5.0 INVESTIGATOR'S BROCHURE: SILIBININ-C-2´,3-DIHYDROGEN SUCCINATE, DISODIUM SALT IN THE PREVENTION AND TREATMENT OF AMATOXIN INDUCED HEPATIC FAILURE

SUMMARY OF PRODUCT CHARACTERISTICS

1. TRADE NAME OF THE MEDICINAL PRODUCT: Legalon® SIL

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

1 rubber-stoppered vial of *598.5* mg dried substance contains: Silibinin-C-2', 3-dihydrogen succinate, disodium salt 528.5 mg [corresponding to 476 mg mono-, dihydrogensuccinate sodium salts (HPLC)] equivalent to 350 mg (315 mg HPLC) of silibinin

3. PHARMACEUTICAL FORM rubber-stoppered vial

4. CLINICAL PARTICULARS

4.1 Therapeutic Indication: The prevention and treatment of amatoxin induced hepatic failure

4.2 Posology and method of administration : lyophilized powder for intravenous use

4.2.1 Dosage

Continuous Infusion method: Initial dose: 5 mg/kg body weight over 1 hour then: 20 mg/kg body weight/day by continuous infusion.

4.2.2 Method and duration of treatment

- Dissolve each of the 4 bottles that are contained in a package in 35 ml of D5NS. When done one will have a total of 140 ml of solution containing the drug.
- Take a 500 ml bottle of glucose and remove 140 ml and replace the removed part with the 140 ml of drug solution.
- The initial loading dose is a one hour infusion of 5 mg/kg.
  After the loading dose the drug should be infused continuously via a 24 hour infusion at a rate of 20 mg/kg/day
- Treatment shall be stopped after 24 hrs if no gastrointestinal symptoms occur.
- In the presence of gastrointestinal signs treatment shall be stopped after 48 hrs if no liver toxicity occurs.
- In case of hepatotoxicity, treatment will continue until PT/INR (if above the upper limit of normal) returns to normal limits, liver function tests decrease significantly, and creatinine (if above the upper limit of normal) also decreases significantly.

4.3 Contra-indications: None known.

4.4 Special warnings and special precautions for use: None.

4.5 Interaction with other medicaments and other forms of interaction: None known.

4.6 Pregnancy and lactation: No data available.

4.7 Effects on ability to drive and use machines: None reported.

4.8 Undesirable effects: The data on undesirable effects are based on the following categories:

Very common: ~ 1/10

Common: ~ 1/100 to < *1/10*

Uncommon: ~ *1/1,000* -< 1/100

Rare: ~ 1/10,000 -< 1/1,000

Very rare: < 1/10,000

Not known: Cannot be estimated from the available data

Very common: heat sensation (flush) may occur during initial infusion.

4.9 Overdose: No toxic effects reported.

5. PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamics

Pharmacotherapeutic group: Antidote, ATC-code: V03AB (Anatomical Therapeutic Chemical Classification by the WHO for class of “antidotes”)

The use of Legalon® SIL (silibinin) for amatoxin poisoning has a well-recognized biochemical mechanism of action: inhibition of OATP1B3 mediated amanitin uptake by hepatocytes. (1, 2) This data also suggests that Penicillin G works by the same mechanism, although with a much less significant effect, and that combining Penicillin G with silibinin may lead to a reduced therapeutic effect. The undesirability of combining Penicillin G with silibinin was the subject of a recent paper published in Germany which suggested less favorable outcomes

when Penicillin G combination therapy was used in comparison to silibinin monotherapy (3).

A second mechanism of action for Legalon® SIL has also emerged. Amatoxin appears to induce fulminant hepatic failure via TNF mediated hepatocyte apotosis. Building on earlier work it now appears that silibinin may inhibit TNF release in the injured liver (4, 5, 6, 7).

5.2 Pharmacokinetic properties

During a 2 hour infusion of Legalon® SIL silibinin ester is detectable in the plasma only in an unconjugated form. Elimination from the blood takes place so rapidly that only small amounts of conjugated silibinin-C-2', 3-dihydrogen succinate, disodium salt are detectable, three hours after the end of infusion. After de-esterification silibinin is also detectable. From blood analyses it may be assumed that silibinin-C-2', 3-dihydrogen succinate, disodium salt is rapidly eliminated and metabolised. Therefore, the intervals between infusions should not exceed 4 hours and a continuous intravenous drip appears to be the most suitable form of administration (8).

5.3 Preclinical Safety Data

In acute testing silibinin-C-2', 3-dihydrogen succinate, disodium salt proved to be practically non-toxic. The LD 50 values after intravenous injection for rats and mice of both sexes are > 1000 mg/kg.

After intravenous, intraarterial or intramuscular injections good local tolerance was observed in rats and rabbits.

In a subacute study over 4 weeks, the active ingredient was well tolerated by male and female beagles in the doses up to 150 mg/kg silibinin per infusion

except for a low-grade transient depressant effect on the circulation in the high dosage group.

Toxicological investigations into the reproduction of rats and rabbits did not reveal any embryo-lethal and/or teratogenic effects after doses of up to 50 mg/kg silibinin. After the administration of higher doses, fetal death due to maternal toxic effects was recorded.

Mutagenicity tests carried out on microorganisms (Ames Test) and mammalian cells in vitro (CHO-and Mouse-Lymphoma Test) were all negative.

Tolerance in rats and rabbits was good after intravenous, intra-arterial or intramuscular injections.

6. PHARMACEUTICAL PARTICULARS

6.1 List of excipients: Inulin

6.2 Incompatibilities : None

6.3 Shelf life: 5 years

6.4 Special precautions for storage

Do not store above 25°C. Once constituted the chemical and physical stability of this ready-to-use preparation has been demonstrated for a period of 12 hours at 20°C. However, to prevent microbiological contamination once constituted the preparation should be used immediately.

6.5 Nature and contents of container

Packs of 4 rubber-stoppered vials containing the dried substance.

6.6 Instructions for use / handling

Hemoperfusion and hemodialysis should be instituted between the Legalon® SIL infusions in order to minimize as much as possible the removal of silibinin from the circulation.

Control of electrolytes, acid-base metabolism and fluid balance is required. Approximately 0.36 mmol sodium per kg body weight are supplied with the recommended daily dose of 20 mg silibinin per kg b.w. and the corresponding amount of sodium chloride used to dissolve it.

7. MARKETING AUTHORIZATION HOLDER

MADAUS GmbH 51101 Koln, Germany

8. MARKETING AUTHORIZATION NUMBER

4178.00.00 (Germany)

9. DATE OF FIRST AUTHORIZATION/ RENEWAL OF AUTHORIZATION

18.04.1984/ 17.01.2005 (Germany)

International Marketing Authorizations

Legalon® SIL with the active substance “Silibinin-C-2´, 3-dihydrogen succinate, disodium salt” is registered or licensed for use in the following countries:

Austria, Belgium, Czech Republic, France, Germany, Hungary, Italy, Luxembourg, Portugal, Slovak Republik, Spain, Sweden, Switzerland

10. DATE OF (PARTIAL) REVISION OF THE TEXT

April 2008
MADAUS GmbH

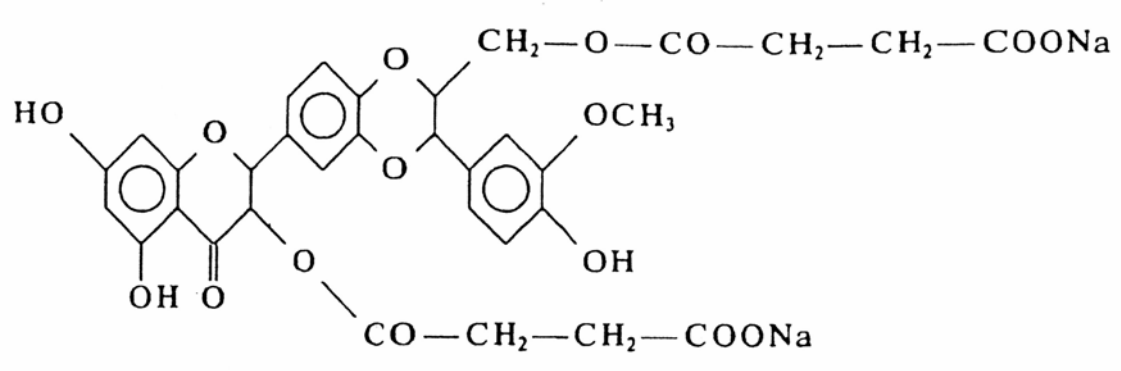
PRODUCT INFORMATION

1. Drug Substance and formulation

1.1 Drug substance

Silibinin-C-2´,3-dihydrogen succinate, disodium salt, is produced by reacting Silibinin obtained by extraction from milk thistle fruit (Ph.Eur.) with succinic anhydride. Silibinin is a mixture of diastereomers Silibinin A and Silibinin B. After the reaction of Silibinin with succinic anhydride, Silibinin-C-2´,3-dihydrogen succinate, disodium salt, is obtained as a mixture of diastereomers of Silibinin dihydrogensuccinate (*trans*-isomer), sodium salt; Silibinin dihydrogensuccinate (*cis*-isomer), sodium salt; Silibinin monohydrogensuccinate, sodium salt.

SILIBININ-C-2´,3-DIHYDROGEN SUCCINATE, DISODIUM SALT
[Corresponding to 90% of mono-, dihydrogen succinate sodium salts (HPLC)]
[Equivalent to 66% (59% HPLC) silibinin]



Molecular formula: $C_{33}H_{28}O_{16}Na_2$

Molecular weight: 678.510

Molecular Mass: 726.56 g/mol

1.2 Drug Product

Drug substance

Silibinin-C-2',3-dihydrogen succinate, disodium salt, 528.5 mg

[corresponding to 476 mg mono-,dihydrogensuccinate sodium salts (HPLC)]

equivalent to 350 mg (315 mg HPLC) of silibinin

Excipients

Inulin 70 mg USP**

Nitrogen (as inert gas) Ph.Eur. **

Total sum per vial **:** 598.5 mg

2.0 PHARMACOLOGY and TOXICOLOGY of AMANITIN

2.1 Amanitin toxicity in liver and kidney

Two different classes of toxins, amatoxins and phallotoxins can be distinguished in most potentially fatal mushrooms. The phallotoxins have a high affinity for intracellular F-actin muscle filaments and block their polymerization reactions. Since they are hardly absorbed by enteral route in animal experiments, phallotoxins are not thought to play a role in human intoxication.

2.1.1 Amatoxins

Toxicity is due to amanitins, which are extremely thermostable, acid and enzyme resistant, and water soluble. Amanitins are bicyclic octapeptides with alpha and beta amanitin accounting for more than 90% of the total amatoxin content in Amanita phalloides. However, with regard to human poisonings alpha, beta and gamma amanitin are roughly equivalent in their biological activity. While the human lethal dose is 0.1 mg/kg body weight, ingestion of 5 to 7 mg of amatoxins, equivalent to less than 50g of fresh mushrooms, may be lethal.

Amatoxins are readily absorbed through the intestinal epithelium, bind weakly to serum proteins and disappear rapidly from the plasma (9, 10). About 60% of absorbed amatoxins are excreted into bile and then enter the enterohepatic circulation (11). Consequently, the exposure of liver cells to the amanitins is prolonged. Human kinetic studies show that amatoxins are detectable in plasma up to 48 hours post ingestion. The toxins are excreted rapidly in the urine and are detectable up to 4 days post ingestion. No correlation has been confirmed between amatoxin plasma concentrations and the clinical severity or intoxication outcomes. Because of the high urinary excretion rate and since the urine reflects the serum situation several hours beforehand, urinalysis can aid in the diagnosis of even mild amatoxin poisonings.

2.1.2 Mechanism of amanitin toxicity

Amatoxins are transported into the liver by a transport system which is responsible for the flux of bile salts under physiological conditions. Penetration into liver parenchymal cells is rapid (12). Experimentally it has been shown that

amanitins bind 1:1 with a subunit of the DNA-dependent RNA polymerase II (polymerase B), which is responsible for the transcription of DNA to mRNA. Thus the formation of mRNA is inhibited and necrosis of the liver occurs due to slow degradation of the mRNA reservoir.

This effect is manifested earliest in tissues with characteristically high rates of protein synthesis and leads to the initial symptoms of diarrhea and vomiting and later hepatic and renal failure. Microscopic examination of intoxicated hepatic parenchyma reveals fatty degeneration, an abnormal concentration of lipid and carbohydrate in the cell nuclei, and a pattern of centrilobular necrosis with hemorrhage (13). Liver hepatocytes are damaged early and hepatic sinusoids are spared. For the hepatocytes amanitin concentrations as low as 3×10^{-7} M can block 90% of mRNA transcription activity. This critical toxin concentration can be reached as quickly as 1 hour after ingestion of large amounts of amatoxins.

2.1.3 Inhibition of amatoxin activity by silibinin

The use of Legalon® SIL (silibinin) for amatoxin poisoning has a well-recognized biochemical mechanism of action: inhibition of OATP1B3 mediated amanitin uptake by hepatocytes. (1, 2) This data also suggests that Penicillin G works by the same mechanism, although with a much less significant effect, and that combining Penicillin G with silibinin may lead to a reduced therapeutic effect. The undesirability of combining Penicillin G with silibinin was the subject of a recent paper published in Germany which suggested less favorable outcomes when Penicillin G combination therapy was used in comparison to silibinin monotherapy (3).

A second mechanism of action of Legalon® SIL has also emerged. Amatoxin appears to induce fulminant hepatic failure via TNF mediated hepatocyte apotosis. Building on earlier work it now appears that silibinin may inhibit TNF release in the injured liver (10, 11, 12, 13).

2.1.4 Animal models of amanita intoxication

Silibinin efficacy in animal experiments is convincing and formed the basis for its subsequent human clinical application. In mice a dose-related reduction in the mortality of alpha amanitin from 80% in control animals to 0% with parenterally administered silibinin (50 to 100 mg/kg) was achieved (14). Histochemical and histoenzymological examinations of the mice livers 2 days after intoxication revealed antagonism of the amanitin induced changes in animals treated with silibinin dihemisuccinate (150 mg/kg). Additionally, adversely affected enzyme activity in nontreated animals was restored to normal. Only the silibinin treated animals had a normal nucleic acid staining reaction and normalized glycogen and lipid reserves (15).

A single oral dose of the lyophilized deathcap fungus Amanita phalloides caused gastrointestinal signs of diarrhea, retching, and vomiting in beagles after 16 hours. Liver damage was maximal 48 hours after poisoning and was evidenced by pathologic lesions, increases in serum transaminase (AST, ALT), alkaline phosphatase, bilirubin, and a prolongation of prothrombin time. Four of twelve dogs given A. phalloides died with signs of hepatic coma within 35 to 54 hours and the biochemical values in the survivors reverted to normal by the ninth day. Silibinin administration (50 mg/kg) 5 and 24 hours after intoxication suppressed the serum changes and the fall in prothrombin time. The degree of hemorrhagic necrosis in the liver was markedly reduced, and none of the silibinin-treated dogs died (16, 17).

Since dogs exhibit enterohepatic circulation of the toxins like humans the dog model is useful in the study of human amanitin intoxication.

Experiments were also conducted to ascertain whether the renal lesions produced by alpha amanitin could also be affected by silibinin. In mice and rats

the renal damage after amanitin poisoning was exhibited via reductions in glomerular filtration rate, decreases in reabsorption of sodium, water, glucose and PAH clearance. Pretreatment with silibinin improved significantly these abnormalities (18).

2.2 TOXICOLOGY

In acute toxicity testing silibinin-C-2´, 3-dihydrogen succinate, disodium proved to be practically non-toxic. The LD50 values after intravenous injection for rats and mice of both sexes are > 1000 mg/kg (19, 20).

In a 4 week subacute study, silibinin was well tolerated by male and female beagles at doses up to 150 mg/kg per infusion. However, a lowgrade transient circulation depressant effect was seen in the high dosage group (21).

Reproduction toxicity studies in rats and rabbits did not reveal any embryo-lethal and/or teratogenic effects after doses of up to 50 mg/kg silibinin. At higher doses fetal death due to maternal toxic effects was recorded (22, 23).

Mutagenicity tests carried out on microorganisms (Ames Test) and mammalian cells in vitro (CHO- and Mouse-Lymphoma Test) were all negative (24).

Tolerance in rats and rabbits was good after intravenous, intra-arterial or intramuscular injections (25).

3.0 Pharmacokinetics

3.1 Pharmacokinetics after single dose in animals

Blood concentration time profiles of non-volatile radioactivity in rats injected intravenously with 5 or 50mg/kg of tritium-labelled Legalon® SIL showed a very rapid decrease in the first two hours with biliary excretion of more than 90% of the dose. First phase half lives were very short, whereas a relatively long terminal half life of 10 to 15 hours was seen. The percentage of renal excretion increased with the dose and may be indicative of a dose-dependent saturation of biliary excretion (26).

In another animal study, anaesthetised dogs with a bile catheter were injected with 5mg/kg Legalon® SIL i.v. Plasma levels and excretion were determined up to 6 hours after injection. Anesthesia was maintained during this time. Comparable to the rat data, decrease of plasma concentrations and excretion were very rapid (84% of the dose in the first two hours). Terminal half life, possibly because of the short experimental interval, was only about 4 hours. Also in the dog, the main elimination pathway was via the bile (84%) compared with only 7% in the urine (27).

3.2 Pharmacokinetics after repeated administration in animals

In a rat study, Legalon® SIL was given in four i.v. doses of 12.5mg/kg each with an interval of 4 hours. The total dose amounted to 50mg/kg. Under these experimental conditions, an increase of Cmin but not of Cmax was observed. The terminal half life did not change compared to single administration (26).

3.3 Organ distribution in animals

Distribution studies in male and female rats revealed a very quick decrease of organ concentrations. At any time, they were lower than plasma concentrations. Only concentrations in the carcass, which included intestine (biliary excretion), were sometimes higher than plasma levels. The very low concentrations in the brain indicate the existence of a brain-plasma-barrier.

Organ concentrations after repeated dosing did not show any peculiarities or special affinities of Legalon® SIL. Fifteen minutes p.i., the concentrations were comparable to those after single dose, whereas 24 hours p.i. they revealed no specific accumulation in any of the organs examined (26).

3.4 Plasma protein binding

Plasma binding of Leglaon® SIL was between 92 and 98% as measured by equilibrium dialysis and ultrafiltration in standardized human serum. Ex vivo determinations in dog plasma, performed by the same methods, revealed binding rates of 91 to 97%. Because of such high binding rates, interactions with other high binding drugs are possible (26).

3.5 Biotransformation in animals

When Legalon® SIL was injected into rats, the succinate ester was split by physiologically available esterases and the resulting silibinin was quickly metabolised to sulphated and glucuronised conjugates. The metabolic pattern of plasma and bile was very similar, except that free silibinin could not be detected in the bile samples. A precise characterization of the conjugates was not performed. After incubation of plasma and bile samples with arylsulfatase/glucuronidase, most of the conjugates could be split, yielding percentages of free silibinin up to 90%. It is hypothesized that all metabolites were phase II metabolites, formed after ester splitting (26, 27).

4.0 CLINICAL PHARMACOLOGY

4.1 Tolerability studies in healthy volunteers

In two open studies 12 male young volunteers received a series of 11 infusions with disodium silibinin dihemisuccinate, dissolved in 500 ml of 50/0 glucose solution. 6 volunteers each were given infusions with 350mg silibinin, equivalent to 5 mg/kg b.w./infusion (20 mg/kg/day) and with 875mg silibinin, equivalent to 12.5 mg/kg b.w./infusion (50 mg/kg/day). Infusion time was approximately 2 hours with intervals of approximately 4 hours between infusions (8).

None of the measured parameters differed significantly from the normal baseline value throughout the study except for the renal sodium excretion after infusion with daily 20 mg silibinin /kg b.w. The above normal sodium excretion of all six volunteers is attributable to the increased intake of sodium resulting from the infusions of disodium silibinin dihemisuccinate (approx. 25 mmol/day). However there were no clinically relevant changes in the electrolyte balance.

The subjective impressions reported by the volunteers revealed that all infusions with 5mg/kg were tolerated without major adverse events. In isolated cases mild headaches were reported, while 1 volunteer reported hot flushes and drowsiness during the first day of infusion.

After infusion with 12.5 mg/kg, 2/3 of the volunteers reported sensation of warmth and flushes in the course of the first infusion which subsided with subsequent infusions. Diarrhoea (soft feces) was reported throughout the entire study period and is attributable to the large fluid intake. Most of the volunteers reported headaches in the course of the study.

4.2 Treatment studies

4.2.1 Course of disease in untreated patients

Patients who consume amatoxin containing mushrooms exhibit symptoms and signs that typically occur in a progression through three stages (13).

There is an initial quiescent period (6-24 hours post ingestion) after which cramping abdominal pain, nausea, vomiting, and watery “cholera like” diarrhea develop. It is at this point that most people seek medical care and may be misdiagnosed as having viral gastroenteritis. Routine laboratory values may reflect dehydration and electrolyte loss, but they are of little value in assessing the magnitude of intoxication or predicting the eventual outcome. Initial diagnosis and treatment of suspected amatoxin poisoning should not be delayed to wait for identification of uneaten mushrooms or detection of the toxin in the patient’s bodily fluids.

The second stage of amatoxin poisoning is characterized by generalized clinical improvement (resolution of gastrointestinal symptoms) that begins 24 to 48 hours after ingestion, but masking the hepatic deterioration that is occurring at the same time. The liver function tests show a progressive elevation of transaminases and an evolving coagulopathy. Early renal damage may be reflected by elevations in the serum creatinine and blood urea nitrogen levels.

Transition into the third stage can occur quite suddenly. The hepatic necrosis may be fulminant and is manifested by a severe increase in serum transaminases and profound coagulopathy. Patients may experience a swift progression from stage I to stage III or IV encephalopathy. The presence of hypoglycemia is a poor prognostic sign reflecting massive hepatic necrosis. Renal failure, due to hepatorenal syndrome and/or direct nephrotoxicity of

amanitins, may result in severe oliguria or anuria. 50% of patients with amatoxin poisoning may also have laboratory evidence of pancreatitis (13).

4.2.2 Prognosis in untreated patients

Grade 1: Patients develop gastroenteritis-like symptoms several hours (6-36) after ingestion but do not develop severe biochemical indications (peak transaminases <1000 without coagulopathy) or renal dysfunction.

Grade 2: Patients manifest a moderate (1000-5000 IU/L) rise in transaminases and a mild (peak INR <2.0) coagulopathy.

Grade 3: Patients develop a marked elevation of transaminases (>5000 IU/L) and significant coagulopathy (INR>2.0). Grade 3 is divided into two subgroups according to bilirubin values. In grade 3a bilirubinemia is mild or absent, while grade 3b shows a steep and continuous rise in bilirubin (greater than 5 mg/dL).

Grade 4: A steep rise in transaminases is accompanied by a corresponding steep decline in clotting function (INR>3.0) and renal dysfunction.

Grade 1 and grade 2 patients have the best chance of surviving amatoxin poisoning and need symptomatic treatment only. Grade 3, particularly grade 3b patients, are at risk and should be transferred to a tertiary care center where liver transplant is available. Grade 4 patients have an extremely poor prognosis. There is a 90% probability of death in spite of intensive therapy. Survival usually requires a liver transplant.

4.2.3 Symptoms listed by organ systems and their prognostic value

Transaminases

Grade 1 patients show mild elevations of liver enzymes. Grade 2 patients develop a moderate rise in transaminases, usually below 1,000 IU/L but sometimes reaching several thousand IU/L. Grade 3 patients show a steep and massive rise in transaminases. AST peaks on day 3, whereas the ALT peaks on day 4. There is no significant difference in grade 4 transaminases as compared to grade 3, although in fatal cases the mean values are generally higher. A decrease of transaminase values on day 4 is either a sign of improvement or a hepatic “burn-out” phenomenon. A massive rise in transaminases (grade 3 and 4) does not necessarily predict a fatal outcome, whereas a mild rise in transaminases (grade 2) always indicates a good chance for survival.

Prothrombin Time

The early and steep prolongation of prothrombin time values is always a bad sign. In Europe, using the Quick’s test (the previous standard before the INR for assessing coagulopathy), those patients in whom prothrombin times could not be improved to values above 20-30% by substitution of clotting factors on day 3-4 had >90% mortality. From 48 hrs after ingestion onwards, there was a significant difference between the prothrombin time of survivors in grade 3 (>30%) and non-survivors in grade 4 (<20%).

Bilirubin

On admission to the hospital, bilirubin values are normal in 90% of all cases. Elevation at this early stage (>3 mg/dL) suggests a poor prognosis. During the course of the intoxication, only about 20% of grade 3 patients (grade 3b) show a marked rise in bilirubin. It is possible to obtain information on the prognosis from the absolute values after day 3. At that time, values <5 mg/dL indicate a

favorable prognosis, even if transaminases are high, while a value > 5 mg/dL indicates a severe or fatal course.

Impairment in kidney function

Prerenal azotemia secondary to dehydration on initial presentation is a common finding that can usually be reversed by aggressive fluid replacement. However early renal dysfunction that does not fully correct after intravenous rehydration signals a poor prognosis. Acute tubular necrosis may also occur in severely dehydrated patients and likely represents a direct cytotoxic effect by the amanitins. A slight elevation of creatinine on days 1 to 2 that does not respond to fluid replacement suggests that a direct toxic effect has occurred. About 70% of fatal cases show this early increase of creatinine. Hepatorenal syndrome occurs late and indicates an extremely poor prognosis.

4.2.4 Discharge criteria and sequelae

If a case of suspected amatoxin poisoning has not been confirmed by the clinical signs or laboratory findings outlined above, patients can be discharged after 24 hrs. Grade 1 and grade 2 patients [patients in whom, 3 days after poisoning, AST/ALT is < 5000U/L, INR <2.0, bilirubin is at most mildly elevated, creatinine is WNL] can be discharged early. Grade 3 patients should remain in the hospital until PT/INR is back to normal and transaminases, bilirubin, and creatinine are improving.

Usually, sequelae are absent in patients surviving amatoxin poisoning. In cases of preexisting liver or kidney damage the patients' condition may worsen after amatoxin intoxication. Chronic active hepatitis and rarely cirrhosis have been reported in some survivors.

4.3 Legalon® SIL treatment recommendations

Initial loading dose of 5 mg/kg over 1 hr, followed by a 20 mg/kg/day continuous infusion for as long as necessary.

4.3.1 Other supportive treatments (28)

Initial measures

- Fluid and electrolyte replacement with central venous pressure monitoring as indicated

Elimination of toxins

- Multidose activated charcoal (MDAC) - 50 grams via gastric tube every 4 hours has been traditionally used.

Retain evidence of poisoning

- Remains of the meal for identification of fungi
- Urine for amatoxin assay if available.

Supportive measures

- Lactulose and intraluminal antibiotics for hepatic encephalopathy
- Intravenous glucose, magnesium and phosphate replacement
- Vitamin K and fresh frozen plasma for coagulopathy

4.4 Silibinin i.v. for amatoxin poisoning

4.4.1 Open labeled clinical trial of 201 patients, 1981-1982 in Europe (29)

Of the 201 patients 96 had firmly established amanita intoxication and the clinical characteristics for these “proven” cases were divided into subgroups as per Tab 2.

Tab. 2 Subgroup of proven cases (n = 96), characterized by ALT/AST and Quick- test (mean values, days after intoxication)

Proven cases	ALT/AST	Quick
Subgroup 1	235 U/l within 4 days	65 % within 2 – 3 days
Subgroup 2	Proven cases 1839 U/l within 3 days	43 % within 2- 3 days
Subgroup 3	5000 U/l within 3 day	23 % within 2 – 3 days
Subgroup 4	atypical clinical course	

[Quick-test = Prothrombin time]

Survival rates for the 4 subgroups of proven patients is in Tab 3.

Tab. 3 Survival rate in patients with proven intoxication (n = 96)

Proven cases	n	death cases n	Survival rate %
Subgroup 1	23	0	100
Subgroup 2	31	3	90
Subgroup 3	35	10	71
Subgroup 4	7	7	0

Of the 20 patients who died, 17 were treated with penicillin and silybinin. Treatment with Legalon® SIL was started in half of these patients on the day of or after the individual minimum in Quick or maximum in transaminases. Early treatment with Legalon® SIL is correlated with lower mortality, as shown in Tab 4.

Tab. 4 **Time period between intoxication and start with LEGALON SIL treatment in patients with proven intoxication (n = 96, data missing for n = 2)**

Patients (n)	Time period (h)	Death cases (n)	%
26	< 24	3	12
43	> 24 - ≤ 48	10	23
13	> 48	5	38
12	> 72	2	17

The 10% mortality rate for all patients given Legalon® SIL, regardless of the severity of intoxication, is lower than the reported 22% before Legalon® SIL was available.

4.4.2 Drug surveillance study, 1983-1992 (30)

Case reports from 1983-1992 for 154 patients treated with Legalon® SIL for suspected amanita intoxication were evaluated for efficacy and tolerability. All patients received Legalon® SIL either as 'antidotal' monotherapy (n = 28) or in addition to penicillin G and/or cephalosporins (n = 126). Laboratory results and mortality rate were evaluated for both treatment regimens, i. e. Legalon® SIL

monotherapy and Legalon® SIL plus penicillin, especially in patients with severe intoxication.

The overall mortality rate for the 154 patients was 9.7 % (n = 15). All but 3 deceased patients (unclassifiable) had severe stage IIIb intoxication. There was one fatality (4 %) in the Legalon® SIL monotherapy group and 14 fatalities (11 %) in the Legalon® SIL plus penicillin group.

The figures below compare laboratory values and death rates for patients on Legalon® SIL alone versus those who were treated with Legalon® SIL plus penicillin. Fig 1 contains the data for the Quick index measure of clotting times. The only patient who died with Legalon® SIL monotherapy was hospitalized 3 days after intoxication with a Quick index of 10 %. Fig 2 contains the data for ALT. Fig 3 contains the data for bilirubin. Fig 4 contains the data for creatinine.

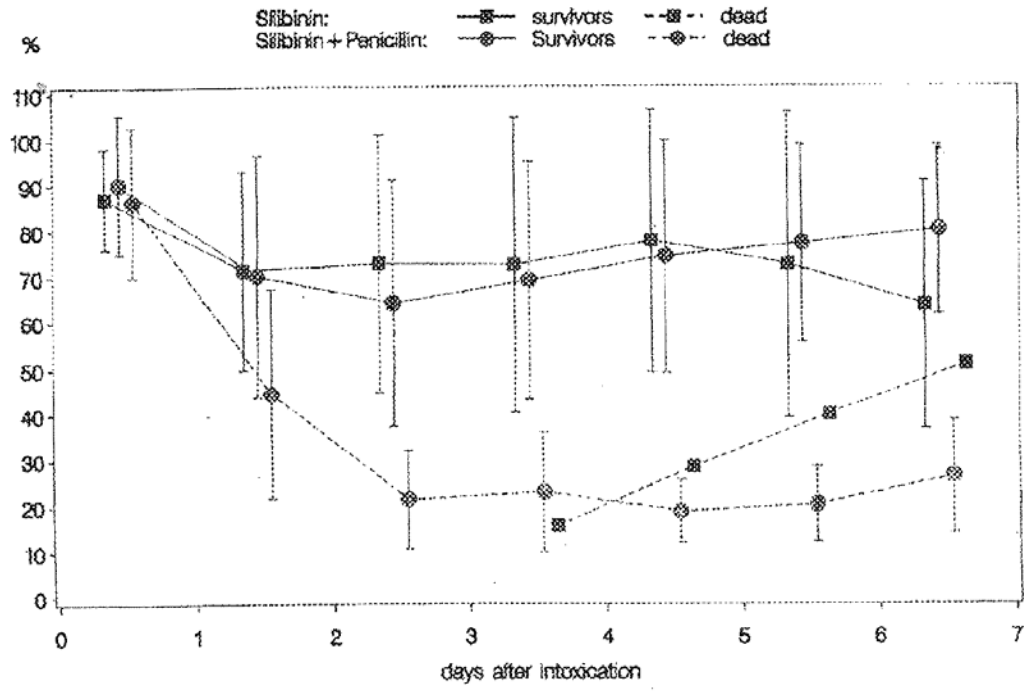


Fig. 1 **Quick index (mean ± SD) during 7 days after intoxication in patients treated with LEGALON SIL monotherapy (n = 28,**

silibinin) and with LEGALON SIL in addition to penicillin (n = 126, silibinin + penicillin)

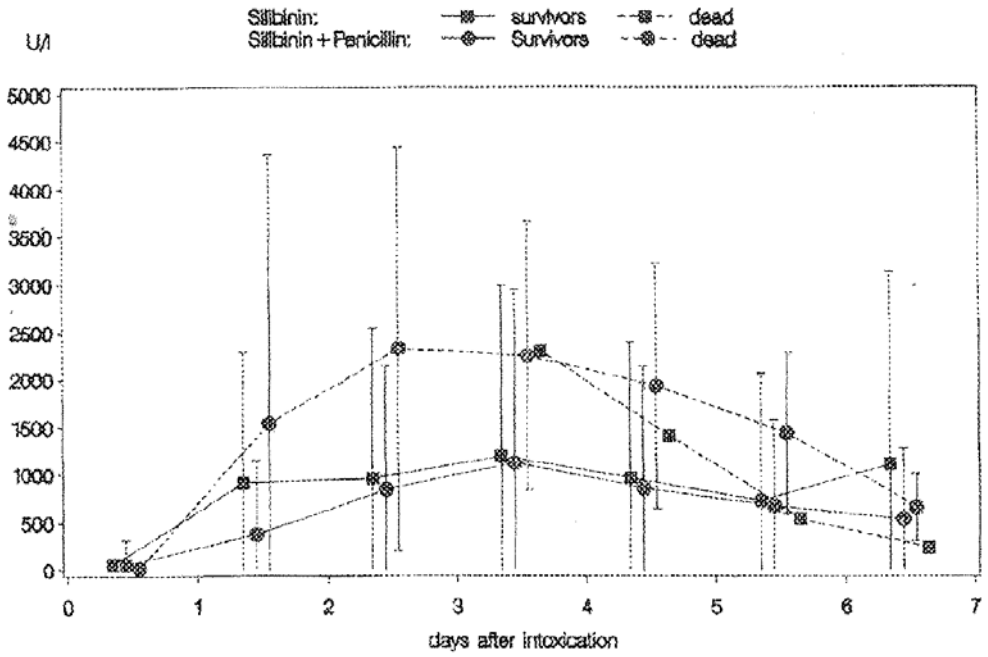


Fig. 2 **ALT (mean ± SD) during 7 days after intoxication in patients treated with LEGALON SIL monotherapy (silibinin) and LEGALON SIL in addition to penicillin (silibinin + penicillin)**

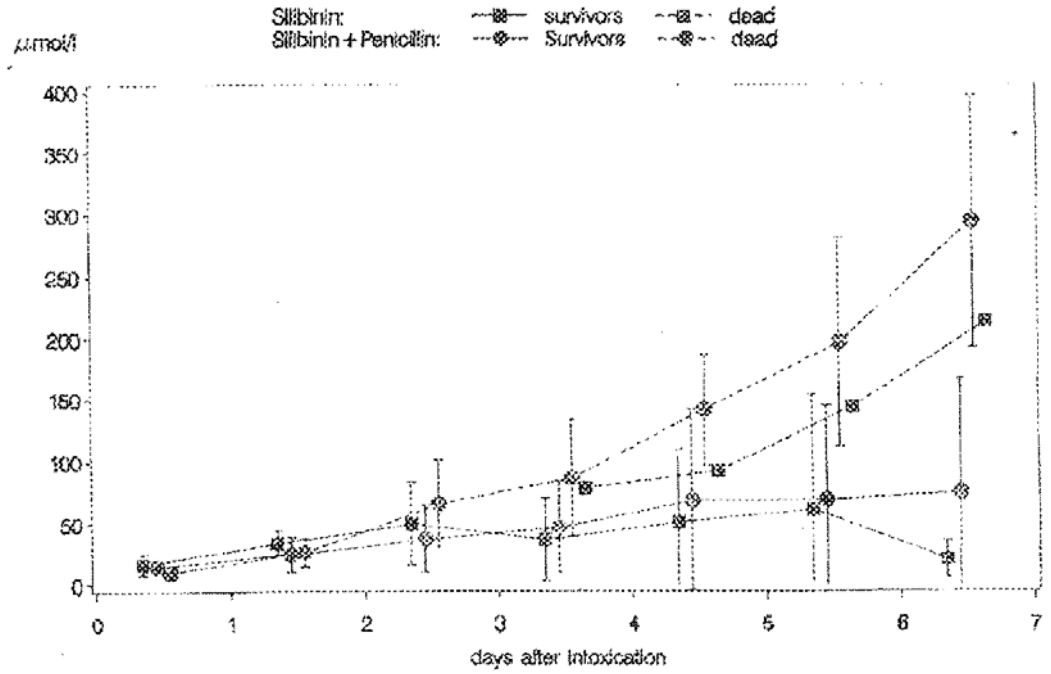


Fig. 3 **Bilirubin (mean ± SD) during 7 days after intoxication in patients treated with LEGALON SIL monotherapy (silibinin) and LEGALON SIL in addition to penicillin (silibinin + penicillin)**

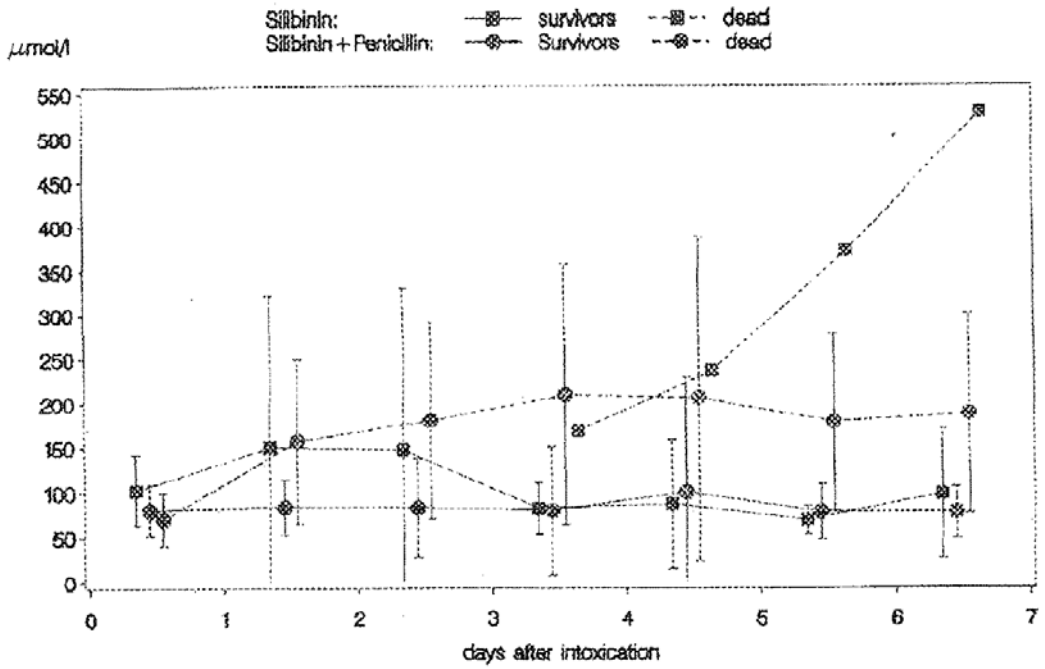


Fig. 4 **Creatinine (mean ± SD) during 7 days after intoxication in patients treated with LEGALON SIL monotherapy (silibinin) and LEGALON SIL in addition to penicillin (silibinin + penicillin)**

4.4.2.1 Pediatric use

Among the 154 intoxicated patients, there were 18 children between 6 and 14 years of age. Eight children presented with stage IIa or IIIb intoxications. Most of the children were treated with Legalon® SIL plus penicillin. There was 1 death among the intoxicated children (stage IIIb).

4.4.3 Retrospective review for the years 1987-2005 published in 2008.

A retrospective review from years 1987-2005 showed 118 patients received Legalon® SIL monotherapy and 249 patients received Legalon® SIL plus penicillin. Both groups had comparable patients with mean ages of 44 yrs vs 39 yrs, time that Legalon® SIL treatment was initiated (32 hrs for both groups; penicillin was started at a mean of 34 hrs), and duration of symptoms (12 hrs in both groups) (3).

The mortality rate in the patients who received Legalon® SIL plus penicillin was 8.4% (21 of 249) and there was a 0.8% (1 of 118) mortality rate in patients treated with Legalon SIL as monotherapy. There was one patient in the Legalon® SIL plus penicillin group and 5 patients in the monotherapy group who required liver transplantation. Therefore the rate of serious morbidity/mortality was 22 of 249 (8.8%) in the combined group vs 6 of 118 (5.1%) in the monotherapy group. In the multivariate model, monotherapy vs combined therapy was not statistically significant (p = 0.28), the time prior to starting silibinin was almost significant (p = 0.059), and the duration of symptoms was significant (p = 0.004).

These data suggest that penicillin is not required if silibinin is administered. These data also point out the importance of rapid recognition of intoxication and early implementation of Legalon® SIL for successful outcomes.

4.4.4 Post marketing surveillance data from 1993-1995

4.4.4.1 Specific postmarketing reports received by Madaus

51 postmarketing patient reports were received by MADAUS AG between 1993 and 1995. When the patients were classified according to severity grades, 19 patients had severe intoxications of stage IIIa (n =14) or IIIb (n =5), and all other patients had milder intoxications of stage O (n =3), stage I (n =16), stage II (n =9) and 4 patients had no severity specified (see Tables 5-7).

There was one fatality classified as IIIa intoxication. This patient received Legalon® SIL and penicillin only on day 3 after intoxication when hepatic and renal deterioration was progressive (Quick-test 48%, ALT/AST 239/317 U/l, bilirubin 2.3 mg%, creatinine 2.4 mg%). The patient died on day 10 after intoxication (bilirubin 14 mg%).

About half of the patients (29/51) received Legalon® SIL in the recommended daily dose of 20 mg/kg/day (± 10 %) (range 13-36 mg/kg/day). Treatment duration ranged from 1 to 7.5 days. The mortality rate among the severely intoxicated patients was 5%. No adverse drug reactions were reported.

Table 5 **Postmarketing surveillance data from 1993**

Pat. no.	Severity	Age y	Sex	Mushroom identification	Latency (h) between fungus ingestion and 1. sympt.	Latency (h) between fungus ingestion and ad. miss.	Latency (h) between fungus ingestion and 1.SIL. inf.	Specific treatment PEN+SIL	Specific treatment SIL	SIL dosage mg/kg	SIL duration d	Laboratory parameters Min./Max. Quick %	Laboratory parameters Min./Max. AST U/l	Laboratory parameters Min./Max. ALT U/l
93/1	0	53	m	RIA+	8	2	2	-	SIL	19	6	100	22	30
93/2	IIIa	46	f	not done	12	15	31	PEN+SIL	-	25	5	50	1490	4020
93/3	IIIa	52	m	not done	13	16	18	PEN+SIL	-	18	5	35	2500	4920
93/4	IIIa	20	m	not done	12	15	30	PEN+SIL	-	23	5	45	2860	4600
93/5	IIIa	62	m	not done	11	24	24	PEN+SIL	-	20	3	52	24	42
93/6*	IIIa	81	f	RIA+	10	46	78	PEN+SIL	-	20	5	16	1353	2299
93/7	II	73	m	RIA+	11	25	32	-	SIL	28	3	55	1848	2700
93/8	-	66	f	RIA+	11	25	32	-	SIL	27	5	27	886	2044

* deceased on day 9 after intoxication

Table 6 **Postmarketing surveillance data from 1994**

Pat. no.	Severity	Age y	Sex	Mushroom Identification	Latency (h) between fungus ingestion and 1. sympt.	Latency (h) between fungus ingestion and ad-miss.	Latency (h) between fungus ingestion and 1.SIL-inf.	Specific treatment PEN+SIL	Specific treatment SIL	SIL dosage mg/kg p.d.	SIL duration p.d.	Laboratory parameters Min./Max. Quick %	Laboratory parameters Min./Max. AST U/l	Laboratory parameters Min./Max. ALT U/l
94/1	0	41	f	not done	0	4	8		SIL	20	0,5	-	-	-
94/2	I	58	f	not done	15	20	24	-	SIL	21	3	-	-	-
94/3	I	52	m	RIA+	10	15	10	-	SIL	19	7	-	-	-
94/4	I	47	f	not done	10	14	16	-	SIL	20	7,5	-	-	-
94/5	I	6	m	RIA+	14	16	28	-	SIL	20	3	-	-	-
94/6	II	40	m	not done	12	14	>96	PEN+SIL	-	18	1,5	-	-	-
94/7	I	3	f	RIA+	19	28	32	-	SIL	20	1,5	-	-	-
94/8	II	4	f	not done	13	19	21	-	-	20	5	-	-	-
94/9	-	10	f	RIA+	12	19	21	PEN+SIL	-	20	5,5	-	-	-
94/10	II	30	f	not done	13	26	31	PEN+SIL	-	23	4,5	44	2860	5620
94/11	0	53	f	not done	6	3	4	PEN+SIL	-	15	1,5	91	13	18
94/12	I	62	m	pos.	16	22	22	-	SIL	18(D)	-	-	-	-
94/13	I	63	f	pos.	8	23	24	-	SIL	21	2	65	25	39
94/14	I	64	m	not done	12	18	68	-	SIL	16	1	82	36	84
94/15	-	26	m	not done	13	18	68	-	SIL	23	1	87	27	58
94/16	-	70	m	pos.	30	52	70	PEN+SIL	-	16	2,5	72	83	69547
94/17	IIIb	71	f	-	10	48	60	PEN+SIL	-	15	4,5	24	3360	0
94/18	II	13	m	not done	16	27	45	PEN+SIL	-	30	4	68	144	134
94/19	IIIa	10	f	not done	16	27	32	PEN+SIL	-	20	3,5	63	1040	880
94/20	IIIa	63	f	pos.	13	45	48	PEN+SIL	-	20	2,5	41	6120	3260
94/21	IIIb	58	m	pos.	16	38	70	-	SIL	30(D)	1,5	26	1500	3400
94/22	IIIb	51	m	pos.	8	20	20	PEN+SIL	-	21	1,5	26	709	1800
94/23	IIIa	61	m	pos.	8	50	52	PEN+SIL	-	-	4	32	13560	12800
94/24	IIIb	21	f	pos.	10	-	45	-	SIL	16	3	16	8210	11500
94/25	I	35	f	not done	6	16	18	PEN+SIL	-	24	2	87	28	30
94/26	IIIa	73	m	not done	8	20	84	PEN+SIL	-	22	2	39	5936	2512
94/27	IIIb	52	m	not done	6	48	96	PEN+SIL	-	23	4	19	4250	4620

Table 7 **Postmarketing surveillance data from 1995**

Pat. no.	Severity	Age	Sex	Mushroom	Latency (h) between fungus ingestion and			Specific treatment		SIL dosage	SIL duration	Laboratory parameters Min./Max.		
				Identification	1. sympt.	admiss.	1.SIL-Inf.	PEN+SIL	SIL	mg/kg p.d.	p.d.	Quick %	AST U/l	ALT U/l
95/1	I	2	m	pos.	40	41	42	-	SIL	20(D)	2,5	68	20	13
95/2	I	9	m	pos.	12	18	20	-	SIL	20(D)	3,5	67	24	17
95/3	I	52	f	RIA+	9	16	16	-	SIL	31(D)	3	89	22	30
95/4	I	29	m	RIA+	11	19	19	PEN+SIL	-	21(D)	5	31	514	1117
95/5	II	15	f	RIA+	9	18	18	PEN+SIL	-	21(D)	4	62	496	1086
95/6	I	33	f	RIA+	11	17	18	-	SIL	36(D)	3	95	16	27
95/7	II	51	f	RIA+	13	24	65	PEN+SIL	-	19	2,5	66	890	1016
95/8	I	23	m	RIA+	15	23	24	PEN+SIL	-	18	2,5	63	-	-
95/9	II	11	m	RIA+	20	22	-	-	SIL	13	6	-	1776	2390
95/10	IIIa	15	f	RIA+	9	16	44	PEN+SIL	-	23	7	-	-	-
95/11	I	39	f	not done	-	-	-	-	-	-	-	-	-	-
95/12	II	52	m	not done	12	16	22	-	SIL	20	-	82	585	673
95/13	IIIa	53	m	not done	12	60	63	PEN+SIL	-	24	-	8	4760	4740
95/14	IIIa	63	f	pos.	11	45	48	PEN+SIL	-	20	2,5	41	3250	6120
95/15	IIIa	36	m	not done	20	48	53	PEN+SIL	-	22,5	5	81	2778	4920
95/16	IIIa	36	m	not done	17	120	122	PEN+SIL	-	-	2,5	50	79	1188

4. 4.4.2 Safety surveillance

Since marketing began in 1984 and up to 1996 the amount of Leglaon® Sil in distribution is enough for 19,926 treatment days and is equivalent to a full course of drug for more than 3,900 patients. Despite this relatively large number of potential patients there have been no reports of serious Legalon® SIL adverse drug reactions (ADR's). The following are summaries of the six non-serious ADR's that have been reported:

Case 1: Flushing was reported for 5 days during an infusion of Legalon® SIL at a daily dosage of 50 mg/kg/day. Flushing is mentioned as a possible side effect in the product profile of Legalon® SIL.

Case 2: A patient with a history of penicillin allergy developed pruritus during a two day course of combined penicillin and silibinin despite concomitant application of cortisone. After withdrawal of penicillin pruritus was no longer mentioned. On day 8 of Legalon® SIL treatment, the patient presented with a rash which disappeared after discontinuation of the drug.

Case 3: A patient presented with a rash on the second day of treatment with penicillin and silibinin. After withdrawal of penicillin there was no further report on the rash.

Case 4: A patient developed a rash on the trunk and extremities six days after withdrawal of penicillin and two days after discontinuation of silibinin.

Case 5: A rash developed at the injection site after infusion of penicillin and silibinin. After treatment with antihistamines the rash disappeared.

Case 6: One patient experienced flushing and developed somnolence during Legalon® SIL infusion. These symptoms disappeared after the withdrawal of Legalon® SIL.

From 1996 until the end of 2001 drug surveillance continued. Reports from another 62 patients after Legalon® SIL infusions were received. No Legalon® SIL specific adverse drug reactions or interactions with other drugs were observed.

4.4.5 Published experience with Legalon® SIL (see Table 8)

Table 8 **Published experience with LEGALON SIL**

Authors	Treatment regimen	No. of. patients treated	deceased	Comments
Floersheim et al. 1982	PEN + SIL	16	0	treatment start 60 h post intoxicationem: severity grade: n = 5 slight n = 6 medium n = 7 severe
Hruby 1987	SIL PEN + SIL	17 42	1 0	severity grade: n = 4 slight n = 6 medium n = 7 severe
Marugg, Reuter 1985	PEN + SIL	12	1	48 h post intox., Quick test 16% ALT > 15.000 U/l, creatinine > 400 µmol/l
Smetana et al. 1986	PEN + SIL	2	0	
Schenke et al. 1987	SIL	2	0	
Kelbel, Weilemann 1989	PEN + SIL	5	1	treatment start 36 h post intox. (Quick test 40 %)
Nagy et al. 1994	PEN + SIL	4	1	child (14) had severe intoxications (Quick test 9 %) and neurological symptoms at admission
Molling et al. 1995	PEN + SIL	2	0	
Kleist-Retzow et al. 1995	PEN + SIL	2	0	
Carducci et al. 1996	PEN + SIL	4	0	treatment with PEN 1st day, SIL treatment day 3-9 severity grade: n = 3 severe n = 1 medium
Alves et al 2001	PEN + SIL	4	0	treatment start 12-48 h after intox. severity grade: n = 4 severe n = 2 orthopic liver transplantation
Boyer,et al. 2001	SIL + N-Acetyl-cystein	1	0	severity grade: medium 11th week of pregnancy No damage to the foetus/child

4.5 Efficacy Summary

There is convincing evidence elucidating silibinin's mechanism for inhibiting the uptake of amanitin into hepatic cells which helps explain the reduced mortality seen in the dog study models that are most applicable to human experience. In addition to mortality, and lab measurements of liver and kidney function, histological analyses of cell damage were improved in these models with the use of silibinin dihydrogensuccinate.

There are more than 700 well documented cases supporting the clinical efficacy of Legalon® SIL as 'antidotal' treatment in patients with amanita intoxication. This represents the largest patient population ever analyzed for this potentially fatal intoxication. The overall mortality rate for Legalon® SIL treated patients regardless of intoxication severity is10%. This represents more than a 50% reduction in the reported mortality rate of 22% before Legalon® SIL was available (31, 32). For severely intoxicated children historic figures show that the mortality rate is higher than with adults at 35%. Therefore it is important to note that the documented cases of severely intoxicated children who were treated with Legalon SIL had a mortality rate of only 13 % which represents a reduction in mortality of more than 60%.

5. Description of possible risks and side effects to be anticipated

In the more than 700 known documented cases where Legalon® Sil was used for amatoxin intoxication there have been no reports of serious adverse drug reactions. Despite enough product in distribution to have treated over 3,900 patients only 6 cases of non serious adverse events have been reported spontaneously or in drug surveillance studies. Flushing, especially during the initial loading dose infusion, can be anticipated in a significant number of patients.

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