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Bioavailability of Interferon Alfa 2b in Chronic Hepatitis C Patients by Using Limited Sampling Strategy

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ABSTRACT

About 5 million people are infected with hepatitis B virus and about 10 million people harbor the hepatitis C virus in Pakistan. Management of chronic hepatitis in Pakistan carries substantial social impact like fear of tolerability of the drug due to its side effects and the most commonly its affordability. Interferon in combination with Ribavirin is used for the treatment of chronic hepatitis C patients. There are about 60 preparations are available in Pakistan imported from different countries with claim of bioavailability more than 90%. Bioavailability of interferon has not been studied yet in Pakistan. This study was designed to find out the first dose bioavailability of the three formulations of interferon alpha 2b which are commonly prescribed by using limited sampling strategy to help the physician to select the drug with maximum bioavailability. **Aim and objectives:** The study was conducted to see the bioavailability of interferon in three formulations of interferon alpha 2b in patients with chronic hepatitis C patients. **Methods**: This was a Quasi – experimental study including sixty patients of either gender. These patients were divided into three groups at random after giving them first dose of three million units of interferon alpha 2b subcutaneously.

Group 1: Uniferon (Getz Pharma Brand)
Group 2: Ceron-alfa (Biocare Pharma)
Group 3: Anferon (CCL Pharmaceuticals)

Blood samples were collected at 00, 08, 20 hours according to limiting sampling strategy. **Results:** The bioavailability was found to be 70%, 60% and 55% in group 1, 2, and 3 respectively. The difference was statistically significant ($p < 0.5$) based on ANOVA and t-test. Almost all patients reported mild interferon side effects (flu-like symptoms, headache). **Conclusions:** Different formulations have variable bioavailability and there is a strong reason to choose the best drug with maximum bioavailability to give the patient maximum benefit.

INTRODUCTION

About 5 million people are infected with hepatitis B virus and about 10 million people harbor the hepatitis C virus in Pakistan[1].

In the last few decades there has been a massive increase in the incidence of Hepatitis B and C infection in Pakistan. However because of the availability of the vaccine for hepatitis B its incidence is almost 50% less than hepatitis C in our population[2].

Management of chronic hepatitis in Pakistan carries substantial social impact like fear of tolerability of the drug due to its side effects and the most commonly its affordability [3].

Pharmaceutically interferon in combination with ribavirin is used for the treatment of chronic hepatitis C patients[4].

There are almost sixty preparations of alpha interferon are available in Pakistan, imported from

different countries like China, Korea, Switzerland, Cuba, & Argentina etc. since interferon is an expensive preparation it is essential to choose the best product on the basis of bioavailability[5].

Bioavailability is defined as the fraction of unchanged drug reaching the systemic circulation following administration by any route [6].

Systemic bioavailability is best described by the measurement of the relative amount of the parameters like Area under the Curve (AUC), the maximum concentration achieved (C^{max}), and the time (T^{max}), at which this occurs [7].

This study was designed to see the bioavailability of locally available three formulations of interferon alpha 2b in chronic hepatitis C patients in Pakistan [8].

AIMS AND OBJECTIVES

To see the bioavailability of three formulations of interferon alpha 2b in chronic hepatitis C patients.

MATERIALS AND METHODS:

Study design

It was a Quasi- experimental study**.**

Setting

Hepatitis clinic Shaikh Zayed Hospital, Lahore. All patients had normal complete blood count and liver function tests.

Duration of study

Study was completed in six months from March 2008 – September 2008.

Sample size

The sample size was estimated by using 0.40 effect size with 5% level of significance and expected bioavailability for Uniferon, Anferon and Ceron alpha above 90% respectively.

The sample size of 60 patients with 20 in each group yielded with 80% power of test.

The treatments were allocated to patients at random by lottery method.

Sampling technique

Purposive non probability sampling.

Sample selection

Diagnosed cases of hepatitis C Patients were enrolled for interferon treatment, meeting the inclusion criteria.

Patient inclusion criteria

- Confirmed case of chronic hepatitis C by Elisa and genotyping.
- Age 20-49 years.
- Gender, either.

Patient exclusion criteria

- With co morbidity of Diabetes Mellitus, Hypertension, Hepatic, Renal, Cardiac failure, and Cancer.
- On special drugs like steroids.
- Addict of alcohol or other drug abuse.

METHODOLOGY

The study was approved by the Institution Review Board, Sheikh Zayed Medical Complex Lahore. Informed written consent was taken from all the patients.

Patients then allocated to different subgroups for using their data in research.

All the selected patients who had chronic hepatitis C virus positive by qualitative PCR test, were identified and divided into three subgroups, Uniferon, Anferon, and Ceron, as group 1, 2, and 3 respectively. Each group had 20 patients, 10 males and 10 females.

Demographic information, Name, Age, Sex, Height, and Weight were recorded.

History of illness was explored regarding types of symptoms, duration and severity.

The patients were given first injection of three million units of alpha interferon 2b (three different products as Uniferon, Anferon, and Ceron) administered subcutaneously as for allocation group.

Venous blood (5 ml) was drawn for complete blood count, in vacutainer containing anti-coagulant and in non heparinized vacutainer for liver function tests and plasma interferon level, at 8 and 20 hours after the first injection assuming that endogenous interferon level is below the quantification level.

Blood samples were taken at 8 after the first

dose of interferon in accordance with limited sample strategy considering the fact that interferon gets maximum plasma concentration(Cmax) at 08 hours when given subcutaneously or intramuscularly and traces remain in the blood after 20 hours (package insert).

Blood samples for plasma interferon level were immediately centrifuged at 3000 revolutions per minute for 5minutes. Plasma was removed and frozen instantly at -80°C and maintained in the frozen state till analyzed.

Liver function tests were analyzed by auto analyzer DADE BEHRING, Dimension Rxl series.

Complete blood count done on auto analyzer Sysmex Kx21.

All samples for plasma interferon level were analyzed by ELISA by DYNEX Human ELISA reader, Best 2000 ELISA system. The procedure followed the instructions of the manufacture, BIOKIT, S.A.08186 Lica d'Amunt, BARCELONE-SPAIN.

Bioavailability was observed, in the review of study [9, 10], AUC and Cmax as the pivotal parameters for bioavailability determination. Cmax was taken as 08 hours; area under cover (AUC) was calculated by trapezoidal rule and by drawing the graph against plasma concentration versus time.

Trapezoidal rule is calculated as under

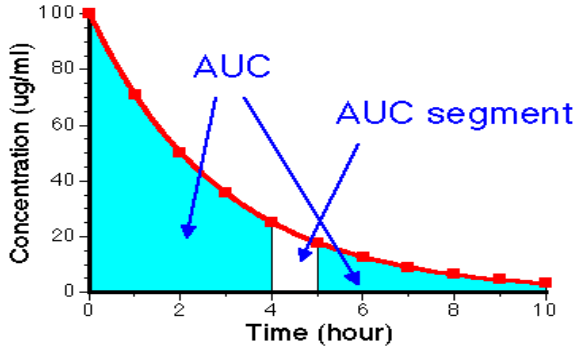

Fig. 1: Calculation of AUC using the trapezoidal rule Linear Plot of Cp versus Time showing AUC and AUC segment

RESULTS

The demographic variable of the 60 cases were not different significantly (Table 1).

The mean age of the three subgroups was not different significantly. The proportion of male to female was equal in three subgroups (Table 1).

Weight, height and body surface area were not different significantly (Table 1).

The complete blood count and biochemical data was not significantly between three subgroups (Tables 2 and 3).

Mean plasma level obtained at 08 hours 71.37, 60.44, and 57.35for groups 1, 2 and 3 respectively (Table 4).

Table1: Demographic and mean parameters of cases summarized.

	Uniferon (20)	Anferon (20)	Ceron (20)	P value
Sex				
Males	10	10	10	-
Females	10	10	10	-
Age (Yrs)	30.4±5.1	29.2±4.7	30.4±4.8	>0.05
Body surface area (m^2)	1.547±0.257	1.558±0.259	1.543±0.257	> 0.05
C max (IU/ml)	60.98±.1	52.8±8.8	49.34± 8.2	*<0.01
T max (hour)	8	8	8	-
AUC (IU/ml/hr)	68.5±11.3	58.5±9.5	52.5±9.0	<0.05
Bioavailability	70%	60%	55%	<0.05

Table 2: Mean value of blood complete of three subgroups

Mean	Group 1 (Uniferon)	Group 2 (Anferon)	Group 3 (Ceron)
Hemoglobin level	10.9±1.8	11.4±1.9	10.9±1.8
White blood count	7.56±1.26	7.09±1.8	7.25±1.2
Polymorphonucleocyte count	67.18±11.19	67.41±10.73	67.63±11.27
Red blood count	4.92±0.82	4.99±0.83,	5.20±0.86
Lymphocyte count	27.58±4.68	28.45±4.74	26.73±4.45
Platelet count	196.63±32.77	196.50±32.75	197.5±32.91

Table 3: Mean value of liver profile of three subgroups

Mean	Group 1 (Uniferon)	Group 2 (Anferon)	Group 3 (Ceron)
Total bilirubin	0.75±0.12	0.72±0.12	0.73±0.12
Alanine amino transferase	75.96±12.66	76.6±12.76	76.05±12.67
Aspartate transferase	49.33±8.22	50.40±8.41	51.05±8.50
Alkaline phosphatase	96.96±16.16	94.30±15.71	97.50±16.25
Total protein	6.98 ± 1.16	6.99±1.16	6.59±1.09
Albumin	3.79 ± 0.63	3.86±0.64	3.66±0.61

Mean plasma level obtained at 20 hours was negligible in three subgroups (Figs. 2 and 3).

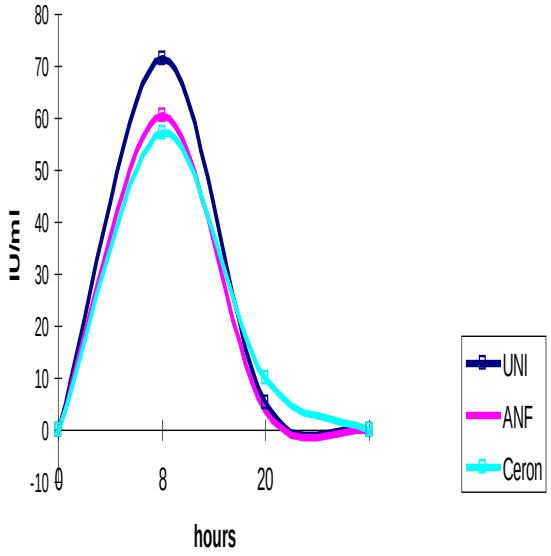

Fig. 2: The means value of plasma interferon level of male patients at 8 hours and 20 hours.

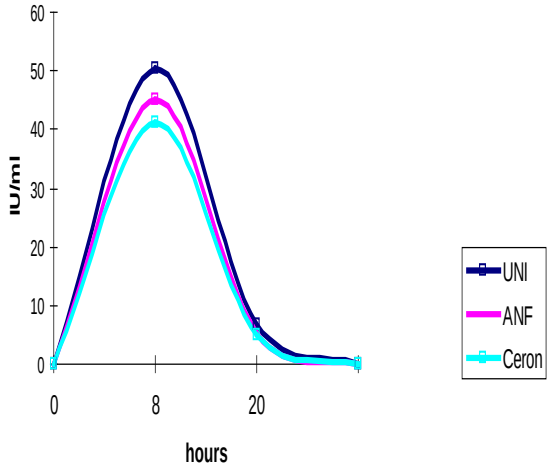

Fig. 3: The means value of plasma interferon level of male patients at 8 hours and 20 hours.

Considering the fact that endogenous interferon level is below the quantification level and the principal findings were that three groups had shown variable plasma level at 8 and 20 hours with significance((Figs. 2 and 3).

Present study observed that at 8 hours in group 1, the Cmax 71.37±11.11 in males and 50.59±9.07 in females with AUC 68.5 of subgroup 1were greater that the Cmax 60.44±10.09 in males 45.16±7.90 and AUC 58.5 of group2 and 3 Cmax 57.35±9.86in males 41.33±7.23 with AUC 52.5 showed a significant difference in Cmax and AUC between three subgroups.

Table 4: Mean plasma level of three subgroups.

Mean plasma level	Group 1 (Uniferon)	Group 2 (Anferon)	Group 3 (Ceron)
08 hours	71.37	60.44	57.35
20 hours	5.75	4.61	4.52

* Between 1 and 3and 1 and 2.

Bioavailability calculated from area under cover, showed a significant difference between three subgroups.

Using the SPSS version 15, all numerical variables were represented as mean ± SEM on the basis of the test described below:

The parameters of the three subgroups were subjected to statistical analysis using one – way ANOVA. p – Value was highly significant between groups 1 and 3 and 1 and 2.

DISCUSSION

The study was based on bioavailability of interferon alpha 2b.

The present study was done for the first time in Pakistan in which plasma concentrations obtained after first, single dose injection of interferon alpha 2b administered subcutaneously were analyzed by Elisa method.

To develop two sampling strategy which was never done in Pakistan, 08 and 20 hours post dose, it was possible to determine the bioavailability with accuracy similar to that of the full sampling time as, two hours interval, 2, 4, 6, 8, 9, 10, 12, 14, 16, 18, 20, 22, and 24 hours after the dose, with minimum inconvenience and pain by reducing pricks and follow ups.

The abundance of preparations which are available in Pakistan it was hypothesized that FDA approved interferon alpha 2b was better in bioavailability.

This also indicated that due to high bioavailability of group 1 (Uniferon) as compared to

of group 2 (Anferon) and group 3 (Ceron) that its function as anti HCV is more strong and effective than other drugs used. Uniferon (group1) had shown maximum bioavailability and cost effectiveness among the three group of injections used followed by Anferon (group 2) and Ceron α (group3) respectively.

Regarding the factors affecting the bioavailability first it was controlled by employing single first dose bioavailability, secondly I employed the limited sampling strategy.

Manufacturers claimed bioavailability of more than 90% for all of the preparations which were tested, but this study showed 70%, 60% and 55% bioavailability for group 1, 2 and 3 which may be variable due to physical factors, climatic conditions, storage and packing techniques.

Uniferon and Ceron are available as reconstituted powder form while Anferon is available in syringes as pre filled ready to use injections.

The study showed that packing as well as active principle or the unchanged drug in the systemic circulation which is important and is seen best in group 1 which is Uniferon although less than the expected bioavailability but still better than the other two products.

This study lastly also proved to be cost efficacy analysis of chronic hepatitis C virus using different interferon. It proved with finality that group 3; (Uniferon) usage decreased the financial burden of the patients without compromising on the bioavailability and the efficacy.

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