Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 10, 2015

Original Article

BIOENHANCEMENT EFFECT OF PIPERINE AND GINGER OLEO RESIN ON THE BIOAVAILABILITY OF ATAZANVIR

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Received: 04 Mar 2015 Revised and Accepted: 20 Aug 2015

ABSTRACT

Objective: The present study was undertaken to investigate the bioavailability enhancing effect of the natural bioavailability enhancers, piperine and ginger oleoresin on the antiretroviral drug atazanvir in combination with ritonavir.

Methods: Sprague-Dawley rats of either sex, were used for the bioavailability studies of ATV and RTV in combination of Piperine. All the drugs were administered orally in the form of suspension to the animals. The bioavailability studies were performed by determination of the drugs concentration in plasma by the HPLC.

Results: The bioavailability of atazanvir was found to be significantly increased in combination with piperine and ginger oleoresin. However, piperine has demonstrated better effect as compared to ginger oleoresin in all the groups. Piperine (30 mg/kg) when administered in combination with atazanvir (ATV), has shown to increase the bioavailibility of both the doses of ATV (6.5 mg/250 gm) and ATV (3.25 mg/250 gm) significantly as compared to the conventional doses.

Conclusion: Results of the investigation proves a significant bioavailability enhancement effect of piperine and ginger oleo resin on atazanavir in lower doses as well as in the absence of rotinavir. These studies places piperine as a mode of increasing activity of atazanvir.

Keywords: Atazanvir, Ritonavir, Bioavailabilty enhancers, Piperine, Ginger oleoresin.

INTRODUCTION

HIV-protease inhibitors play a very important role in antiretroviral drug therapy for the treatment of HIV-AIDS. The aspartic protease of human immunodeficiency virus is responsible for the cleavage of the viral Gag and Gag-Pol polyprotein precursors into mature, functional viral enzymes and structural proteins. This process, called viral maturation, which leads to the final morphological rearrangements, is indispensable for production of infectious viral particles [1]. If HIV-PR is inhibited, the nascent virions cannot go on to attack other cells and the spreading of HIV is therefore stopped.

The agents in this category inhibit HIV aspartic protease which is essential for post-translational proteolysis of gag and gag-pol polyproteins. Inhibition of that proteolysis results in the release of immature HIV that is no longer infectious [2, 3]. The protease inhibitors, compared to nucleoside analogues, undergo a very different metabolism in humans. In general, similar to non-nucleoside reverse transcriptase inhibitors, protease inhibitors are substrates for cytochrome P450 oxidation. Thus, a number of clinically relevant drug–drug interactions have been documented with regard to the metabolism of these agents. Oral bioavailability of these agents is poor [4]. They are competitive inhibitors of HIV PR and all but one are peptidomimetics of the polyprotein cleavage sites.

Currently available anti-HIV protease inhibitors have relatively short half-life, low bioavailability, poor CNS penetration and retention, and undesirable side effects. These drawbacks give us opportunities to design and develop novel drug delivery systems to overcome transport barriers and inherent elimination/metabolism problems associated with these anti-HIV drugs. The delivery systems of these anti-HIV drugs have been developed to compensate the short half-life, circulation time, increase bioavailability, improve CNS penetration and retention, and deliver the drugs to target cells/tissues [5]. Many of these protease inhibitors have been studied for their bioavailability enhancing activity with piperine. As such studies have not been performed using Atazanvir, its detailed bio-enhancing activity has been studied in this paper in combination with piperine and ginger oleoresin.

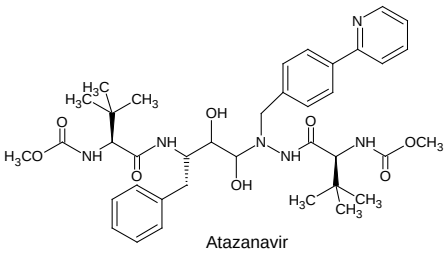
Atazanavir

Atazanavir (ATV) methyl N-[(2S)-1-[2-[(2S,3S)-2-hydroxy-3-[[(2S)-2-(methoxycarbonylamino)-3,3 dimethyl- butanoyl]amino]-4-phenyl butyl]-2-[(4-pyridin-2-ylphenyl)methyl]hydrazinyl]-3,3-di methyl-1-oxobutan-2-yl]carbamate with molecular formula C38H52N6O7 and molecular weight 704.8 is an antiretroviral drug of the protease inhibitor (PI) class. Like other antiretrovirals, it is used to treat infection of human immunodeficiency virus (HIV) and is used in combination with other HIV medications. It is the first protease inhibitor approved for once-daily dosing, and also appears to be less likely to cause lipodystrophy and elevated cholesterol as side effects. It may also not be cross-resistant with other protease inhibitor. The U. S. The food and Drug Administration (FDA) approved atazanavir with three different strengths such as 100 mg, 150 mg and 200 mg, on June 20, 2003. On October 20, 2006, the FDA approved a new formulation of ATV (300 mg capsules) to be taken as part of combination drug therapy [6]. This formulation should reduce pill burden, as one 300 mg capsule may replace two 150 mg capsules.

Dose of Atazanavir alone-400 mg orally once a day (OD) while Atazanavir+Ritonavir-300 mg orally OD+100 mg orally OD.

Cytochome P (CYP) enzyme CYP3A4 is the major isoenzyme responsible for ATV metabolism. ATV is a competitive inhibitor of CYP3A4 (Inhibition rate constant (Ki) = 2.35 μM) and UGT1A1 (Ki = 1.9 μM) at clinically relevant concentrations. ATV also competitively inhibits CYP1A2 and CYP2C9, but the Ki values (Ki ≥ 12.2 μM) were higher than steady-state plasma concentrations, suggesting that ATV is unlikely to inhibit CYP1A2 and CYP2C9 substrates at the recommended dose [7, 8].

The most common side effects of ATV include diarrhea, nausea or vomiting, headache, and fatigue. Others include circumoral paresthesia (tingling sensation around the mouth), and changes in liver function, which can be detected and monitored with laboratory tests. An increase in bilirubin (by product of red blood cell break down) has been associated with ATV. Sometimes the increase in bilirubin results in jaundice (yellowing of skin and eyes). ATV has demonstrated a low incidence of lipodystrophy [9, 10].

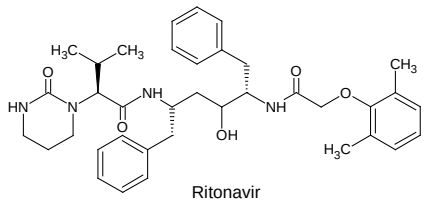


Atazanavir

Ritonavir

Ritonavir (RTV) 1,3-thiazol-5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4-yl] methyl}) carbamoyl] amino} butanamido]-1,6-diphenyl-hexan-2-yl] carbamate with molecular formula $C_{37}H_{48}N_6O_5S_2$ and molecular weight 720.9. Ritonavir is the first and only co-formulated HIV-1 protease inhibitor (PI). Large clinical trials have demonstrated RTV's clinical efficacy in both antiretroviral-naive and experienced patients. The immunologic and virologic benefits of treatment with this agent have been proven in HIV-infected adults, adolescents, and children. Smaller studies support the use of RTV monotherapy as a therapeutic option in certain patients. The drug is characterized by a high genetic barrier to resistance, and appears to be more forgiving of non-adherence than earlier, unboosted PIs. RTV is frequently prescribed with highly active antiretroviral therapy (HAART), not for its antiviral action, but as it inhibits the same host enzyme that metabolizes other protease inhibitors. This inhibition leads to higher plasma concentrations of these latter drugs, allowing the clinician to lower their dose and frequency and improving their clinical efficacy. The Dose of RTV alone is 300 mg orally twice a day while RTV in combination with ATV is 100 mg orally OD+300 mg orally OD.

It undergoes rapid first-pass metabolism in the liver by CYP3A4 and CYP3A5. It inhibits the CYP3A4 isoenzyme in the human liver microsomes and results in drug interaction [11].



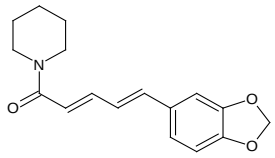
Ritonavir

The most frequent side effects of ritonavir includes diarrhea, nausea, and vomiting. These gastrointestinal adverse effects are generally mild to moderate. Immune reconstitution syndrome has been reported in patients treated with antiretroviral regimens containing RTV, manifested as an inflammatory response to indolent opportunistic infections [12, 13]. The above data reveals that the adverse effects of both ATV and RTV are higher and it can be reduced by lowering the doses of the drugs. But if we reduce the dose of the drugs, then it is also necessary that the same plasma concentration should be achieved to have the same therapeutic effects. This problem can be resolved if we use the bioenhancer with the lower dose of the drug.

Bioavailability enhancers

Piperine, an amide alkaloid obtained from the mature fruits of *Piper nigrum* and *P. longum,* is the first and till date the most potent bioenhancer to be discovered. The bioenhancing activity of piperine and the concept of bioavailability enhancement using piperine both were discovered and scientifically validated in 1979 in India [14]. It was studied successfully to reduce the dose of the drug and cost of the treatment. Collaborative studies conducted by Cadila Labs Ltd. at Regional Research Laboratory, Jammu, led to successful launching of the well known antitubercular drug Rifampin (200 mg) along with bioenhancer piperine (10 mg) under the trade name 'Risorine', in 2009. In the above case, Rifampicin's conventional dose of 450 mg has been reduced to 200 mg with the same bioavailability [15].

Piperine brings about its bioenhancing activity by inhibition of P-glycoprotein drug efflux pump (PGP_2) and by inhibiting cytochome P enzymes such as $CYP1A_1$, $CYP1B_1$, $CYP1B_2$, $CYP1E_1$ and $CYP3A_4$. All the drugs metabolized by these enzymes are influenced by bioenhancer piperine [16]. All categories of drugs like cardiovascular, respiratory, anticancer, immunomodulatory drugs, drugs acting on the central nervous system and gastrointestinal tract, antibiotics and several other classes of drugs and nutraceuticals are greatly influenced by piperine. It is interesting to note that piperine brings about its bioenhancing effect in a dose of 10 mg in all formulations irrespective of the dose of combination drug.



Piperine

Ginger oleo-resin

The drug consist of oleo-resin isolated from the rhizome of the plant *Zingiber officinalis* Roscoe, family Zingiberaceae, commonly known as ginger. Many drugs are found to be more active when used in combination with bio enhancer products developed from oleo-resin of ginger. The effective range for ginger oleo-resin as a bioenhancer is 10-150 mg. Class of drugs which has shown enhanced activity are drugs acting on the cardiovascular and central nervous system, anti retrovirals, anti-inflammatory, antiarthitic, antitubercular, antileprotic, anti ulcer and many other therapeutic agents [17, 18].

MATERIALS AND METHODS

Plant material, chemicals and reagents

All crude drugs namely Black pepper, *Piper nigrum* and Ginger, *Zingiber officinalis,* was procured from reliable sources. Fenugreek seeds, Black pepper and Ginger rhizomes were procured from local market of Bilaspur. The drug samples of atazanavir and ritonavir used in the present study were procured as the gift sample from Cipla Laboratories, Mumbai.

Isolation of Piperine: Dried ripe fruits of black pepper, *Piper nigrum* were defatted with petroleum ether (60-80 °) in soxhlet extractor for 24 h. The extract was dried and further extracted with ethyl alcohol (95%) for 48 h. The total ethyl alcohol extract was cooled and filtered to remove fine particles if necessary, and concentrated under reduced pressure to yield total alcohol extract in 2.5% yield. The concentrated solution was kept in an ice bath, and water was added drop wise (about 30 ml will be required) to precipitate piperine. Piperine was collected on a sintered glass funnel. It was further recrystallized from acetone: hexanes (3:2) to afford pale yellow crystals of piperine (7%). Thin layer chromatographic study of isolated piperine along with the authentic primary standard has shown a single spot of R_f 0.52, in solvent system toluene-ethyl acetate (7:3) when spread with Dragendorff's reagent [14, 15].

Isolation of Ginger Oleo Resin (GOR): About 250g of completely dried ginger was powdered in a mechanical grinder, finely shifted and subjected to continuous hot percolation process using soxhlet apparatus, by using solvents petroleum ether and then with ethanol for 24 and 48 h respectively. The extracts thus obtained were concentrated to a thick brownish yellow semi-solid mass using Rotary vacuum evaporator or water bath. The thick pasty mass was added to water to precipitate oleo-resin. The practical yield was found to be 2.9 %. The oleo-resin extract was subjected to thin layer chromatography using silica gel G and petroleum ether: ethyl acetate (7:3) as a solvent system. The Rf values of the oleo-resin constituents were found as per the reported values in the literature [19].

Bioavailability studies

Animals

Sprague-Dawley rats of either sex, weighing 200±50 gm, of almost same age, were used for the bioavailability studies of ATV and RTV in combination of Piperine. The animals were provided with food and water before and during the study protocol. Ethical approval

was obtained from the Committee for the purpose of control and supervision of experimental animals (CPCSEA), Chennai, India. The study was performed as per the standard operating procedures for Institutional animal ethics committee of the CPCSEA. In each group, three animals were used the blood was collected five times. The weight of the rats was recorded in each group on the day of the study, before the commencement of the study.

The dose of the drugs-ATV (H-6.5 mg/250 gm), ATV (L-3.25 mg/250 gm) and RTV (2.13 mg/250 gm) and the dose of the bioenhancers-piperine (30 mg/kg) and gingerol (30 mg/kg), was calculated according to the body weight of the rats. For the doses given in above brackets, 'H' and 'L' stands for 'Higher' and 'Lower', respectively.

For the control group, saline was administered first and then the drug after an interval of 30 min. For the treated group, bioenhancers were administered first and then the drug after an interval of 30 min. The drug and bioenhancer samples for oral administration to the animals were prepared in 2% tragacanth solution.

The drug bioavailability studies were done by determinations of the drugs concentration in plasma by the High performance liquid chomatography (HPLC). The solvents required for the HPLC studies are Acetonitrile, Ammonium formate and water [20-22].

Sigma-Aldrich-Ammonium formate buffer of pH=3, 10 mM was required for the HPLC studies. Ammonium formate, 0.63 gm, was weighed and dissolved in 900 ml of HPLC grade water. The pH of the solution was maintained to 3 with orthophosphoric acid, where orthophosphoric acid was diluted with water in the ratio of 1:5. When the pH of the solution was maintained, then the volume of the buffer was made to 1000 ml. The acetonitrile and ammonium formate buffer was mixed in the ratio of 45:55 v/v. This solution was used as the mobile phase for the HPLC of the drugs, ATV and RTV.

HPLC studies

The HPLC was used for the detection of the retention time, peak area and concentration of the drugs. HPLC maker & model: Shimadzu 2010 CHT with a column of 250 mm X 4.6 mm X 5 micron (Princeton) was used for the present study.

After 30 min of drug administration, the blood was withdrawn from the rats. Prior to blood withdrawal, the rats were anaesthetized with ketamine, at a dose of 0.6 ml/kg. Then the blood was collected at regular intervals and 5 samples were withdrawn from each group. The blood was withdrawn as follows after every 30 min, 1, 3, and 6 h.

The blood was kept in the refrigerator, till its centrifugation. The plasma separation was done in the cooling centrifuge. The blood was centrifuged at 2000-3000 rpm for 20 min. After the separation, the plasma samples were stored at-20˚C, till the HPLC studies [22].

For HPLC studies, the plasma samples were centrifuged again, with the addition of the mobile phase. The supernatant liquid obtained after the centrifugation of the samples was injected into the column. The method followed for centrifugation is as follows:

2M sodium carbonate (400 µl), n-hexane: ethyl acetate (50:50) (800 µl) and vortex the plasma sample carefully Plasma samples and centrifuge the samples at 10,000 rpm, at temperature-10 to-20˚C. Two layers were obtained, the upper organic layer and lower aqueous layer. The organic layer was separated for further process and the aqueous layer was discarded. The organic layer was left for overnight for complete evaporation of the solvent. To the residue, mobile phase was added and centrifuged the samples at 10,000 rpm, at temperature-10 to-20˚C. The supernatant liquid obtained was carefully collected and subjected to HPLC studies. The drug ATV, with the help of UV set at 210 nm.

Besides the plasma samples, the HPLC of ATV-8 µg/ml, was also done. This served to be the standard with the peak area and retention time of 166732 and 12.469 respectively. This peak area was considered to be 100 %. The concentration of the samples was determined from peak area of the respective samples with the help of data of the standard sample.

HPLC analysis

The plasma samples were analyzed with HPLC for the drug ATV, with the help of UV set at 210 nm. After the extracts were obtained, the animal studies were done. The animals were divided in ten groups where each group contained two animals. The control groups were treated with saline and drug, and treated groups with piperine and GOR along with the drug. The rats were pre treated with saline in case of control and with piperine and GOR in case of treated. The blood samples were withdrawn from the rats. From 0.30 to 2 hour, thee samples were withdrawn from one rat and from 2 to 6 h, two samples were withdrawn from other rat of the same group in the anticoagulant added tubes. After collection of blood samples, they were centrifuged and plasma was collected. This plasma before injecting into the column of the HPLC was centrifuged with the mobile phase again. The resulting supernatant liquid so obtained was then injected into the column. The plasma samples were then analyzed for the drug ATV. Besides the plasma samples, the HPLC of ATV-8 µg/ml, was also done. This served to be the standard with the peak area and retention time of 166732 and 12.469 respectively. This peak area was considered to be 100 %. The concentration of the samples was determined from peak area of the respective samples with the help of data of the standard sample.

Statistical analysis

The results were presented as the means S. E. M. One way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons.

RESULTS AND DISCUSSION

ATV and RTV were subjected to bioavailability studies in the presence of natural bioavailability enhancer's piperine and GOR. For ATV group, higher dose (6.5 mg/250 gm), ATV (H) and lower dose (3.25 mg/250 gm) ATV (L) were selected to examine the bioavailability of both the conventional doses in combination with RTV (2.13 mg/250 gm), piperine and GOR. The results of the studies have been given in table 1,2 and fig. 1,2.

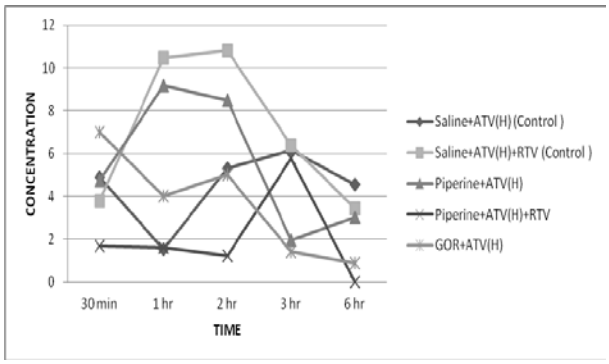


Fig. 1: The concentration of ATV (6.5 mg/250 gm) in all the plasma samples as analyzed by the HPLC

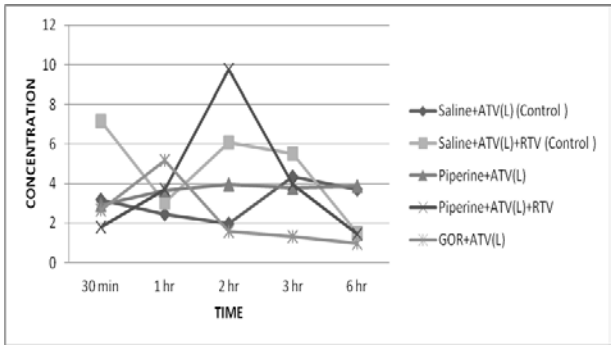


Fig. 2: The concentration of ATV (3.25 mg/250 gm) in all the plasma samples as analyzed by the HPLC

Table 1: Mean retention time, peak area and plasma concentration of ATV (6.5 mg/250 gm) in all the plasma samples as analyzed by the HPLC

Sample	Time	Mean retention time (Average)	Mean peak area	Mean Plasma conc. (µg/ml)
Control	30 min	12.348	103143	4.90
Saline+	1 h	12.426	31733	1.52
ATV(6.5 mg/250 gm)	2 h	12.386	110584	5.30
	3 h	12.411	128194	6.15
	6 h	12.317	94639	4.54
Control	30 min	11.111	78575	3.77
Saline+	1 h	11.178	218016	10.48
ATV(6.5 mg/250 gm)+	2 h	11.127	225026	10.82
RTV (2.13 mg/250 gm)	3 h	11.133	132572	6.37
	6 h	11.150	71839	3.45
ATV(6.5 mg/250 gm)+	30 min	12.287	99370	4.76
Piperine (30 mg/kg)	1 h	12.373	190849	9.16
	2 h	12.456	177023	8.49
	3 h	12.377	40566	1.94
	6 h	12.384	63344	3.04
ATV (6.5 mg/250 gm)+	30 min	11.072	34809	1.67
RTV (2.13 mg/250 gm)+Piperine (30 mg/kg)	1 h	11.110	33158	1.59
	2 h	11.099	25771	1.24
	3 h	11.185	120059	5.77
	6 h	No readings as animal died		
ATV(6.5 mg/250 gm)+	30 min	12.401	145840	7.0
GOR (30 mg/kg)	1 h	12.267	83584	4.01
	2 h	12.256	104696	5.02
	3 h	12.321	29655	1.42
	6 h	12.268	18406	0.88

Table 2: Mean retention time, peak area and plasma concentration of ATV (3.25 mg/250 gm) in all the plasma samples as analyzed by the HPLC

Sample	Time	Mean retention time (Average)	Mean peak area	Mean plasma conc. (µg/ml)
Control	30 min	12.574	66834	3.20
Saline+	1 h	12.445	51404	2.46
ATV (3.25 mg/250 gm)	2 h	12.512	41023	1.96
	3 h	12.246	90882	4.36
	6 h	12.401	76684	3.68
Control	30 min	11.176	148392	7.14
Saline+	1 h	11.059	63867	3.07
ATV (3.25 mg/250 gm)+	2 h	11.236	126701	6.09
RTV (2.13 mg/250 gm)	3 h	11.106	114630	5.51
	6 h	11.029	31509	1.515
ATV(3.25 mg/250 gm)+	30 min	12.427	60832	2.91
Piperine (30 mg/kg)	1 h	12.447	76302	3.66
	2 h	12.491	82236	3.94
	3 h	12.278	79321	3.80
	6 h	12.354	81061	3.89
ATV(3.25 mg/250 gm)+	30 min	11.090	37372	1.79
RTV (2.13 mg/250 gm)+Piperine (30 mg/kg)	1 h	11.120	76466	3.68
	2 h	11.060	203218	9.77
	3 h	11.143	82691	3.98
	6 h	11.119	30619	1.47
GOR (30 mg/kg)+ATV(3.25 mg/250 gm)	30 min	12.471	55452	2.66
	1 h	12.379	107991	5.18
	2 h	12.276	33120	1.58
	3 h	12.364	27757	1.33
	6 h	12.361	20209	0.97

First selecting the conventional 6.5 mg dose, the groups to be compared are Saline+ATV(H), ATV(H)+Piperine and ATV(H)+GOR. In the control group, the initial conc. is 4.90 µg/ml at 0.5 h, minimum conc. is 1.52 µg/ml at 1 h and maximum conc. obtained is 6.15 µg/ml at 3 h. After 3 h, the conc. of the drug decreases and is 4.54 µg/ml at 6 h. The treated group, ATV(H)+Piperine, the initial conc. is 4.76 µg/ml at 0.5 h, it then rises to 9.16 µg/ml at 1 h. Then the conc. starts decreasing, 8.49 µg/ml at 2 h and 1.94 µg/ml at 3 h.

Thus it is clear from the above data, that the maximum conc. of the drug is 9.16 µg/ml at 1 h with the Piperine treated group. The conc. as well as the time, i.e., Cmax and Tmax both is higher for the piperine group in comparison to the control group. Graph-1 clearly shows the two-fold increase in the plasma concentration of the drug.

The second treated group, ATV(H)+GOR, the initial conc. of the drug is 7 µg/ml at 0.5 h, then at 1 h it decreases to 4.01 µg/ml, at the 2 h, it again increase to 5.02 µg/ml, then it decreases to 1.42 µg/ml at 3 h and finally to 0.88 µg/ml at 6 h. The maximum conc. for the GOR treated group is 7 µg/ml at 0.5 h. Thus this maximum conc. is more than the control group, which is 6.15 µg/ml at 3 h. It indicates that the GOR also enhanced the bioavailability of the drug and that too the tmax is achieved earlier than that of the piperine group. However, among the two, piperine has more bioavailability enhancement effect. Thus the ATV(H)+piperine, has two times more bioavailability as compared to all the other groups of the higher dose.

In case of ATV (3.25 mg) dose as compared to control group, Saline+ATV (L), the initial conc. of drug is 3.20 µg/ml at 0.5 h, then it

decreases to 1.96 µg/ml and then increases to 4.36 µg/ml. This is the maximum concentration of this group at 3 h. After this, the conc. again starts declining and reaches to 3.68 µg/ml at 6 h. In piperine treated group, the initial conc. of the drug is 2.91 µg/ml at 0.5 h, then the conc. increases to 3.66 µg/ml in 1 h, then to 3.94 µg/ml in the 2 h. At 3 h, the conc. drops to 3.80 µg/ml and then again rises to 3.89 µg/ml at 6 h. The maximum conc. among the above groups is 4.36 µg/ml, for the control group at 3 h. The concentration obtained with piperine group is below 4.36 µg/ml. Fig. 2, shows the maximum conc. among the two, however in the case of control group, the concentration shows a considerable decrease after 3 h, whereas it is maintained in case of piperine. In ATV(L)+GOR, the initial conc. of the drug is 2.66 µg/ml at 0.5 h, at 1 h it is maximum with 5.18 µg/ml. It then decreases to 1.58 µg/ml at 2 h, 1.33 µg/ml at 3 h and 0.97 µg/ml at 6 h. On comparing it with the control and piperine group, the conc. 5.18 µg/ml is maximium and at 0.5 h.

For ATV+RTV groups, the conventional higher and lower doses of ATV are taken with 2.13 mg dose of RTV. The study of this combination is done with piperine. In the control group, Saline+ATV(H)+RTV, the initial conc. at 0.5 h is 3.77 µg/ml, and then it rises to 10.48µg/ml at 1 h and further to 10.82 µg/ml at 2 h, which is the maximum conc. Then at 3 h, it starts decreasing and is 6.37µg/ml and at 6 h it is 3.45 µg/ml. In piperine treated group, the initial concentration at 0.5 h is 1.67 µg/ml, at 1 h it reaches 1.59µg/ml, and then decreases to 1.24µg/ml at 2 h, which is the minimum conc. At 6 h, it is maximum 5.77 µg/ml. The blood sample at 6 h was not withdrawn as the animal died. On comparing the above results, the maximum conc. is 10.82 µg/ml at 2 h from the control group. In this case, the results show that the piperine is not able to have enhanced the conc. of the drug, since the maximum conc. in the piperine treated group is 5.77 µg/ml, or it can also be possible that the piperine treated group would have given the higher conc. at the 6 h.

In the group Saline+ATV(L)+RTV, the initial conc. is 7.14 µg/ml at 0.5 h, then it decreases to 3.07 µg/ml at 1 h. At 2 h, the conc. Increases to 6.09 µg/ml, and then decreases again to 5.51µg/ml at 3 h and further to 1.515 µg/ml at 6 h. The maximum conc. is 7.14 µg/ml at 0.5 h and minimum is 1.515 µg/ml at 6 h. For ATV(L)+RTV+piperine, the initial conc. is 1.79 µg/ml, then it increases to 3.68 µg/ml at 2 h. At 3 h, the concentration is 9.77 µg/ml, which is maximum. After this, it starts decreasing, 3.98 µg/ml at 3 h and 1.47 µg/ml at 6 h. Here, on comparing both the groups, the maximum concentration obtained is 9.77 µg/ml at 3 h for the piperine group. From fig. 1, it is clear that the ATV(H)+RTV at higher doses gives the maximum concentration at 10.82 µg/ml which clearly indicates that RTV inhibits the host enzymes that metabolizes ATV as a protease inhibitors. However, ATV(L)+RTV+piperine group shows the highest concentration 9.77 µg/ml which indicates that a combination with the piperine has more advantage as compared to the conventional combination. The conventional dose of ATV(H) with piperine gave maximum conc. of 9.16 µg/ml at 1 h and the group, ATV(L)+RTV+piperine gave the maximum concentration 9.77 µg/ml at 3 h. Therefore, it is rather safer to use ATV(H) with piperine to produce the effective plasma concentration rather than the ATV(L)+RTV+piperine group, as the patient would avoid the adverse effects caused due to RTV.

CONCLUSION

Ritonavir (RTV) is generally given along with atazanavir (ATV) during the HIV treatment not for its antiviral action, but for its inhibitory effect on the host enzyme that metabolizes ATV and other protease inhibitors. Piperine and other natural bioavailabily enhancers have also the great potential to inhibit these host enzymes. ATV-piperine combination, both in higher and lower doses indicated maximum plasma concentration of the drug in blood, comparable to ATV-RTV combination. Further more extensive bioavailability studies are needed on this combination to confirm their role and use in the treatment of HIV-AIDS.

CONFLICT OF INTERESTS

Declared None

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