There are no translations available.

5.c. Molecular homology between Hepatitis C Virus (HCV) core, Hepatitis B Virus (HBV) pre-S1 and Phallotoxin of Amanita phalloides: Interest of Silymarin of Silybum Marianum.

EXTENDED VERSION /VERSION LONGUE

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INTRODUCTION.

In France, AIDS haemophiliacs and drug addicts and 600,000 patients were infected by HCV, with the risk of cirrhosis and cancer. This constitutes a major health problem. Interferon-alpha and ribavirin could resolve this problem only partially in about 6/10 cases.

No HCV vaccine is available to date.

In Egypt, an epidemics of hepatitis C spread after the mass campaign of emetin intravenous injections with contaminated syringues for bilharsiosis (Schistosomia Mansoni and Haematobium) (Franck C., 2000).

OBJECTIVE.

Our objective is to analyze the HCV mechanism of action on hepatocytes. Such an analysis of the port of entry permits the definition of an epitope, for designing a vaccine and developping drugs directed in the blockade of this entry.

We try also to understand the activity of interferon alpha in hepatitis C. We hypothetized that a molecular mimicry exists between the virus and interferon. [However the results seem too preliminary and are not completely convincing (sequence AVAYYRGLDV of Hepatitis C and AVKRYFQRITL of interferon alpha 2 and LKRYYGRIL of interferon beta)]. It may be that another interferon type was more mimetic to hepatitis C.

METHOD.

We used amino acid (AA) sequences comparison. The most important point is to select a correct probe to screen the different proteins. One of the most interesting one is the phallotoxin of the mushroom Amanita Phalloides, which is a very short heptapeptide able to kill a human of 70 kg.

The second probe is Fas ligand (GenPept GI 7512421), because fulminant hepatitis can occur after Fas-triggered apoptosis. Fulminant hepatitis can be blocked by Bcl-2, an anti-apoptotic oncogene (Lacronique V., 1996). A role for Fas/Fas ligand in hepatitis C and B infections was discovered, based on the up-regulation of Fas expression by hepatocytes (Hiramatsu N., 1994), and Fas ligand by the liver-infiltrating mononuclear cells (Mita E., 1994 ; Hayashi N., 1997).

Interestingly, there is a clear alignment between these 2 probes, phallotoxin and Fas ligand, because both possessed the tripeptide WAP (Try Ala Pro, or Tryptophane Alanine Proline). The asterisk * means the presence of an hydroxylated OH grafted on the residue. The motif WSP is thus homologous to the motif WAP*, because the Proline is hydroxylated: So the Alanine of WAP looks like the Serine of WSP, the consensus hepatocyte adhesion.

Phallotoxin	**T**	**A***	**D***	**A***	**L**	**W**	**A**	**P***	**-**	**C**	**T**	**V**
Fas ligand (S18-28)		**S**	**S**	**P**	**W**	**A**	**P**	**P**	**G**	**T**	**V**

RESULTS.

There is a significant homology between HCV core (Muller H.M., 1993), HBV pre-S1 and hepatotoxic or hepatotropic proteins & viruses: Yellow fever virus (a flavirus like HCV) ns2a (Rice C.M., 1985), Frog virus 3 (NCBI : NC_003407), Amanita phalloides phallotoxin, Plasmodium falciparum TRAP & CS (circumsporozoite) (Good M.F., 1987), thrombospondin (a hepatocyte ligand) (Roberts D.D., 1985), complement properdin. We centered especially on phallotoxin, a very short (7 AA) cyclopeptide able to induce, in Amanita phalloides intoxication, a fatal fulminant hepatitis. The dipeptide Ser-Pro (SP) is homologous to Ala-OH-Pro (AP*).

The hepatocyte adhesion motif is "W S P" (Nolan K.F., 1993):

HCV core (1T0-10P in reverse sense & P125-129)						**..**		**T**	**L**	**T**	**-**	**C**
												G
Hepatitis B virus pre-S1 (107-112S & 119-120Q)						**..**		**T**	**L**			
Yellow Fever virus ns2a (1224-9 & S1262-1259)						**..**		**.**	**V**	**S**	**L**	**C**

Plasmodium falciparum TRAP & SCS			**P**		**C**		**S**	**V**	**T**	**-**	**C**	**G**
Thrombospondin	**W**	**S**	**S**	**C**			**S**	**V**	**T**	**-**	**C**	**G**
Complement Properdin	**W**	**S**	**P**	**C**	**S/**		**T**	**V**	**T**	**-**	**C**	
Frog virus 3 DNA-dependent RNA polymerase												
Phallotoxin (covalent bond between W and C							**T**	**V**				

(L** = diOH-L ; asterisk is an hydroxyle OH).

CONCLUSION.

HCV core & HBV pre-S1 mimick a powerful hepatotoxic peptide of 7 AA, phallotoxin from Amanita phalloides, as well as many other hepatotoxins.

CLINICAL TRIAL WITH SILYMARIN.

Such a molecular homology opens a new avenue for clinical trials in HCV and HBV infections with silymarin (Legalon) (see review in: Legalon, from Laboratory Madaus), an anti-phallotoxin and anti-frog virus 3 drug, almost devoid of any major toxicity and currently authorized (see the french Vidal dictionary) since 1974 for benign functional hepatic troubles. Legalon is protective against Amanita Phalloides intoxication in mice and dogs ; furthermore, in humans, this drug has been used for the same intoxication in Germany in reanimation at very high (20mg/kg/day) intravenous doses (Scheen A., 1987). Many authors obtained an increase in survival and a decrease in severity if Legalon is given precociously in the first 48 hours (Floersheim G.L., 1982 ; Hruby K.,1983-85 ; Csomos G.,1986 ; Flammer R., 1988 ; Daoudal P., 1989).

One of us (G.M.) tried Legalon in 2 patients. The first is 49 years old, with a chronic hepatitis C since 1998 (4 years), his transaminases rose from 39 to 158 (SGOT) and from 89 to 302 (SGPT), he then received 6c./day since March 2002 and, in 2 months only, his hepatic enzymes fell down from 158 to 97 (SGOT) and from 302 to 162 (SGPT). The second patient is a drug-induced hepatitis, whose hepatic enzymes normalize after 3 years of treatment, whereas they were never normal before Legalon. These 2 cases were promizing, with no side effects and a patient well-being. A larger randomized, double-blind clinical trial in 110 patients with hepatitis C with HIV-1 seronegativity, is on going since 1998. The double blind will be broken in 2 months (August 2002) and the results will be published in 6 months. The drug used is Silibinin, a more biodisponible form of Legalon (Pr Poynard T., Hospital Pitié, Paris). A chemically similar drug, Arcapilline, from Artemisia Capillaris, is empirically used since millenaries in China as traditional herbs against hepatitis and icterus (Tang W., 1992).

VACCINE DESIGN

The HCV core epitope with its WSP hepatotropic motif can contribute to ameliorate the HCV vaccine (Major M.E., 1995). An interesting design is to present the epitope by a chaperone, like the heat shock Hsp protein family, to the dendritic cells, in order to amplify the immune response, both cellular and humoral (Srivastava P., 2002).

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