The present invention relates to the use of an Enterococcus faecium for the preparation of a medicinal product suitable for the therapeutical treatment of chronic hepatitis. In particular, the present invention takes into consideration the Enterococcus faecium belonging to that strain defined as SF68. Such a use may also be advantageously performed with an associated bile acid. The present invention furthermore discloses, and relates to, pharmaceutical compositions directed to such a use.
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<td>Viet Nam</td>
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"PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF CHRONIC HEPATOSES"

Italian Patent N. 1,112,479, in the name of Giuliani S.A., relates to a pharmaceutical composition particularly suitable for therapeutic treatment of infective enterites and diarrhoeas in general, characterized in that it contains, as the active principle, a cultivation of bacteria belonging to Enterococcus faecium species.

In particular, said patent relates to compositions in which Enterococcus faecium bacteria belong to the bacterial SF 68 strain.

The SF 68 bacterial strain is the active principle of patent medicine BIOFLORIN®, a well documented biological preparation containing this particular strain of Enterococcus faecium in dry form, widely used for the treatment of acute diarrhoeal-enterocolitic forms.

Enterococcus faecium, already classified, since 1984, in Lancefield's D group of Streptococcus genus within the family of Streptococcaceae, is considered as belonging to Enterococcus genus and has the appearance of a spherical coccus having a diameter of about 1 μm, generally existing as chains of various lengths. It is gram-positive, aerobic, facultative anaerobic, non-sporiferous, non-mobile.

The optimal growth temperature is of 36°C-37°C, and optimal growth pH value is 7. Preferred cultivation media for achieving an optimal growth are MRS broth (Difco) and horse blood agar. The
growth can be estimated from a uniform cloudiness in liquid medium and small, round, slightly convex, smooth colonies on solid medium. A slight alpha-hemolysis takes place on colonies growing on blood agar.

In TSA blood agar (Difco) with 5% erythrocytes of rabbit, sheep, man, guinea pig and horse, an alpha-hemolysis is obtained in the presence of horse, sheep and man blood, but not with rat, rabbit and guinea pig blood.

The SF 68 strain was initially isolated from human bodies and it was used also in food industry, in particular, for cheese fermentation.

The SF 68 strain was deposited by the Company Giuliani with DSM (Deutsche Sammlung Mikroorganismen und Zellkulturen): the deposit code is DSM No. 8912.

The preparation containing Enterococcus faecium SF 68 designed for clinical purposes had its origin in 1978; the same strain was studied and used also in the veterinary field.

Giuliani Company developed the preparation during years 1978-1980, thanks to a very large amount of toxicological, microbiological and clinical studies; almost all of these clinical studies were carried out as double-blind controlled studies versus placebo or versus active controls.

The review by Loizeau E. (published on "Revue Médicale de la Suisse Romande" 114; 651, 1994) summarizes the biological and clinical properties of the preparation.
The primary biliary cirrhosis (PBC according to Anglo-Saxon short nomenclature) is the best known cholestatic type hepatosis: in these forms the stasis of bile acids, caused by the destruction of intrahepatic ducts, causes, in its turn, a series of hepatocellular and hepatocanalicular injuries, various biochemical alterations at hematic level and clinical symptoms or disorders, among which jaundice (in cases with high hyperbilirubinemia) and pruritus appear to be the most frequent ones.

The evolution into cirrhosis is slow but progressive as time goes by; generally, the prognosis is fatal in the long term.

The effect of treatments is evaluated:

- In the short term, by checking the hematochemical indices of hepatocellular injury and biliary stasis, by checking the symptoms evolution.
- In the medium term, by means of histological assays at hepatic level (serial biopsies) and from the improvement in life quality.
- In the long term, from the extension of survival and from the decrease in the need for, or from the possibility of delaying the liver transplantation, which is the solution in the advanced stages of the disease.

The available pharmacological treatments have complementary or symptomatic character, in the sense that to date no one of them proved to be capable of substantially modifying the natural evolution of the disease.
During the past years, ursodeoxycholic acid (UDCA) proved to have a good clinical usefulness in bland to moderate forms of the disease, at least for the control of alterations in hematochemical indices of hepatic injury (transaminase, alkaline phosphatase, γ-GT levels) and of subjective symptoms such as pruritus, as well as to stop or to slow down the evolution of the histologic damage at hepatic level. Still insufficient and discussed, even though encouraging, are the results relevant to the effects on survival and on reduced or delayed need for liver transplantation.

The clinical experience on the therapeutic use of UDCA in these forms is now very wide, also as regards the control of the functional recovery of liver after liver transplantation.

The effect of UDCA in such forms can depend on the choleretic effect, but, above all, it depends on the fact that it is less detergent (and thus it does not cause damages to hepatocyte membranes) than endogenous bile acids, such as chenodeoxycholic and cholic acids. The administration of UDCA inhibits the ileal absorption of endogenous bile acids, thus modifying the bile acids pool; UDCA becomes the prevailing bile acid and thus reduces the detergent power and the capability of damaging liver displayed by the pool.

We have now surprisingly found, and this constitutes the object of the present invention, that for the treatment of chronic hepatoses, such as
primary biliary cirrhosis (PBC), pharmaceutical compositions, containing, as their active principle, an *Enterococcus faecium*, in particular of SF 68 strain, alone or in combination with ursodeoxycholic acid (UDCA) or with one or more other therapeutically active bile acid(s), proved to be particularly efficacious.

Patients affected by PBC were treated with a pharmaceutical preparation in the form of capsules containing *Enterococcus faecium* of SF 68 strain (known on the market as BIOFLORIN®) owing to enteric disorders (diarrhoeal phenomena with discontinuous abdominal pains and febrile events), in wait for carrying out proper checks and for beginning the specific treatment required for the hepatic form in question.

A brief description of the clinical observations is reported in the following.

The experiments were carried out at the Gastroenterology Division of the University of Bologna and relate to 5 subjects, 4 female and 1 male patients, from 35 to 70 years old.

The characteristics of the patients subjected to the study are reported in the following table:
<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (Years)</th>
<th>Sex</th>
<th>Duration (Years)</th>
<th>Stage</th>
<th>AMA</th>
<th>Bilirubinemia (%) mg</th>
<th>ALT* (IU/L)</th>
<th>AST* (IU/L)</th>
<th>ALP* (IU/L)</th>
<th>γ-GT* (IU/L)</th>
<th>Cholesterol (mg/dL)</th>
<th>Diarrhoea</th>
<th>Fever</th>
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<tr>
<td>VT</td>
<td>54</td>
<td>F</td>
<td>5</td>
<td>I</td>
<td>+</td>
<td>2.0</td>
<td>2.8</td>
<td>2.4</td>
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<td>12.4</td>
<td>270</td>
<td>moderate</td>
<td>absent</td>
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<tr>
<td>LM</td>
<td>70</td>
<td>F</td>
<td>6</td>
<td>II</td>
<td>+</td>
<td>2.1</td>
<td>3.0</td>
<td>3.1</td>
<td>5.1</td>
<td>14.8</td>
<td>260</td>
<td>severe</td>
<td>moderate</td>
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<tr>
<td>GG</td>
<td>49</td>
<td>F</td>
<td>3</td>
<td>I</td>
<td>+</td>
<td>2.3</td>
<td>3.1</td>
<td>3.3</td>
<td>5.8</td>
<td>15.1</td>
<td>289</td>
<td>severe</td>
<td>high</td>
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<tr>
<td>ER</td>
<td>35</td>
<td>F</td>
<td>7</td>
<td>II</td>
<td>+</td>
<td>2.0</td>
<td>2.3</td>
<td>2.5</td>
<td>5.3</td>
<td>13.7</td>
<td>189</td>
<td>moderate</td>
<td>moderate</td>
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<tr>
<td>OP</td>
<td>55</td>
<td>M</td>
<td>6</td>
<td>II</td>
<td>+</td>
<td>2.4</td>
<td>3.5</td>
<td>3.0</td>
<td>4.1</td>
<td>15.1</td>
<td>310</td>
<td>moderate</td>
<td>bland</td>
</tr>
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</table>

* The values of serum enzymes levels are reported as multiples of the highest limit value of the range of normal reference values (xN).
All patients displayed typical clinical and biochemical patterns of PBC; the histologic situation had evidenced a I or II stage disease with increase in alkaline phosphatase (ALP) level up to values at least four times as high as normal values, as well as positive results for γ-GT, ALT, IgM and anti-mitochondrion antibodies.

The patients in question had arrived to the observation procedure following clinical checks and for implementing a suitable therapeutical protocol; no patients had undergone any specific treatments for PBC for some months.

The appearance of an acute aspecific diarrhoeal disease had suggested establishing the biological treatment with the product containing Enterococcus faecium SF68 strain; such a diarrhoeal form could be indirectly reconducted to the base hepatic disease, also on considering a concurrent steatorrhea.

The treatment with Enterococcus faecium SF68 strain was carried out with the marketed preparation BIOFLORIN® (containing at least 75 millions of alive microorganisms per each capsule) administered at the rate of 2 capsules, three times a day.

The evolution of the acute diarrhoeal pattern was very favourable, i.e., fever and diarrhoea disappeared within 1-3 days in all cases.

On considering that it is advisable to continue the treatment for some days in order to consolidate the obtained results, the treatment with SF 68 was continued also after the remittance of the
diarrhoeal pattern, obtaining positive effects on general clinical pattern of the patients.

A first hematologic check performed after one week of treatment with SF 68 strain supplied considerably interesting results as to the hepatic disease (not connected to diarrhoea), in particular as regards a rapid considerable decrease in alkaline phosphatase (ALP) level in some cases; this result induced us to continue administering SF 68 during at least 1 month.

The results observed after 1 month of treatment with SF 68 strain were as follows:

- The explored hematochemical indices displayed surprising improvements: we were able to evidence a considerable decrease in alkaline phosphatase levels, which in three from five patients reached only slightly higher values than normal (from 850 to 530 U/L); normal values = 40-190 U/L) and in γ-GT levels in two from the five patient; the overall “composite biochemical index” decreased from 1 down to values comprised within the range of from 0.6 to 0.9 in the five treated cases; we remind that the “composite biochemical index” is the weighed average of changes, expressed as ratios based on baseline values, in bilirubinemia, AST, γ-GT, alkaline phosphatase or ALP levels, according to the following relationship (see the work by the Gruppo Multicentrico Italiano per lo studio dell'UDCA nella PBC (Italian Multicenter Group for study of UDCA in PBC), published on
"Journal of Hepatology", 17, 332-338, 1993, in which one of the inventors of the instant patent participated as Co-author

\[
\frac{2 \times (\text{bilirubinemia ratio}) + (\text{AST ratio}) + (\text{ALP ratio}) + (\gamma-\text{GT ratio})}{5}
\]

- bilirubinemia levels displayed an average decrease of 20-30 %, in particular in those patients who displayed higher initial values;
- itch intensity displayed a reduction in practically all cases;
- total cholerestolemia (total level of cholesterol in blood) displayed a considerable decrease (20-30% on the average), in those 4 patients who displayed higher than normal initial values, whereas HDL cholesterol levels displayed a slight increase in the same patients;
- besides the fast disappearance of diarrhoeal events, a decrease in severity of dyspeptic disorders, when present, was observed;
- owing to the absolutely occasional and preliminary stage of the study, finer hematochemical measurements, as those relevant to the hematic pattern of bile acids, the serum protein pattern and the conditions of the immunocompetent system, were not carried out;
- no side effects were observed on treated patients.

Summing-up, the results of the evolution of the clinical and hematochemical indices relevant to the hepatic pattern of PBC following the treatment with SF 68 alone were decidedly surprising in the sense
that both the explored hematochemical indices and clinical symptomatology displayed a relevant and fast improvement (in only 2-3 weeks, up to 1 month, of treatment), with at least equivalent effects to those which are usually observed with UDCA or other drugs used for PBC treatment.

Furthermore, the surprising effects have to be remarked on the situation of hypercholesterolemia present in 4 from the 5 patients; this index is not usually influenced by the treatment with UDCA.

For comparison purposes, we regard it meaningful to synthetically remind the clinical results obtained by means of the treatment with UDCA in the already cited multicenter controlled clinical study performed according in double blind mode versus placebo ("Journal of Hepatology", 17; 332-338; 1993) performed on 88 patients with substantially comparable PBC diseases, as to stage and extent of disease, to the small group of cases which were treated in the pilot test described above, carried out with the SF 68 strain containing preparation.

In the following table, a synthesis (average values) is reported of the results obtained with UDCA, after 6 months of treatment, relevant to the same main parameters also explored in the original pilot study with SF 68 reported above:
<table>
<thead>
<tr>
<th>Group treated with UDCA</th>
<th>Itch (score)</th>
<th>Bili-rubinemia (µg)</th>
<th>Composite biochemical index</th>
<th>Cholesterol-olemia (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At the beginning</td>
<td>2.3</td>
<td>2.67</td>
<td>1</td>
<td>263</td>
</tr>
<tr>
<td>After 6 months</td>
<td>1.4</td>
<td>2.24</td>
<td>0.82</td>
<td>263</td>
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</table>

For further comparison, we regard it useful reporting the average results obtained in another group of PBC forms which were substantially comparable, as to stage and extent of diseases, to those present in the other patients taken into consideration and treated with UDCA during approximately 1 month.

In general, we were able to observe a detectable and meaningful decrease in enzyme levels, even if in no cases did they reach their normal levels; the order of magnitude of the observed changes is as follows:

- alkaline phosphatase or ALP level: from approximately 900 to about 450 U/L;
- γ-GT level: from about 250 to about 130 U/L;
- ALT level: from 75 to 40 U/L.

As stated above, it appears that the results obtained with Enterococcus faecium SF 68 strain are substantially equivalent, if not better, as compared to UDCA, even if to-date, obviously, obtaining a statistically reliable evaluation from this limited number of observations was not possible.

It is also important to underline the absence
of any effects displayed by UDCA on hypercholesterolemic levels, differently from what observed in the cases treated with the SF 68 containing biologic preparation.

In order to better evaluate the effect of SF 68 on PBC, a further pilot study was performed on 5 further patients affected by PBC with clinical patterns and disease stages equivalent to as displayed by the previously studied groups (four females and one male, stage I or II) and associating with the known treatment with UDCA (10-12 mg/kg-day) the same treatment with SF 68 which had previously supplied surprising results when administered alone.

A summary of these experiences is reported in the following.

Also these observations were carried out at the Servizio di Gastroenterologia (Gastroenterology Service) of University of Bologna; they relate to five further patients with clinical history and biochemical profile which are typical of primary biliary cirrhosis with bilirubinemia levels of about 2 mg/dl.

The patients in question had arrived to the observation procedure following diagnostic checks; they had not been treated with drugs for PBC treatment for some months. Contrarily to the first group, they did not display diarrhoeal diseases or dismicrobial alterations to the damage of intestinal flora.

The characteristics of these patients follow:
<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (Years)</th>
<th>Sex</th>
<th>Disease duration (Years)</th>
<th>Stage</th>
<th>AMA</th>
<th>Bilirubinemia (% mg)</th>
<th>ALT*</th>
<th>AST*</th>
<th>Alkaline phosphatase (ALP)*</th>
<th>γ-GT*</th>
<th>Cholesterol (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB</td>
<td>52</td>
<td>M</td>
<td>6</td>
<td>I</td>
<td>+</td>
<td>2.2</td>
<td>2.7</td>
<td>2.1</td>
<td>4.5</td>
<td>13.4</td>
<td>290</td>
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<tr>
<td>MR</td>
<td>58</td>
<td>F</td>
<td>3</td>
<td>II</td>
<td>+</td>
<td>2.8</td>
<td>3.1</td>
<td>3.2</td>
<td>5.4</td>
<td>16.3</td>
<td>275</td>
</tr>
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<td>49</td>
<td>F</td>
<td>5</td>
<td>II</td>
<td>+</td>
<td>2.7</td>
<td>3.0</td>
<td>3.2</td>
<td>5.9</td>
<td>15.2</td>
<td>195</td>
</tr>
<tr>
<td>LM</td>
<td>40</td>
<td>F</td>
<td>4</td>
<td>I</td>
<td>+</td>
<td>2.5</td>
<td>2.2</td>
<td>2.4</td>
<td>4.3</td>
<td>12.9</td>
<td>289</td>
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<tr>
<td>FC</td>
<td>56</td>
<td>F</td>
<td>5</td>
<td>II</td>
<td>+</td>
<td>2.8</td>
<td>3.4</td>
<td>3.5</td>
<td>4.0</td>
<td>15.7</td>
<td>300</td>
</tr>
</tbody>
</table>

* The values of serum enzymes levels are reported as multiples of the highest limit value of the range of normal reference values (xN).
The treatment was carried out with *Enterococcus faecium* SF 68 strain, by using the marketed preparation BIOFLORIN® (containing at least 75 millions of alive microorganisms per each capsule), administered at the dosage rate of 1-2 capsules, three times a day, associated with ursodeoxycholic acid (UDCA) administered at the dosage rate of 10-12 mg/kg-day (in general, 1 capsule of 300 mg twice a day); the associated treatment was performed during 1 month.

The results observed after 1 month of associated treatment were the following:
- the explored hematochemical indices displayed detectable favourable changes; a summary of the changes occurred in main parameters is reported in the following table:

<table>
<thead>
<tr>
<th>Group treated with UDCA +</th>
<th>Itch (score)</th>
<th>Bili-rubinemia (µg)</th>
<th>Composite biochemical index</th>
<th>Cholesterol oleemia (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF 68 At the beginning</td>
<td>2.3</td>
<td>2.60</td>
<td>1</td>
<td>270</td>
</tr>
<tr>
<td>After 6 months</td>
<td>1.3</td>
<td>2.10</td>
<td>0.70</td>
<td>210</td>
</tr>
</tbody>
</table>

The hematochemical enzymatic indices displayed a considerable decrease with trend to reach borderline values relatively to normality:
- alkaline phosphatase (ALP) levels: considerable decrease in 4 from the 5 patients with average values from 850 down to 300 U/L;
• γ-GT levels: marked decrease observed in 3 from the 5 patients, with average values from 285 down to 95 U/L;

• ALT levels: also in this case, marked decrease, with average values from 80 down to 30 U/L.

The overall "composite biochemical index" decreased from 1 to values comprised within the range of from 0.6 to 0.8 in the 5 treated cases;

• Bilirubinemia levels showed average decreases of 20-30%, in particular in those patients who displayed higher initial level values;

• itch: itch intensity displayed a reduction in practically all cases;

• the total level of cholesterol in the patient's blood considerably decreased in particular in those 4 subjects who displayed higher than normal initial values (from 270 down to 210 mg %); in parallel, HDL cholesterol displayed an increase in the same patients;

• also in this study, owing to its being in an absolutely preliminary stage, finer hematochemical measurements, as those relevant to the hematic pattern of bile acids, the seral protein pattern and the conditions of the immunocompetent system, were not carried out;

• no side effects were observed on treated patients.

Summing-up, the results of the evolution of the clinical and hematochemical indices relevant to the hepatic pattern of PBC following the combined treatment with UDCA at the dosage rate of 600 mg/day
with the SF 68 biological preparation, were very interesting, because they are even better than those obtained with UDCA alone and those, already per se decidedly surprising, as already obtained with SF 68 strain alone.

Furthermore, the surprising effects on the situation of hypercholesterolemia present in 4 from these further 5 patients were confirmed -- which effects have to be ascribed to the treatment with SF 68, because UDCA alone (see above) did not display any effects.

The results obtained with the combined treatment with UDCA + SF 68, when compared to the already cited experiences gained with SF 68 alone and with UDCA alone, allow us to think that a considerable synergy exists between both approaches, which evidently take place according to substantially different mechanisms from each other, to which an effect of reduction in hypocholesterolemia is to be added which, on the contrary, seems due to SF 68 alone.

The study of the possible mechanism of action of *Enterococcus faecium* SF 68 strain

The clinical results obtained with *Enterococcus faecium* SF 68 strain were so interesting, that they suggested us to perform at once a clinical study of clinical pharmacology on a panel of volunteers in order to verify which the possible mechanism of action of the biological preparation would be in these forms and to try to validate the hypothesis
that the surprising clinical-therapeutical in cholestatic hepatoses, of which PBC is a typical representative, could be due, like for UDCA, to a change in circulating pool of bile acids in the direction of a lower detergent, lesser hepatocyte-injuring, action.

The target the present study aimed at was of evaluating the pharmacokinetics of cholic acid (CA) and the speed and effectiveness of its biotransformation into deoxycholic acid (DCA) in healthy subjects and studying the effect of Enterococcus faecium SF 68 strain on such metabolic steps.

The logical base was of verifying whether Enterococcus faecium SF 68 strain modifies the transformations of CA, among which the most important one is 7-alpha-dehydroxylation into DCA through its activity on intestinal flora.

The kinetics of CA and its transformation into DCA was determined on 6 healthy volunteers (23-35 years old, 3 male, 3 female volunteers) before and after treatment with Enterococcus faecium SF 68 strain in the commercial form marketed under BIOFLORIN® trade name, containing at least 75 millions of CFU per each capsule.

The dosage rate was of 3 capsules, three times a day, during 3 weeks (9 capsules/day).

On each subject, in both cases, a baseline blood sample collection was performed, 150 mg of $^{13}$C-CA (carboxy $^{13}$C) were administered per os, and further
blood sample collections were carried out after 8, 24, 48 and 72 hours.

The serum samples were treated in order to analyse them by gas chromatography-mass spectrometry in order to measure the abundance of $^{13}\text{C}/^{12}\text{C}$ isotopes in endogenic CA and formed DCA.

In particular, the bile acids were extracted from serum by solid-liquid extraction, the so obtained bile acids were deamidated, derivatized as methyl trimethylsilyl-ether esters to be separated by gas chromatography and analysed by electron impact mass spectrometry (70 eV) with SIR acquisition (m/z 458/459 for CA and 370/371 for DCA).

From the experimental data and, in particular, from the value of $^{13}\text{C}/^{12}\text{C}$ isotopic ratio, by using the principle of isotopic dilution, the pool of CA, the fractional turnover and its synthesis from cholesterol and the speed and effectiveness of transformation of CA into DCA could be calculated.

The treatment with *Enterococcus faecium* SF 68 strain caused meaningful changes in CA metabolism and kinetics in all studied subjects.

From the kinetics of $^{13}\text{C}$-CA disappearing from plasma, the constant ("fractional turnover"), the CA pool and the hepatic synthesis were calculated.

From such data (Table 1) one will observe that an increase in fractional turnover and a decrease in CA pool occur.

In Table 2, the observed percent increase in $^{13}\text{C}$-DCA during the first 24 hours is reported: one
will observe that in all studied subjects, such a value is practically unchanged after the treatment with *Enterococcus faecium* SF 68 strain, whereas before the treatment the increase was highly meaningful.

The above demonstrates a nearly complete blockade of 7-alpha-dehydroxylation of CA to yield DCA caused by the administration of *Enterococcus faecium* SF 68 strain.

The qualitative analysis of all bile acids by GC-MS evidenced also, after administration of *Enterococcus faecium* SF 68 strain, a meaningful decrease in DCA level, a slight decrease in CA level and an increase in CDCA level, associated with the presence of only LCA traces.
<table>
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<tr>
<th>Subject</th>
<th>K (days⁻¹)</th>
<th>CA pool (mmoles)</th>
<th>Synthesis</th>
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</thead>
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<tr>
<td></td>
<td>before</td>
<td>after</td>
<td>before</td>
</tr>
<tr>
<td>FED</td>
<td>0.21</td>
<td>0.25</td>
<td>1.19</td>
</tr>
<tr>
<td>FRA</td>
<td>0.20</td>
<td>0.28</td>
<td>1.70</td>
</tr>
<tr>
<td>MT</td>
<td>0.43</td>
<td>0.34</td>
<td>2.14</td>
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<tr>
<td>PIE</td>
<td>0.31</td>
<td>0.37</td>
<td>3.74</td>
</tr>
<tr>
<td>CAR</td>
<td>0.33</td>
<td>0.49</td>
<td>1.09</td>
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</table>

Table 1
Fractional turnover constant, cholic acid pool, and synthesis before and after treatment with Enterococcus faecium SF 68 strain.
Table 2
Kinetics of formation of $^{13}$C deoxycholic acid derived from the first 24 hours metabolism of administered $^{13}$C cholic acid (before and after treatment with Enterococcus faecium SF 68 strain)

<table>
<thead>
<tr>
<th>Subject</th>
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<th>after</th>
</tr>
</thead>
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<tr>
<td>FED</td>
<td>11.0</td>
<td>0.3</td>
</tr>
<tr>
<td>FRA</td>
<td>15.9</td>
<td>0.6</td>
</tr>
<tr>
<td>MT</td>
<td>32.7</td>
<td>0.8</td>
</tr>
<tr>
<td>PIE</td>
<td>6.0</td>
<td>0.3</td>
</tr>
<tr>
<td>CAR</td>
<td>4.9</td>
<td>0.3</td>
</tr>
</tbody>
</table>

- Data expressed as change in $^{13}$C/$^{12}$C ratio during the first 24 hours.

The obtained data demonstrate that chronic administration of Enterococcus faecium SF 68 strain inhibits the bacterial 7-alpha-dehydroxylation (probably by modifying enteral bacterial flora) and therefore, slackens down the metabolism of CA with consequent lesser formation of DCA.

On the other hand, CA is then rapidly excreted by virtue of its physical-chemical characteristics, whereas the other primary bile acid, CDCA, is better retained in the enterohepatic loop.

The end result is an increase in primary bile acids over the secondary, more toxic, bile acids.

From the whole of performed observations, firstly casually detected and then developed according to a rational approach, it can be inferred that Enterococcus faecium SF 68 strain displays
surprising properties which are clearly novel from several viewpoints; these are decidedly surprising and novel aspects also as regards the area of possible therapeutical applications.

The biological product appears to be promising as an important and advantageous base treatment in all those clinic situations in which reducing the detergent power and the cellular membrane damaging potential displayed by the pool of bile acids, with possible reflexes both at hepatic level (as we displayed in the particular case of primary biliary cirrhosis) and in other situation, without excluding the same colon cancer (it should be reminded that secondary bile acids are co-carcinogenic), seems to be useful. The preparation can constitute an original and innovative approach, also on considering the low cost of the biological preparation and its widely documented safety of use, also in those cases in which the suitability of long-lasting administrations is envisaged.

All these aspects will be even more interesting if one would take into consideration also the possibilities of application for preventive purposes.

Furthermore, it seems to us that the observed action sinergy with UDCA should be not neglected which, above all as regards the uses in chronic hepatopathies with or without symptoms of cholestasis, can represent an original and innovative starting point which may be highly
interesting if one will consider the possibility of both increasing the therapeutical effectiveness of the product, and reducing the necessary doses of UDCA in order to achieve equivalent therapeutical effects. All the above can take place through a slowing down action on possibly conjugated UDCA biotransformations (deconjugation and subsequent 7-alpha-dehydroxylation) with relationship to its hepatic metabolites (glyco-UDCA, tauro-UDCA) at the entero-hepatic loop level.

In this regard, the high cost should be kept in mind of the treatment with bile acids and consequently the reflexes and advantages, not only clinical, but also financial, which one can achieve.

As regards the dosage rates to be proposed with relationship to the novel applications proposed, it should be stated in detail that *Enterococcus faecium* microorganisms used should display vital characteristics and the number of CFU (colony forming units) to be administered daily may range from 10 millions up to more than $10^5$ millions; such a wide range is justified on considering that the essential aspect for the biological effect is that inside the body conditions exist which allow bacteria to undergo growth -- which, as its typical feature, is very fast (doubling every 19 minutes).

In a similar way, the extemporaneous or preformed association of SF 68 strain (or other equivalent *Enterococcus faecium* strains) with other bile acids having therapeutically useful
characteristics (for example, CDCA, tauro-UDCA, taurodeoxycholate, fluoroUDCA, and so forth) shall be regarded as falling within the scope of the protection of the present patent.

In order to better understand the features of the present invention, some exemplifying embodiments thereof are reported which relate to the pharmaceutical forms to be used for the purposes of the invention.

Such examples are only illustrative and are not limitative of the invention.

EXAMPLES

Pharmaceutical forms

The pharmaceutical forms containing Enterococcus faecium SF 68 strain to which the present patent relates, are all those which are normally useable for clinic use.

In particular, as compositions, the following examples can be cited:

1. Hard gelatin capsules
Per each capsule:
- Enterococcus faecium SF 68 strain ≥ 75 millions
- Magnesium stearate 5.5 mg
- Precipitated silica 0.8 mg
- Lactose 257.7 mg

2. Powder in vial with metering spoon
Per 100 g of powder:
- Enterococcus faecium SF 68 strain ≥ 7.5 milliards
- 25 -

- Dextrose 75 mg
- Dextrin 25 mg

3. Granules packaged in bags
Per each bag containing 1 g:
- Enterococcus faecium 
  SF 68 strain > 75 millions
- Mannitol 150 mg
- Sodium saccharin 10 mg
- Xanthan gum 100 mg
- Natural flavour 40 mg
- Sorbitol q.s. to mg 1500

4. Pellets in bags
Per each bag containing 1.2 g:
- Enterococcus faecium 
  SF 68 strain > 75 millions
- Lactose 120 mg
- Saccharose 702 mg
- Starch 78 mg
- Methyl cellulose 200 mg
- Polyvinylpyrrolidone 60 mg
- Natural flavour 40 mg

5. Pellets in capsule
Per each capsule with a content of 380 mg:
- Enterococcus faecium 
  SF 68 strain > 75 millions
- Lactose 38 mg
- Saccharose 257 mg
- Starch 64.25 mg
- Polyvinylpyrrolidone 18.75 mg
- Magnesium stearate 2 mg
The pellets, if so desired, can be made gastroresistant by coating them with films of methacrylic acid polymers.

As regards the reference pharmaceutical form for ursodeoxycholic acid (UDCA) used in our test, also with reference to the possible associations covered by patent, the composition is as follows:

**Capsules of hard gelatine**

Per each capsule
- Ursodeoxycholic acid (UDCA) 300 mg
- Starch 44 mg
- Magnesium stearate 3 mg
- Colloidal silica 4 mg

Any other pharmaceutical forms of UDCA useable for clinic uses, including the controlled release forms, fall within the scope of instant patent protection for the possible extemporaneous association with *Enterococcus faecium*, e.g., of SF 68 strain.
Claims

1. Use of an Enterococcus faecium for preparing a medicinal product suitable for therapeutic treatment of chronic hepatoses.

2. Use according to Claim 1, characterized in that said Enterococcus faecium belongs to SF 68 strain.

3. Use according to Claim 1, characterized in that said Enterococcus faecium is used in association with one or more therapeutically useful bile acids.

4. Use according to Claim 3, characterized in that said therapeutically useful bile acids are selected from the group comprising CDCA, tauroUDCA, tauroiodeoxycholate, fluoroUDCA.

5. Use according to Claim 1, characterized in that it is directed to the treatment of primary biliary cirrhosis.

6. Use according to Claim 1, characterized in that it is directed to the treatment of a chronic hepatosis with symptoms of cholestasis.

7. Use according to Claim 1, characterized in that it is directed to the treatment of a chronic hepatosis with no symptoms of cholestasis.

8. Use of an Enterococcus faecium, as the Enterococcus faecium SF 68 strain, for treating each clinic condition possibly induced by drugs, in which reducing the level of secondary or detergent bile acids both at local intestines level and at circulating level, including the prevention of the
intestinal neoformations, is useful.

9. Pharmaceutical composition for treating the chronic hepatoses, characterized in that, as active principle, it comprises an *Enterococcus faecium*.

10. Pharmaceutical composition according to Claim 9, characterized in that said *Enterococcus faecium* belongs to SF 68 strain.

11. Pharmaceutical composition according to Claim 9, characterized in that, as a further active principle, it comprises a therapeutically active bile acid.

12. Pharmaceutical composition according to Claim 11, characterized in that said bile acid is selected from CDCA, tauroUDCA, tauroiodeoxycholate, fluoroUDCA.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K35/74 //A61K35/74,A61K31:19

According to International Patent Classification (IPC) or to both national classification and IPC.

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the international search (name of database and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>EP 0 196 858 A (ADVANCE KAIHATSU KENKYUSHO) 8 October 1986 see page 7 - page 8 see page 13 - page 14</td>
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Further documents are listed in the continuation of box C.

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"I" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another invention or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"S" document member of the same patent family

Date of the actual completion of the international search

18 April 1997

Date of mailing of the international search report

2 9 0 4 9 7

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx: 31 651 epo nl, Fax (+31-70) 340-3016

Authorized officer

Fernandez y Branas, F
## INTERNATIONAL SEARCH REPORT

### DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>DIGESTION, vol. 23, 1982, pages 80-88, XP000673022 SALVIOLI G. ET AL: &quot;Bile acid transformation by the intestinal flora and cholesterol saturation in bile&quot; see the whole document ---</td>
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INTERNATIONAL SEARCH REPORT

Box I  Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 8
   because they relate to subject matter not required to be searched by this Authority, namely:
   
   Remark: Although claim(s) 8 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:  
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:  
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II  Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims, it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)
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