Current Health Sciences Journal
Vol. 36, No. 3, 2010

Updates on the Treatment of Chronical Hepatitis B
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ABSTRACT Chronic infection with hepatitis B (HBV) is a major public health problem worldwide, with important socio-economic implications. It is estimated that over 2 billion people have been infected with HBV, of which 350-400 million chronic HBV carriers remain. Chronic HBV infection can lead to the development of liver cirrhosis and liver failure. Moreover, HBV is the most common cause of CHC and it is considered an oncogen virus. The goal of therapy in chronic viral hepatitis B is to improve patients’ quality of life and increase survival by preventing progression to cirrhosis, liver cancer and death. Achieving the desired goal can be done through sustained serum HBV DNA suppression, which is accompanied by reducing the histological activity of chronic hepatitis and cirrhosis, leading to the decrease of risk of cancer. HBV infection can not be completely eradicated because of persistence of covalently circular DNA (cccDNA) in the nucleus of infected hepatocytes. But reducing viral load by treatment (studies with interferon or nucleoside analogues) have shown to reduce the risk of liver cancer. Treatment of chronic hepatitis B virus (HBV) is currently an exciting topic because of the progress in this direction. Controversy is tied primarily to the choice of first line treatment - pegylated interferon vs. nucleoside analogues / nucleotide, but also the best way to approach therapy of viral resistance development. There are data that argue the choice of pegylated interferon as initial therapy in patients with chronic viral hepatitis B, both in patients with HBe Ag positive and those with negative HBe Ag. On the other hand, nucleoside analogues (Lamivudine, Entecavir) and the nucleotide (Adefovir, tenofovir) have advantages that make them preferred in certain situations as initial treatment of chronic hepatitis patients with HBV.

KEY WORDS chronic viral hepatitis B, therapy, PEG-interferon alpha 2a, lamivudine, entecavir

Introduction
The infection with VHB is a major health problem worldwide and in our country as well, being one of the most usual causes for hepatic cirrhosis, hepatic carcinoma and death. It is estimated that there are 400 million carriers of the disease and among these 350 million have chronic hepatic disease [1-2]. It is also estimated that 1 million persons die every year because of HVB infection. The endemicity of the infection with HBV varies a lot worldwide being highly influenced by the age of appearance of the infection and the economic development of that certain region. The high prevalence of the infection is found in under developed countries where 7-8% of the population is HBs Ag positive in comparison to the highly developed countries where, due to the vaccin campaigns and other prevention measures, prevalence is under 1% of the population. In Romania the prevalence is considered to be medium although there are few studies related to this [2-3].

Due to the increasing importance of the HBV infection, the identification of certain therapies which would diminish the progress towards cirrhosis and further complications is an essential matter [1-3].

The current treatment of HBV chronic hepatitis and also the criteria for selecting the patients are clearly rated by guide treatments. Nevertheless, the sustained virus response is obtained with only 30% of the patients included in the treatment [3].

Pegylat Interferon
The proteic pegilation is an addition process of polietilenglicol (PEG) to a protein thus achieving a molecule that has the advantage of a protracted plasmatic half-life time, reduced renal clearance and lower immunogenicity compared to the standard formula. The characteristics of the new protein depend on: PEG structure (ex: size,type,force of the bond) and the place of link to the proteic structure [3].

The attachment of the PEG to the interferon molecule is the most recent innovation of the virus B chronic hepatitis therapy. PEG is an inert molecule from a biological point of view and by linking to the proteins, it reduces their degrading process, thus prolonging their biological activity. The purpose of the pegylation is to optimize the pharmacokinetics, to reduce the clearance, to improve tolerance, compliance and efficiency. The pharmacokinetic profile and the activity of pegylated proteins depend on the structure and the
characteristics of PEG, but also on the number and position of the waiting situs.

40 kDa PEG - IFN a-2a (Pegasys) – IFN is being linked to a ramificated molecule of PEG with molecula weight of 40 kDa, the dose recommended by the current treatment guides being 180 µg/week.

Much like standard IFN, the pegylated interferon also has his side effects; these mainly consist of flu type effects that give in to antipiretic drugs and also of hematological effects (anemia, thrombocytopenia, leucopenia) that imply monthly careful monitorising of complete blood count [1 3].

**Nucleozide analogs in virus B chronic hepatitis**

They represent a group of substances with a chemical formula that resembles the azotic bases which are part of the nucleous acids’ structure (adenin, guanin, citidin, timidin, uracil). The alteration of the genetic information together with the functional blocking or the inhibition of viral replication can be obtained by the repalcement of natural components with different analogs [4 5].

According to their chemical structure the nucleozide analogs can be clasified in the following way:

- **purin analogs** - adenozin (adefovir dipivoxil) and guanozin (ribavirina, ganciclovir, famciclovir, levovirin, viramidină, entecavir)
- **pirimidine analogs**: citidin (lamivudin, emtricitabin) and timidin (b-L-deoxitimidin).

**Lamivudine**

**Mechanism of action**

From a chemical point of view Lamivudine is the negative enatiomer of the 2'-deoxy-3'-thiacitidine. The drug is metabolised at the hepatocytes’ level by the addition of phosphat group thus resulting the 5'-triphosphat derivat. There are 2 main antiviral ways of action of the Lamivudine [6 7]:

- the first mechanism is the stopping of the assembly of the DNA viral chain by attaching itself to the viral DNA molecule due to the structure’s resamblance to the triphoshat deoxicitidina. The consequence is the inhibition of the DNA chain synthesis by stopping the proviral DNA chain.
- the second mechanism is the inhibition of the DNA or RNA relieing DNA-polymerases (ex: viral reverse-transcriptase) both in vivo and in vitro.

Lamivudine doesn’t supress the mitocondrial DNA or the stem spine cells at the doses recommended as treatment for virus B hepatitis. The administration of Lamivudine is followed by the fast decrease of the serum HBV-DNA towards undetactable levels. 20 mg/day doses lead to the incomplete supression of the HBV-DNA and 100mg/day doses produce a complete supression followed by the elimination of HBV-DNA 4 weeks after the initial point of the treatment [7 8].

**Virus effects of the treatment**

Virus B DNA supression is the first virus modification that is observed during the Lamivudine treatment. The viral DNA level detected by PCR becomes undetectable 4 weeks later at all the patients who have recieved 100 mg/day or more. The complete serum supression of the virus DNA takes a certain amount of time according to the administrated dose but more than 100mg/day do not produce an increase of the response (68%). At the end of the treatment, the viral DNA serum level returns to detectable levels in the great majority of cases but the levels are half of the initials ones at the 100mg/dy doses.

Patients with sustained response also had simultanous normalised levels of aminotranferases and the negative AgHBe, the effect still being there 36 weeks after the treatment [4 5].

Part of the patients have different values of the viral DNA at the detectable limit along the treatment; these ones had a refall at the end the therapy but with favourable answer at the re-initiation of the treatment [6].

The return to detectable values of the viral DNA is slower as the period of the treatment grows longer [7]. Measuring the HBe antigen during the Lamivudine treatment shows the disappearance of this antigen at various levels depending on the period of the treatment [8 9].

Stopping the treatment leads to the reappearance of the Hbe antigen in almost half cases which implies a longer period of the treatment. The HBe antigen-anti-Hbe antibodies seroconversion is much more rare and it is met in 16-17% of cases 12 months later. The high level of the serum alanil-amanitotransferase level before the treatment represents the major prediction factor for the seroconversion. HBs getting negative and the HBs antigen-anti HBs antibodies seroconversion are rather rare [9].
Side effects

Generally speaking, Lamivudine is a well tolerated product along the treatment, studies have shown minor side effects such as:

- fatigue, headache, dizziness
- nausea, dry mouth
- abdominal discomfort
- rash, dizziness
- peripheric neuropathy (rare);
- biochemical anomalies : amylase , lipase, creatin-kinase increase [8 9]

The most important side effect of the treatment is the development of resistance as a result of genetic modifications. This modification implies a substitution of the metionine with valine or izoleucine placed in the YMDD region of the HBV DNA polymerase [9]. The incidence of the resistant mutants grows along with the period of the treatment: 17% in one year, 67% in 4 years of antiviral therapy. The mutation is clinically observed by the reappearance of the detectable levels of the viral DNA through PCR techniques and sometimes by the increase of aminotransferases levels. Rare cases of acute attacks due to mutants have also been notified. There are cases in which the mutation is associated with low level of viral DNA and moderate levels of aminotransferases. Also in rare cases the mutation acute attacks lead to hepatic insufficiency and death [5 6].

Entecavir

It is a ciclopentil guanidin analog with important inhibition effect upon the viral replication. In this sense it is superior to Lamivudine (decrease of the viral quantity by 0,07-1,28 log copies/ml more) at doses of 0,1-0,5 mg/day [9 10].

It is efficient in blocking the viral replication , in turning the Hbe antigen negative and in decreasing the transaminase values. It inhibits the HBV replication at 3 levels: it blocks the HBV DNA polymerase, the HBV-DNA ngative chain revers transcription and the positive chain synthesis [10].

The usual dose is 0,5 mg/day at naive patients and 1 mg/day at those who have already developed resistance to lamivudine and it must adjusted to those patients who have an altered kidney function (creatinine function < 50 ml/min) [9].

Vitro studies have shown that entecavir is more efficient than lamivudine specially with mutant viruses that already have resistance. The rate of sustained response is superior to lamivudine [11]. Refalls have been noticed at 3-7% patients and apparently those patients who show resistance to entecavir are sensitive to the action of adefovir-dipivoxil [10 11].

6 months afer the treatment with entecavir HBV-DNA becomes undetactable with usual methods. The drug produses the seroconversion in the HBe system and it’s comparable to lamivudine. Up to now published studies did not show any mutants [11].

Tolerance is good and the side effects mentioned by the published studies are related to an increase of lung and brain tumors [10 11].

Telbivudine

It is a nucleozide analog with an intense activity of inhibition upon the HBV replication but also with high resistance rate to treatment through mutants (mechanism of crossed resistance with lamivudine). That is why the monotherapy with telbivudine has a limited role in HBV chronic infection treatment [9].

Nucleotid analogs

Adefovir dipivoxil

It is a precursor of a adefovir, which is an analog of adenosin-monophosphat. The drug is converted to the active intra-cell metabolit, adefovir diphosphat, which inhibits viral DNA polymerase to concentrations that are inferior to those which inhibits human DNA polymerase.

It is active on wild forms of the virus and also on mutant ones incuding the ones which are resistant to lamivudine, in doses of 10-30 mg/day. The administration of adefovir dipivoxil led to a decrease of the aminotranspherase levels along the treatment both at patients with positive HBe and negative HBe ones and the normal values of serum AT have ben noted at 48-55% patients. The use of adefovir dipivoxil was followed by a hysthological improval at more than 50% of patients [12].

Side effects

The main side effects were:

- headache, dizziness, fatigue
- abdominal pain, nausea, dispeptic syndrom, diarrhea, anorexia
- flew syndrom, pharyngitis, coughing;
- torax pain [9]

Certain modifications of biochemical tests have also been reported: creatinine values increased by 0,2mg% at patients that recieved 30mg/day but in all cases the renal function returned to normal after ending the treatment. An increase of phosphat levels by 0,1mg% has also been notified [13]. So far studies have not shown
any adefovir dipivoxil resistant mutants that might have appeared in the HBV population.

The drug is also efficient on resistant lamivudine, including YMDD mutation viruses. The efficiency resembles the lamivudine's one and the safety profile is similar to placebo except the high rate of fatigue and diarrhea. Creatinine increased only when 30 mg/day were used and it implied the dynamic monitoring of creatinine; the modification was reversible when reducing or blocking the dose and the peak values were minor.[9].

Criteria for being included in the antiviral treatment and choice of therapy at adult patients with viral B chronic hepatitis

1. Viral B chronic hepatitis with positive AGHBe and anti-HBe negative antibodies

Clinic and biological evaluation tests when initiating the treatment at patients with Virus B chronic hepatitis

When initiating the treatment the following investigations will be needed:
- complete blood count;
- protrombine activity;
- protrombine time;
- AST and ALT;
- AgHBe, anti HBe antibodies;
- serologic testing for other types of chronic hepatitis (VCH, VDH);
- HIV serologic test at those with high risk;
- hepatic biopsy punctum indicated according to treatment guides at certain patients;
- HBV-DNA measuring method with detection limit a 10U/ml (50 copies/ml);
- superior abdomen ultrasound;
- alphafetoproteine (AFP)

General criteria for being included in the treatment:

- biochemical: ALT twice increased or the superior limit of normal value for more than 6 months
- virusological: AgHBs positive ≥ 6 months; AgHBe positive and negative anti-Hbe antibodies; HBV-DNA more than 100 000 copies/ml; negative anti HDV IgG;
- Patients with a viremy of more than 100.000 copies/ml but with ALT within normal limits or less than 2X LSN will be included in the treatment only if biopsy punctum detects lesions ANI > 4 (Knodell score) or if the Fibrotest result shows a value over 7.1 Kpa. If the test result is confusing a hepatic biopsy is recommended.

Therapy protocols in use:

Alpha 2a Peginterferon

Additional criteria for being included in the treatment: age under 65, HBV-DNA under 10^9 copies/ml
Dose: 180 micrograms/week.
The strategy and the monitorising of the treatment
- ALT and complete blood count monthly check up
- AgHbe at 24 weeks and at the end of the treatment
- HBV-DNA 24 and 48 weeks after the beginning of the treatment
The treatment stops in 24 weeks if HBV-DNA didn't decrease by more than 2 log_10 or is being maintainced at over 10.000copies/ml
The period of the treatment: 48 weeks if 24 weeks after the starting point the HBV-DNA goes under 2 log 10 and has a value of less than 10.000 copies/ml
The treatment evaluation: it is performed at the end of the 48 weeks of using peginterferon and 24 weeks after the end of treatment. The responsive patients are considered to be those whose HBV-DNA is less than 10.000 copies/ml and for whom the AgHBe seroconversion has been obtained (anti HBe antibodies have appeared). The seroconversion can also be obtained 12 months after the treatment.

Nucleozid/nucleotidic analogs: Lamivudinum, Entecavirum, Adefovirum dipivoxilum

Additional criteria for being included in the treatment: HBV-DNA viremy more than 10^4 copy/ml
The choice of the medicine: Lamivudinum is used with patients who are sensitive to Lamivudin. Entecavirum and Adefovirum dipivoxilum are used with patients who are primarily resistant to Lamivudinum or to those who have become resistant after the initial treatment with Lamivudinum.
Lamivudinum dose: 100 mg/day; the initial response is evaluated after 6 months of therapy by measuring the ALT. If ALT has not reached normal values, HBV-DNA should be evaluated. If
this value has not dropped by more than 2 log_{10} we should consider primar resistance to Lamivudin and the treatment should be stopped. Later on, ALT, AgHBe and anti-HBe antibodies should be checked every 6 months. According to the biochemical and virusological response, the treatment will be stopped or continued up to 5 years. The evaluation of viremy is imposed by the increase of transaminases along the treatment and the increase of viramy along the treatment is considered to show resistance and will lead to the change of therapy. The resistance and the lack of response lead to the reevaluation of the patient and to a new therapy decision. In case of response, the treatment continues for 6 months after the AGHBe seroconversion (this one is checked 3 and 6 months later)

**Entecavirum dose:** 0.5 mg/day at naive patients and 1 mg/day at the ones who have already developed resistance to Lamivudinum. The evaluation of the response is made after 6 moths of therapy by measuring the HBV-DNA. If this one has not decreased by more than 2 log_{10} it is considered to be primar resistance and the treatment stops. Later on, ALT,AgHBe, anti HBe antibodies and HBV-DNA will be evaluated every 6 months. According to the biochemical and virusological response, the treatment will be stopped or continued up to 5 years. The evaluation of viremy is imposed by the increase of transaminases along the treatment and the increase of viramy along the treatment is considered to show resistance and will lead to the change of therapy. The resistance and the lack of response lead to the reevaluation of the patient and to a new therapy decision. In case of response, the treatment continues for 6 months after the AGHBe seroconversion (this one is checked 3 and 6 months later)

**Adefovirum dipivoxilum dose:** 10 mg/day. The evaluation of the response is made after 6 moths of therapy by measuring the HBV-DNA. If this one has not decreased by more than 2 log_{10} it is considered to be primar resistance and the treatment stops. Later on, ALT,AgHBe, anti HBe antibodies and HBV-DNA will be evaluated every 6 months. According to the biochemical and virusological response, the treatment will be stopped or continued up to 5 years. The evaluation of viremy is imposed by the increase of transaminases along the treatment and the increase of viramy along the treatment is considered to show resistance and will lead to the change of therapy. The resistance and the lack of response lead to the reevaluation of the patient and to a new therapy decision. In case of response, the treatment continues for 6 months after the AGHBe seroconversion (this one is checked 3 and 6 months later)

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**2. Virus B chronic hepatitis with negative AGHBe and anti-HBe positive antibodies**

Clinic and biological evaluation tests when initiating the treatment at patients with Virus B chronic hepatitis

When initaiting the treatment the following investigations will be needed:

- a. complete blood count;
- b. protrombine activity;
- c. protrombine time;
- d. AST and ALT;
- e. AgHBe, anti HBe antibodies;
- f. serologic testing for other types of chronic hepatits (VCH, VDH);
- g. HIV serologic test at those with high risk;
- h. hepatic biopsy punction indicated according to treatment guides at certain patients;
- i. HBV-DNA measuring method with detection limit a 10U/ml (50 copies/ml);
- j. superior abdomen ultrasound;
- k. alphafetoproteine (AFP)

Genera criteria for being included in the treatment:

- biochemical: ALT superior limit of normal value twice increased for more than 6 months
- virusological: AgHBs positive ≥ 6 months; AgHBe negative and positive anti-Hbe antibodies; HBV-DNA more than 100 000 copies/ml; negative anti HDV IgG ;
- Patients with a viremy of more than 100.000 copies/ml but with ALT within normal limits or less than 2X LSN will be included in the treatment only if biopsy punction detects lesions ANI > 4 (Knodell score) or if the Fibrotest result shows a value over 7,1 Kpa. If the test result is confusing a hepatic biopsy is recommended.

**Therapy strategies in use:**

**Alpha 2a Peginterferonum**

Additional criteria for being included in the treatment: age under 65, HBV-DNA under 10^9 copies/ml

Dose: 180 micrograms/week.
Therapy period: 48 weeks

The strategy and the monitorising of the treatment
6 months later: normal ALT, HBV-DNA decreases by 2 \log_{10} but still stands below 10^3 copies/ml - treatment continues for 24 more weeks

- normal or increased ALT but unmodified or decreased by 2 \log_{10} HBV-DNA - resistant genotype (D) and the treatment stops.

**Nucleozid/nucleotid analogs:**

- **Lamivudinum**, **Entecavirum**, **Adefovirum dipivoxilum**

  Additional criteria for being included in the treatment: HB-DNA over 10^4 copies/ml.

  No superior age limit and inferior limit according to each drug.

  The choice of the medicine: Lamivudinum is used with patients who are sensitive to Lamivudin. Entecavirum and Adefovirum dipivoxilum are used with patients who are primarily resistant to Lamivudinum or to those who have become resistant after the initial treatment with Lamivudinum.

  **Lamivudinum dose:** 100 mg/day; the initial response is evaluated after 6 months of therapy by measuring the ALT. If ALT has not reached normal values, HBV-DNA should be evaluated. If this value has not dropped by more than 2 \log_{10} we should consider primar resistance to Lamivudin and the treatment should be stopped. Later on, ALT, AgHBe and anti-HBe antibodies should be checked every 6 months. According to the biochemical and virusological response, the treatment will be stopped or continued up to 5 years. The evaluation of viremy is imposed by the increase of transaminases along the treatment and the increase of viramy along the treatment is considered to show resistance and will lead to the change of therapy. The resistance and the lack of response lead to the reevaluation of the patient and to a new therapy decision. In case of response, the treatment continues for 6 months after the AGHBe seroconversion (this one is checked 3 and 6 months later).

  **Entecavirum dose:** 0.5 mg/day at naive patients and 1 mg/day at the ones who have already developed resistance to Lamivudinum. The evaluation of the response is made after 6 months of therapy by measuring the HBV-DNA. If this one has not decreased by more than 2 \log_{10} it is considered to be primar resistance and the treatment stops. Later on, ALT,AGHBe, anti HBe antibodies and HBV-DNA will be evaluated every 6 months. According to the biochemical and virusological response, the treatment will be stopped or continued up to 5 years. The evaluation of viremy is imposed by the increase of transaminases along the treatment and the increase of viramy along the treatment is considered to show resistance and will lead to the change of therapy. The resistance and the lack of response lead to the reevaluation of the patient and to a new therapy decision. In case of response, the treatment continues for 6 months after the AGHBe seroconversion (this one is checked 3 and 6 months later).

  **Adefovirum dipivoxilum dose:** 10 mg/day. The evaluation of the response is made after 6 months of therapy by measuring the HBV-DNA. If this one has not decreased by more than 2 \log_{10} the treatment stops. Later on, ALT,AGHBe, anti HB antibodies and HBV-DNA will be evaluated every 6 months. According to the biochemical and virusological response, the treatment will be stopped or continued up to 5 years. The evaluation of viremy is imposed by the increase of transaminases along the treatment and the increase of viramy along the treatment is considered to show resistance and will lead to the change of therapy. The resistance and the lack of response lead to the reevaluation of the patient and to a new therapy decision. In case of response, the treatment continues for 6 months after the AGHBe seroconversion (this one is checked 3 and 6 months later).

  If HBV-DNA is undetectable or drops below 1000 copies/ml the therapy continues for 24 more weeks.

  Treatment for virus B chronic hepatitis is currently an exciting topic due to all the progress made in this regard. Controversy is mainly related to the choice of the first line of treatment - Interferon versus nucleozid/nucleotid analogs but also to the optime therapy strategy in case of viral resistance [15 16].

**Conclusions**

There is data that favours the choice of interferon as first line treatment at patients with virus B chronic hepatits both with positive and negative AgHBe. This way the AgHBE seroconversion and the AgHBe is superior to the results obtained by using nucleozid analogs. Pegylat interferon has the advantage of not leading to resistance.

On the other hand, nucleozid analogs also have their advantages which make them eligible in certain situations during the initial treatment of patients with virus B chronic hepatitis. The effect does not depend on genotype and the level of transaminases and the AgHBe seroconversion, although much more reduced than with Peg-
interferon, is being obtained in time. The side effects are less frequent and the oral administration is more comfortable in comparison with the injectable therapy.

According to current data, 1 year after the treatment, out of the nucleozid/nucleotid analogs the best response for positive AgHBe patients is obtained with Entecavir and Tenofovir.

As for the nucleozid resistance after 5 years of therapy, it reaches 70% for Lamivudine and only 1% for Entecavir and 30% for Adefovir.

References

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