Antiviral combination for treatment of HCV and HBV

Technical Field

Pharmaceutical combination for treatment of virus hepatitis HCV & HBV Composed from Two types of monoclonal antibodies as a human source And one of antifibrinolytic drug to use them in new form as antiviral drug.

Background art:

There are a number of multi trails for viral hepatitis treatment like Combined treatment with peg interferon (IFN) and ribavirin is the current standard of care for HCV & HBV infection - based combination therapy for Immune activation as Antiviral effect,
Oligonucleotides which act by destruction or incapacitation of HCV RNA in the cytoplasm to prevent or to reduce attachment of RNA to the ER and impair translation of large ORE that produce the HCV polyprotein ,Protease inhibitors VX-950 and SCH 503034 have recently entered phase II clinical trails, Interleukins -2 and interleukins -12 ,Immune modulators (Thymosin-levamisole), Herbal Therapy Phyllantus amaurus, Nucleoside analogs (Lamuvidine-Famcyclovir) , Therapeutic vaccines, we filled an application about using of antistreptolysine antibodies as clonal antibodies with tranexamic acid as synthetic amino acids in patent office of Egypt in3/9/2003 no. 865 Under the name of (New treatment and vaccine for hepatitis virus c by using antibodies Against virus consciousness) But there is no doubt that all of the above not give actual treatment With ought the great list of side effects; With Interferon therapy The majority of patients develop significant adverse Symptoms Like (flu-like symptoms), fatigue, Headache, Alopecia, nausea, Abdominal pain, Thrombocytopenia , Auto antibodies, In actual practice the clinical application Of Oligonucleotides have unfortunately been problematic. These agents Face a Number of hurdles, including the opportunity for drug Inactivation by host Nucleases prior to Target delivery, inhibition of the HCV NS3/NS4A protease has proven difficult Since its protease binding groove is shallow and the Affinity For bound substrate is weak, Also Cytokines, Interleukins and Interferon are members Of hormones proteins, so it produced the same side effects Of interferon, The vaccines not succeeded till now because the virus Has the great ability to change itself via Large open reading frame , The Previous combination for me needs another type of antibodies to the give the immune cells highly expression mechanism, for that it's not Give good results
For the acute and chronic patients.

Disclosure of the invention

We looked for all of thee above and we start in understanding the consciousness OF the viruses and how can we design a new antiviral therapy with ought all of the above list. We build my hypothesis depends on that any biologic processes it require a Balance between the proteases that initiate the proteolysis pathways essential to Life and the inhibitors that limit excessive protease activity. Coagulation and fibrinolysis the two processes form and dissolve fibrin, respectively. These Two processes are exquisitely regulated and protect the body from excessive Blood loss or excessive fibrin deposition.
We hypothesize that hepatitis patients have defect in the inhibitors Mechanism,

which play important role in suppress the consciousness of the Immune cells, so the over usage of a fibrininogen for multi repeating functions with ought the actions of the plasmin Will stimulate the cell to produce
Antiplasmin Abs, anti polymerase Abs. My hypothesis depend on hypothesize That hepatitis patients must have three types of antibodies Antiplasmin Abs & Anti polymerase antibodies and anti fibrin abs for that we foreword my trails in Detections of the three antibodies in HCV patients.
We obtain a good proof for the presence of the three Abs in serum of Infected Patients with compare to control normal persons
If we look for the liver of the infected person and what's means by fibrosis and Cirrhosis , we found Fibrin

Fibrin is made from its zymogene fibrinogen, a soluble plasma glycoprotein

That is synthesized by the liver. Processes in the coagulation cascade activate

The zymogene prothrombin to the serine protease thrombin, which is responsible

For converting fibrinogen into fibrin. Fibrin is then cross linked by factor XIII

To form a clot.

Fibrinogen is a 340 kDa glycoprotein synthesized in the liver hepatocytes and megakaryocytic, which normally has a concentration between 1.5 - 4.0 g/L

(Normally measured using the Clauss method) in blood plasma. In its natural

Form, fibrinogen is useful in forming bridges between platelets, by binding to

Their GpIIb/IIIa surface membrane proteins; though fibrinogen's major use is

As a precursor to fibrin in the normal liver, sinusoids are formed.

By a fenestrated endothelium, which is separated from hepatocyte by sub? Endothelial space within the sub endothelial space lie hepatic satellite cells, Which are quiescent in Normal liver, In the injured liver, satellite cells activate Into proliferating My fibroblasts that produce scar,
While at the same time releasing TIMP-1 which Bind and inactivate the Metalloproteinase's needed for scar degradation
Thus scar accumulate through increased production and decreased degradation For example the low plasma alpha-antiplasmin aggregates in the endoplasmic Reticulum of hepatocytes as inclusions at their site of synthesis in the Endoplasmic reticulum, accumulation of the antiplasmin reflect the severity of Liver cirrhosis, that means the viruses acts to stimulate the
Auto antibodies against the units of serine protease, like stimulation of Anti-Antiplasmin antibodies and anti fibrin Abs Fibrin is our aim in this idea It enter in the formation of all cement Material of the cells It represent the Gravity and the mass of the cell, All the Micro fibrils, actin filaments , microtubules acts as cell form and architecture units.
For all of that we start my trails firstly to proof the presence of the two Antibodies In viral infected hepatitis patients
Then if we proofed that we will go to use another mechanism acts against Them in Trail to eradicate the virus.

1 We searched for Anti-plasmin Abs in samples for patients infected By HCV RNA
2 We searched for Anti-polymerase Abs in samples for patients infected by HCV RNA
3 we searched for Anti-fibrin Abs in samples for patients infected by HCV RNA

To test the hypothesis that there are anti-plasmin Abs & Anti-polymerase

Abs And Anti fibrin Abs in hepatitis HCV infected patients firstly

We examine patients,

PHASE 1 34 Hepatitis patients and 10 normal Controls. All samples Were analyzed.

Results

We found ratio between the Quantities measure for HCV RNA (Moderate to highly viremia) And the value of the antibodies and the level of liver enzymes *High value for HCV RNA by PCR correlates with Low Anti- polymerase Abs & low Anti plasmin Abs and high anti fibrin Abs With slightly elevation of liver enzymes about one to two folds than normal Values In value of low to V.low viremia HCV RNA by PCR we found high titer For anti- polymerase Abs & High titer for and low anti fibrin Abs Anti-Anti plasmin Abs with marked elevation of liver enzymes to three to five Folds than normal in controls patients we found in three cases slightly Increasing in titer of Anti plasmin with no viremia and no Anti polymerase Abs the results showed that hepatitis patients have more IgG anti-plasmin Abs than normal controls, these findings define a novel autoantibody in hepatitis.

Second phase

This work was carried out on 100 HCV infection patients including (78 males and 22 females). And 33 HBV infection patients, all of them males Their ages ranged from 30-60 years who have been already diagnosed previously As HCV & HBV- infected patients (Risky and non Risky) And confirmed by PCR. HCV-PCR and HBV PCR Was done; quality and quantity before and after each therapeutic trail which Up to (77%) of all studied HCV- (85 %) of all HBV infected patients reached Become to have undetectable level using HCV-PCR & HBV PCR whom quantaitivly while the remaining cases still positive with variable results-using the same method –but at lower level than in the beginning of the study.

From this work it can be believed that the current therapeutic trail may have an effect on HCV & HBV infection.

After 6 months from the last trail; out of (77) studied cases, only (60) were followed while remaining (17) cases did not come.

And out of (18) studied cases from HBV infected patients and the remaining (15) Cases did not come. On using HCV-PCR all of (60) cases showed undetectable Level, except (3 cases) were still showing (+ve) results but at lower level.

On using HBV-PCR all of (18) cases showed undetectable level

Conclusion; this is a new approach for therapeutic trails for

HCV & HBV infected patients showed good results without any complications.

(It is an excellent therapeutic trail for human being)
Therapeutic trail. As well as after 6 months from last trail.

Reagents
Materials provided;
Chemical part (1st. part take the validity as drug used in medicine therapy) non specific poly clonal Abs Of human source (2 nd. components) .The preparation was protein in nature, so, desensitization test Must be done for each patient before administration of this preparation.
Composition and standardization;
A mixture formed of 2 components : (100 mg/ ml) under complete aseptic Conditions the following were done 1 Vol. Of first component were mixed With 1 Vol. Of second component the resulting preparation was tested for; HIV,HIV2,HCV and HBV The resulting mixing solution was sterilized by filtration and carefully put in sterilized ampoule sealed after this.

Storage at; +2 to +8 c°

Preparation of the Ampoule containing The previously mentioned (reagents) 2 components; The prepared mixture is ready to be Used without additional

Procedure ;
Note;
the ampoule containing mixture should be brought to room temperature (+ 15 c° to + 25 c°) The mixture must be mixed well immediately Prior to use.
Injection was done;
gradually –according to patient weight as following
-1st. Dose 15 cc ampoule of the mixture; one hour after light meal (2 times daily with 2 hours interval) 7 days rest for patient to start the 2 nd. ampoule but with increasing the dose up to 1.5 cc (2 times daily with 2 hours interval) -3 rd. dose; 7 days rest to start the 3 rd. Ampoule same manner mentioned in 2 nd. dose
-4th. Dose; 7 days rest to start the 4th. one but with increasing the dose to2 cc (2 times daily with 2 hours interval)
Evaluation
Rest 20 days after which ;HCV-PCR HBV –PCR was done as well as the other Mentioned previously investigations before these trails Were done
Dosage;
It is given I/M.
-1st. (dose) trail 15 cc daily dose, 2 cc I/M
-2nd.(dose) trail 15 cc daily dose, 3 cc , I/M
-3 rd. (dose) trail 15 cc daily dose, 3 cc I/M
4th. (dose) trail 15 cc daily dose, 4 cc I/M

Pharmacokinetics of the trail

The chemical part is easily absorbed via GIT with peak plasma concentration Occurring after 2 hours with very low protein binding. It is excreted in the urine Mainly as unchanged drug. For Abs. Renal absorption were occur as any protein Particular molecules .It is excreted in the bile, urine and saliva.

Adverse reactions
The mixture appears to be well tolerated ,Toxic side effect; No
Experimental animals;
We classified 15 white mice in 3 groups every group has 5 mice Every Component of the mixture is injected separately in -1st. and -2nd. group

while the -3 rd group injected by the mixture of the two components for about 15 days for all group

No side effect or toxic effect was detected and the mouse is still alive.

In vitro Experiment for;

1-this therapeutic trail was infected packet of blood bank blood) (infected HCV Blood of moderate viremia patients and infected HBV Of donner By PCR the virus can not be detected in about 10 samples (Undetectable level). While other samples show decreasing the viremia to v.mild number

2-We examined 15 patients infected by (HCV ,11 patients)and (HBV ,4 patients) for the action of the component of the mixture separately, The first five patients(3 ,HCV patients)and(2 , HBV patients) injected by the drug (First component) for 10 days by I/M injections for 2 c.c two times , two hours interval , the second 5 patients (5, HCV patients) injected by the monoclonal Abs. I/M for 1.c.c two times ,two hours interval ,and the third group(3 ,HCV patients) and(2 , HBV patients) By the mixture trail for 10 days firstly before we reach to final equation for the mixture.

After 6 months;

-All studied cases(77) were reexamined after (6 months) "as follow up "

Sixty patients came for this follow while the remain (17 patients) did not come.

-Using (HCV-PCR &HBV-PCR) ; 57 persons are still with the level (Undetectable) While the remaining there patients showed lower viremia which may be due to Relapse on one hand or due to re infection (After asking patient about if he exposed to infected blood or products.

- We have very good prognosis for patients clinical data, improvement in Biochemical data like liver functions (enzymes, synthetics functions), complete changes in hematological data especially no. Of blood platelets and leukocyte count

And increasing in the titer value of HCV Abs titer as immunological data.

In the last group after 15 days

2 ,cases(1 ,HBV and 1,HCV patients) show undetectable level while the other show marked drop in the viral load ,and when we reexamined them again after 3 months; No change in the results were recorded, from this point We go to use the combination.

We using the (HCV-HBV by PCR) we found complete eliminations in 3, patients (2,HCV and 1, HBV) cases and marked drop in the viremia in the other two cases, while after one month with reexamined them by using the same technique ,we found slightly increasing in the viral no in all of HCV infected patients.

In the second group with using the Abs. Alone ,we found by using(HCV-HBV by PCR) Marked decreasing in the viral no. And when we reexamined them after one month the same no. Of the virus still found.

NB.(We examined the hepatitis B virus pre and after for every patients for HBV-DNA by PCR only ,we not include the other markers like; (HB S Ag,HB S Ab,HB c Ag,HB c Abs HB e Ag and HB e Abs)

Response of 100 studied cases for therapeutic trails

HCV infected / Case No of Therapy. Trail	Responded cases to therapeutic trails			
	NO	%	% to total studied cases	
1st Trial	$^{30}/_{100}$	30%	30%	70
2nd Trial	$^{37}/_{100}$	52,86%	37%	33
3rd Trial	$^{9}/_{100}$	27.3%	9%	24
4th Trial	$^{1}/_{100}$	4.17%	1%	23
Total	$^{77}/_{100}$		77%	$^{23}/_{100}$

Follow up after (6) months for all studied (77) cases with (Undetectable level)

Studied cases	NO. of cases
Cases still with undetectable level	57
Cases with lower level;	3
- Relapse	1
- Re infection	2(Exposed to infected blood material)
Cases don't complete the follow up	13

Best mode for carrying out the invention

1- Using of polyclonal non specific antibodies and synthetic Amino acids drug in mixture form, to induce the antiviral effects for All Hepatitis virus and HIV.

2- The new mixture give exact explanation about the Mechanism of the virus and how can we overcomes it.

3- Trying to understanding the relation between HCV and Memory system

Claims

1- New Antiviral combination of polyclonal Abs and synthetic amino acid For Treatment of (HCV, HBV).
According to the first claim we need to claim using specific new combination For virus hepatitis HCV & HBV treatment formed from
a- Monoclonal antibodies for Streptolysine Ag (Antistreptolysine)
B-Monoclonal antibodies for streptokinase Ag (Ant streptokinase)
C-Tranexamic acid a (synthetic amino acid) as anti fibrinolytic.

2- Antibodies that formed as a result of streptococcus infection for Treatment of viral Hepatitis HCV & HBV.
According to the second claim we need to claim using antibodies of streptolysine And Antibodies for streptokinase as antiviral depending on the ability of this Two types of clone antibodies in suppress the plasmolysis activity of the HCV and to stabilize the fibrin in blood circulation.

3- Antifibrinolytic drug for virus hepatitis (HCV & HBV).
According to the third claim we need to claim using tranexamic acid (Synthetic amino acid) Depends on their power in preventing the fibrinolysis Of fibrin to Fibrin Degradation products.

4- Anti streptococcus Abs and their relation to HCV & HBV
According to the Forth claim we need to claim the relation between actions Of Streptococcus M.O, on fibrin, collagen and cells membranes And the affects of the HCV & HBV on the immune system and the liver cells.

5- Three types of auto-immune Abs found in serum of HCV patients.
According to the Fifth claim we need to claim the relation between HCV and Presence of three types of auto-immune Abs, a- anti plasmin Abs, b-Antifibrin Abs And anti polymerase Abs, we found that HCV infection induce our immune System to attacks plasmin factor, fbrin, and polymerase.

6- Coagulation and fibrinolysis.
According to the sixth claim we need to claim the relation between HCV & HBV And the two processes in blood.