ORIGINAL ARTICLE

IN VITRO AVAILABILITY OF ATORVASTATIN IN PRESENCE OF LOSARTAN

M. SAEED ARAYNE, NAJMA SULTANA* UROOJ HAROON, FAIZA QURESHI AND SYED ASIF ALI*

Department of Chemistry, University of Karachi, Karachi-75270, Pakistan
**Department of Pharmaceutical Chemistry, Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, University of Karachi*

ABSTRACT

Hydroxymethylglutaryl-coenzyme A reductase inhibitors (statins) are a group of cholesterol lowering agents that have become the largest selling drugs in the world. They are of proven clinical benefit in coronary heart disease, at least in those patients who do not have overt chronic heart failure (CHF). Co-administration of statins with angiotensin II receptor blockers (ARBs) is most common, since there is strong synergy between hypertension and hypercholesterolemia in terms of risk factors for the development of cardiovascular diseases. In present paper, we describe the *in vitro* availability of atorvastatin, a potent HMG-CoA reductase inhibitor, in presence of losartan potassium, which is a non-peptide angiotensin II receptor antagonist. These studies were carried out at 37, 48 and 60°C in different pH environments simulating human body compartments. It was observed that in pH 1, 7.4 and 9 the availability of atorvastatin was very high while losartan was not at all available. However in pH 4 these effects were reversed and atorvastatin was not available at all. At 48°C the availability of atorvastatin was high and that of losartan was depressed at pH 9, whereas the later was not available at pH 1, 4 and 7.4 at all. Likewise at 60°C, the availability of atorvastatin at pH 7.4 and 9 was high, whereas the charge-transfer complex formed between the two drugs was broken at pH 1 at this temperature and the entire drug was available. On the other hand the availability of losartan at pH 4 and 9 was high while it was not available at pH 1 and 7.4. The availability of atorvastatin was maximum in simulated gastric juice as compared to buffer of pH 7.4 and 9. This high availability of one drug in presence of other is attributed to the formation of a charge-transfer complex, which was stable at elevated temperatures, except at 60°C in pH 1.

Keywords: UV, atorvastatin, losartan, drug interactions.

INTRODUCTION

Atorvastatin, (R-(R*R*))-2-(4-fluorophenyl)-β,δ-dihydroxy -5-(1-methylethyl)-3-phenyl-4-((phenylamino)carbonyl)-1H -pyrrole-1-heptanoic acid (figure 1), mimics the activity of HMG-CoA reductase blocking the rate-limiting step of cholesterol biosynthesis (Agostino and Rodolfo 1999). It reduces LDL cholesterol, apolipoprotien B and triglycerides and increases HDL in the treatment of hyperlipidaemia. Atorvastatin can also be effective as adjunctive therapy in patients with homozygous familial hypercholesterolemia who have LDL receptor function (Malinowski, 1998; Malhotra and Goa, 2001; Ohashi *et al.,* 2005).

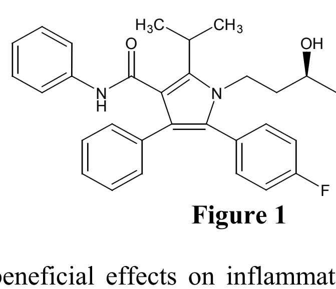

Figure 1

It has beneficial effects on inflammation, oxidative stress, and lipid profile in patients with hyperlipidaemia (Kumar *et al.,* 2004). Atorvastatin is rapidly absorbed after oral administration, maximum plasma concentration occurs within 1 to 2 hours and half- life in humans is ~ 14 hours (Hans-Ulrich *et al.,* 2004; Frisen *et al.,* 2004; Kannan *et al.,* 2004; Wenji *et al*., 2004; Lennernas, 2003; Remington, 2000).

Losartan, 2-butyl-4-chloro-l-((2'-(lH-tetrazol-5-yl)(l,l'-bi-phenyl)-4-yl)methyl)-lH-imidazole-5-methanol (figure 2) monopotassium salt, is a potent, orally active antagonist that binds selectively and specifically to the AT_1 subtype of angiotensin II receptor antagonist (Chiu *et al*., 1990; Goldberg *et al*., 1993). Oxidation of the 5-hydroxymethyl groups on the imidazole ring results in the active metabolite of losartan.

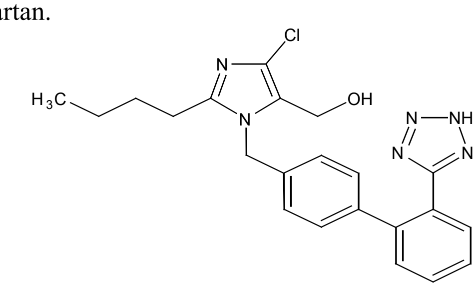

Figure 2

Corresponding author: *Email: arayne@gawab.com*

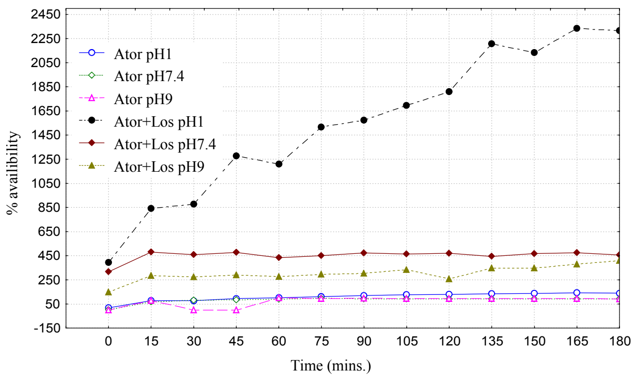

Figure 3: Availability of atorvastatin in presence of losartan in different medium at 37°C.

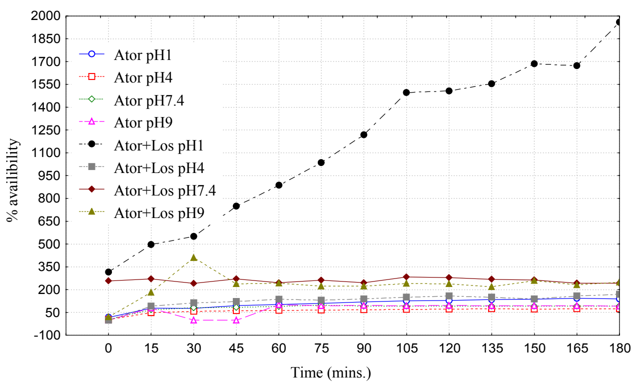

Figure 4: Availability of atorvastatin in presence of losartan in different medium at 48°C.

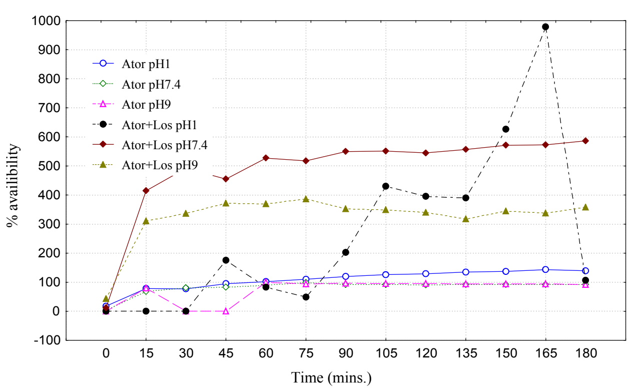

Figure 5: Availability of atrovastatin in presence of losartan in different medium at 60°C.

Atorvastatin is metabolized by the cytochrome P450 iso enzyme CYP3A4, interactions may occur with drugs that inhibit this enzyme (Black, 1998), including cyclosporine (Asberg, 2001), intraconazole (Kantola, 1998; Mazzu *et al.* 2000), erythromycin (Siedlik, 1999), clarithromycin (Amsden, 2002), HIV protease inhibitors (Hsyu, 2001), troglitazone (Ditusa and Luzier, 2000), and diltiazem (Lewin, 2002). There may also be a similar interaction with grape fruit juice (Lilja, 1999; Arayne *et al.,* 2005).

In addition to their primary mode of action, statins and (ARBs), possess common additional properties (Agostino and Rodolfo, 1999). HMG co-reductase inhibitors (statins) are of proven clinical benefit in coronary heart disease, at least in those patients who do not have overt chronic heart failure (CHF). However, as there have been no prospective clinical trials of statins in CHF patients, the question arises as to whether the benefits observed in the absence of CHF can be necessarily inferred in those patients in whom CHF is established. The theoretical considerations as to why these agents may not necessarily be of benefit in this setting will depend on the formation of association or charge-transfer complex which has not been studied so far. Moreover, there are evidences that beneficial potential of statins clearly relates to their plaque stabilization properties and associated improvements in endothelial function, which together should reduce the risk of further infarction and, perhaps, the ischemic burden on the failing ventricle (Krum and McMurray, 2002).

Furthermore, these agents may have beneficial effects independent of lipid lowering. These include actions on neoangiogenesis, downregulation of AT_1 receptors, inhibition of proinflammatory cytokine activity and favorable modulation of the autonomic nervous system, which may be disturbed by the formation of an association or charge-transfer complex. The potential adverse effects of statins to exacerbate oxidative stress and loss of the protection that lipoproteins may provide through binding and detoxifying endotoxins entering the circulation via the gut, may be reduced. It is envisaged that combination therapy of an ARB and a statin would find utilization in people with cardiovascular risk factors. However at present no trials have tested a combination of ARBs with statins (Georg, 2004).

In present paper, we describe the *in vitro* availability of atorvastatin in presence of losartan potassium. The later drug is most commonly co-administered with statin drugs and atorvastatin is the most common drug of choice. These interactions were carried out in different pH environments simulating empty and full stomach juice (pH 1 & 4) intestinal juice (pH 9) and blood pH (7.4) at physiological and elevated temperatures. The results show formation of a charge-transfer complex as the availability of atorvastatin was greatly increased, while availability of losartan was depressed.

EXPERIMENTAL

Materials and equipment

Atorvastatin reference standard was a gift from National Institute of Cardiovascular Diseases Karachi. Losartan potassium reference standard was a gift from Pharmevo Karachi. Atorvastatin and Losatan potassium (Atorscot® 10mg and Tancin® 50mg) tablets were purchased from the market. All the reagents used were of analytical grade. All the glassware were washed with chromic acid followed by a thorough washing with water and finally rinsed with de-ionized water which was freshly prepared in the laboratory.

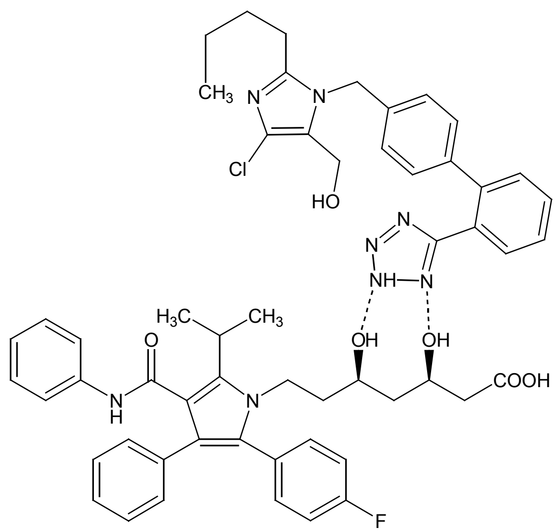

Fig. 6

Dissolution test apparatus manufactured according to B.P 2003 (British Pharmacopoeia, 2003) standards, with little modification, (Iftikhar *et al*., 2005) was used for interaction studies. UV/Visible spectrophotometer (Shimadzu 1601) was used to quantitate the drug contents.

Quantitation of interacting drugs

Reference standards

Atorvastatin and losartan potassium both obeyed Beer's law at their respective λ max at 241-242 and 205-206 nm respectively in the concentration range of 10^{-4} - 10^{-5} moles in all buffer systems under study. Molar absorbtivities of both the drugs were calculated (table1) for their quantitation from unknown solutions.

Availability studies

In the first set of experiments, *in vitro* availability of atorvastatin and losartan potassium were studied in different dissolution mediums, each being 1000 ml of simulated gastric juice, buffers pH 4, 7.4 or simulated intestinal juice. Samples were withdrawn periodically at an interval of 15 minutes for 180 minutes. The volume of dissolution fluid was maintained by adding an equal amount of dissolution fluid withdrawn, which had previously been maintained at the same temperature in the same bath. The absorbance of the sample withdrawn was measured at the λ_{max} of each drug and quantitated with the help of the simultaneous equation.

Interaction studies

The *in vitro* interaction of atorvastatin with losartan potassium was carried out in simulated gastric juice, at pH 4, 7.4 and 9 at 37°C, 48°C and 60°C. In the first set of experiments atorvastatin tablet was added at zero time to the dissolution medium already maintained at specified temperature (37°C) while losartan potassium was added after 15 minutes time interval. Aliquots were withdrawn and assayed for both the drugs. The above procedure was repeated at 48 and 60°C keeping the other experimental conditions constant. Graphs were plotted for % availability of drug versus time in each set of experiment.

RESULTS AND DISCUSSION

Although there are number of methods reported in the literature for atorvastatin (Shum *et al*., 1998; Jemal *et al*., 1999; Bullen *et al*., 1999) and losartan (Hillaert and Van den Bossche, 2003; Williams *et al*., 1996; Hertzog *et al*., 2002; Prabhakar and Giridhar, 2002). In present studies, we employed UV spectroscopic methods for quantitation of both drugs (Nevin, 2003; Prabhakar and Giridhar, 2002). The absorption maximum of atorvastatin reported to be at 241 nm is due to the π-π* transition between the carbonyl oxygen and the unsaturated carbon of the pyrrole ring, the lone pair of nitrogen further results in the extension of conjugation. Similarly the reported absorption maxima of losartan at 206nm is due to the presence of tetrazole ring. For quantitation, molar absorptivities of both the interacting drugs were calculated using their reference standards at the λ_{max} of each other in methanol and in other mediums of interaction (table 1).

During the analysis, it was observed that the absorption maxima of each drug interfered with the other drug (fig. 7). As the λ_{max} of both the drugs was quite far apart (206 and 241), hence following simultaneous equations were developed to quantitate each drug from the solution, while countering the interference of the other drug.

$$C_a = \frac{A_{241.} b_2 - A_{206}. b_1}{a_1 b_2 - a_2 b_1} \qquad (1)$$

and

$$C_b = \frac{A_{241.} a_2 - A_{206}.a_1}{a_2 b_1 - a_1 b_2} \qquad (2)$$

Where Ca and Cb were the concentration of atorvastatin and losartan, a_1 and a_2 were the molar absorptivites of atorvastatin at 241 and 206 nm, while b_1 and b_2 were the molar absorptivites of losartan potassium at 241 and 206 nm. These equations were used to measure the quantities of losartan potassium and atorvastatin simultaneously present in solution. Prior to these studies, the validity of the

Table 1: Molar absorptivities of atorvastatin and losartan potassium

S. No.	Dissolution medium	<——— Atorvastatin ———>		<——— Losartan ————>	
		λ	(ε $moles^{-1}Lcm^{-1}$)	λ	(ε $moles^{-1}Lcm^{-1}$)
1	Simulated gastric juice	241 nm*	23659	241 nm	15539
		206 nm	43021	206 nm*	22303
2	Buffer of pH 4	241 nm*	23500	241 nm	43021
		206 nm	15539	206 nm*	22303
3	Buffer of pH 7.4	241 nm*	24000	241 nm	50949
		206 nm	14492	206 nm*	21218
4	Buffer of pH 9	241 nm*	24000	241 nm	50894
		206 nm	15543	206 nm*	20382

* = λmax of drug

Table 2: Availability (%) of atorvastatin and losartan in independent dosage forms at 37°C

S. No.	Time (min)	<——pH 1——>		<——pH 4——>		<——pH 7.4——>		<——Ph 9——>	
		Ca	Cb	Ca	Cb	Ca	Cb	Ca	Cb
1	0	17.9	13.7	2.59	0.00	3.8	10.9	79.0	8.78
2	15	78.2	69.5	49.8	67.2	68.2	60.4	79.0	86.5
3	30	77.3	81.4	56.8	65.8	81.1	73.7	0.0	97.4
4	45	95.0	99.5	62.4	72.3	83.5	77.2	0.0	94.5
5	60	101	99.7	63.2	74.2	89.8	79.8	99.7	93.8
6	75	101.2	87.3	66.1	74.3	98.3	84.7	94.3	98.4
7	90	102.8	86.2	68.6	75.3	91.8	87.0	96.6	99.5
8	105	102.6	92.8	69.8	75.1	91.8	95.0	94.9	99.5
9	120	102.9	94.5	71.6	75.5	91.1	90.8	95.2	93.5
10	135	102.3	95.3	73.7	73.3	91.6	90.9	94.0	97.4
11	150	102.7	99.1	73.7	75.3	92.4	90.6	94.3	95.4
12	165	102.4	98.1	75.5	71.3	91.6	92.0	94.4	95.6
13	180	102.4	96.3	74.9	72.9	92.3	90.8	91.5	91.5

Ca = atorvastatin; Cb = losartan K

spectrophotometric assay methods for individual drugs was checked with the reference drugs, and molar absorptivities of both the interacting drugs at the λmax of each drug were calculated (table 1), where a correlation coefficient of 0.999 was obtained.

The availability of both the drugs in individual dosage formulations at different pH are given in table 2. The availability of atorvastatin and losartan potassium in presence of each other at 37, 48 and 60°C are given in tables 3-5 and are plotted in figures 3-5.

The % availability of atorvastatin in simulated gastric juice after 3 hrs. was found to be 102.4. At pH 4, 7.4 and 9 it was 74.9, 92.3 and 91.5 % respectively. Availability of atorvastatin in presence of losartan potassium after 3hrs at 37ºC in simulated gastric juice, pH 4, 7.4 and 9 was found to be 2314.74, zero, 455.78 and 409.38% respectively. This high availability was due to the formation or association of charge-transfer complex, molar absorptivity of which was greater than atorvastatin. On the contrary, the non availability of losartan indicates that the drug immediately underwent complexation through its chromophoric site. In the proposed charge-transfer complex (figure 6), hydrogen bonding exists between the alcoholic group at the heptanoic acid side chain of atorvastatin and tetrazole nitrogen of losartan.

To study the Arrhenius parameters of the reactions, similar studies were carried out at 48 and 60ºC. After 3hrs, in simulated gastric juice availability of atorvastatin was found to be 1958.48 and 106.45% respectively. This decrease in the availability of atorvastatin with the increase in temperature is due to the dissociation of the charge-transfer complex with the increase in temperature. Similar results were observed in pH 7.4 and 9. On the other hand, availability of losartan in simulated gastric juice and in pH 7.4 at all temperatures was found to be 0% in presence of

Table 3: Availability (%) of atorvastatin and losartan potassium after interaction at 37°C

S. No.	Time (min)	<——atorvastatin——>			losartan
		pH1	pH 7.4	pH 9	pH 4
1	0	394	317	150	0
2	15	843	480	284	386
3	30	877	459	274	361
4	45	1276	478	291	402
5	60	1210	434	276	367
6	75	1517	451	296	4.8
7	90	1573	474	305	423
8	105	1695	464	333	397
9	120	1811	471	257	428
10	135	2207	444	349	430
11	150	2133	468	347	432
12	165	2335	474	381	405
13	180	2315	456	409	418

Concentration of atorvastatin at pH4 and of losartan at pH 1, 7.4 and 9 were zero.

Table 4: Availability (%) of atorvastatin and losartan potassium after interaction at 48°C

S. No.	Time (min)	<—— atorvastatin ——>				losartan
		pH 1	pH 4	pH 7.4	pH9	pH 9
1	0	317	0	257	1.21	1.65
2	15	498	91.8	271	70.4	106
3	30	550	114	241	129	81.7
4	45	749	122	271	137	71.3
5	60	886	136	246	138	75.1
6	75	1035	130	262	152	76.5
7	90	1218	139	246	167	76.6
8	105	1497	150	283	160	77.6
9	120	1508	159	280	155	75.4
10	135	1908	150	268	158	75.4
11	150	1687	139	264	147	69.5
12	165	1673	159	243	158	69.2
13	180	1959	168	243	159	72.7

Concentration of losartan at pH 1, 4 and 7.4 were zero

atorvastatin. At 48^{o}C losartan was available in pH 9 only and at 60^{o}C losartan was available in pH 4 and 9 only. The rate of atorvastatin-losartan charge-transfer complex formation was pH dependent and was less in basic medium as compared to acidic medium.

There are reports on the interaction of losartan with statins. Combined treatment using losartan with pravastatin provided synergistic effects in attenuating inflammatory and fibrotic processes in a rat model of chronic CsA-induced nephropathy, and this effect was independent of their hypolipidemic and hypotensive actions (Li *et al*., 2005). In another study, losartan had synergistic effects with pravastatin (Tsung-Ming *et al*., 2005). Losartan, when coadministered with simvastatin, had additive effects on endothelial function (Koh *et al*., 2004).

CONCLUSION

Interactions of atorvastatin in presence of losartan revealed that the availability of atorvastatin either decreased or increased which reflects its complexation with this drug.

When these drug-drug interactions were carried out at different temperatures and pH, the availability of atorvastatin in presence of losartan was maximum in simulated gastric juice followed by buffer of pH 9 and 7.4 respectively and least in buffer pH 4. The influence of temperature on the energetics of these reactions reveals that complexation increases in the basic medium while decreases in acidic medium with the increase in temperature. From these observations, it is concluded that co-administration of atorvastatin and losartan leads to a formation of charge-transfer complex depleting both the interacting drugs to bind to their respective receptors. Until and unless this complex is broken down *in vivo*, both the drugs will remain unavailable. A considerable time gap between administrations of these drugs may avoid such interactions.

On the basis of these evidences, it is proposed that atorvastatin forms association complex with losartan, through its alcoholic -OH group on the side chain with the tetrazole nitrogen of losartan. Due to the bond formation, the molar absorptivity of atorvastatin is increased which is evident from its %availability. Furthermore due to these bond formations, the π- π* of losartan at the tetrazole ring is disturbed as a result of which the availability of losartan is enormously decreased. This is also due to the fact that electron cloud of losartan is delocalized over a larger area including both the –OH group of atorvastatin which may possibly extend over the carboxylic group to a certain extent. The proposed charge transfer complex is given in figure 6.

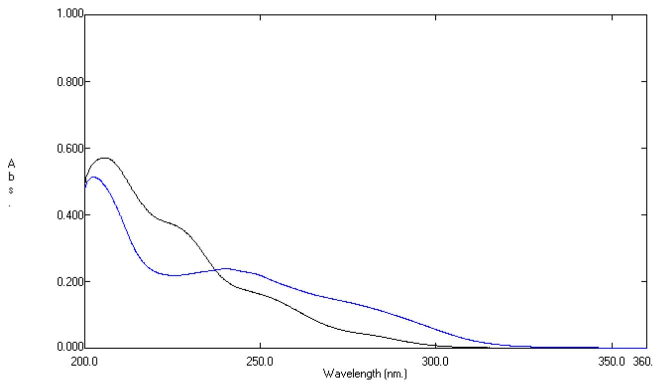

Fig. 7: UV absorption spectrum of atorvastatin and losartan potassium showing interference with each other.

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Table 5: Availability (%) of atorvastatin and losartan potassium after interaction at 60°C

S. No.	Time (min)	<——— atorvastatin ———>			<—losartan—>	
		pH 1	pH 7.4	pH 9	pH 4	pH 9
1	0	0.00	11.7	9.62	416	2.33
2	15	0.00	415	374	360	177
3	30	0.00	492	469	393	120
4	45	176	455	456	368	119
5	60	83.4	527	459	347	120
6	75	49.3	518	471	335	120
7	90	202	550	469	327	120
8	105	430	551	475	319	120
9	120	395	545	472	305	119
10	135	390	557	476	303	120
11	150	626	571	472	298	119
12	165	978	573	470	295	119
13	180	106	587	476	292	120

Concentration of atorvastatin at pH4 and of losartan in pH 1 and 7.4 were zero.

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