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Short communication

In vitro interaction study of retinoic acid isomers with telmisartan and amlodipine by equilibrium dialysis method using UV spectroscopy

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ABSTRACT

The *in vitro* protein binding of retinoic acid isomers (isotretinoin and tretinoin) and the antihypertensive drugs (amlodipine and telmisartan) was studied by equilibrium dialysis method. In this study, free fraction of drugs and the % of binding of drugs in the mixture to bovine serum albumin (BSA) were calculated. The influence of retinoic acid isomers on the % of protein binding of telmisartan and amlodipine at physiological pH (7.4) and temperature (37 ± 0.5 °C) was also evaluated. The *in vitro* displacement interaction study of drugs telmisartan and amlodipine on retinoic acid isomers and also interaction of retinoic acid isomers on telmisartan and amlodipine were carried out.

1. Introduction

Plasma protein binding properties are considered as the primary determinants of the pharmacokinetic properties of drugs. Any physiological condition that causes the alteration in the albumin binding of the drugs might lead to change in the pharmacokinetic and pharmacological properties of the drugs. Drug–drug interactions thus play a vital role in the extent of plasma protein binding and consequently the therapeutic effect of the drugs [1].

Most drugs have multiple pharmacological effects in patients, especially the newer, more complex drugs being marketed. Clinically significant drug interactions can occur when two or more drugs are taken in combination. Some drug interactions do not need to be avoided or reversed because they are unlikely to seriously harm the patient.

The current study has been carried out to understand *in vitro* displacement interaction of drugs telmisartan and amlodipine on retinoic acid isomers and also interaction of retinoic acid isomers on telmisartan and amlodipine. Bovine serum albumin (BSA) and human serum albumin (HSA) have structural similarity [2]. BSA was used in lieu of HSA, because of its low cost and easy availability. The *in vitro* protein binding of retinoic acid isomers and the selected antihypertensive drugs (amlodipine and telmisartan) has been conducted by equilibrium dialysis method. In this study, the free fraction of drugs and the % of binding of drugs in the mixture to BSA were calculated. The influence of retinoic acid isomers (isotretinoin and tretinoin) on the % of protein binding of telmisartan and amlodipine at physiological pH (7.4) and temperature (37 ± 0.5 °C) has been evaluated.

2. Experimental

2.1. Chemicals and reagents

Isotretinoin, and tretinoin reference standards were provided by Systopic Laboratories Pvt. Ltd. (Bangalore, India). Amlodipine, and Telmisartan pure samples were provided by USV Pvt. Ltd. (Himachal Pradesh, India). Bovine serum albumin was procured from Himedia Laboratories Pvt. Ltd. (Mumbai, India). Potassium dihydrogen phosphate (AR grade) was supplied by S.D. Fine Chemicals Ltd. (Mumbai, India). HPLC grade water was prepared by use of a Millipore Milli-Q Academic water purifier (Bangalore, India).

2.2. Instrumentation

The UV–vis spectra were recorded on a Jasco V-530 UV/Vis spectrophotometer equipped with 1.0-cm quartz cells. All weighing was done on a Shimadzu electronic balance (Japan), Model BL-220H.

2.3. Preparation of reagents and solutions

2.3.1. Buffer solution pH 7.4

To 50 ml of potassium dihydrogen phosphate solution was added 39.1 ml of sodium hydroxide solution and finally distilled water was added to produce 200 ml.

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Table 1
Calibration data.

Drugs	Linearity range ($\mu g\,ml^{-1}$)	λ_{max} (nm)
Isotretinoin	1–10	351
Tretinoin	1–10	353
Amlodipine	1–10	228
Telmisartan	5–50	239

2.3.2. Potassium dihydrogen phosphate solution

27.22 g of potassium dihydrogen phosphate was dissolved and diluted with distilled water to produce 1000 ml.

2.3.3. Sodium hydroxide solution 0.2 N

8 g of sodium hydroxide was dissolved and made up to 100 ml with water.

2.3.4. Bovine serum albumin (1×10^{-4} M)

0.165 g of bovine serum albumin was dissolved in 25 ml of buffer solution of pH 7.4 and the volume was made up to 25 ml.

2.3.5. Preparation of standard solutions

5 mg each of isotretinoin, tretinoin, amlodipine, and telmisartan were dissolved separately in 10 ml of methanol and the volume of each flask was made up with phosphate buffer (pH 7.4) to 50 ml.

2.3.6. Preparation of standard curve

For the determination of drug concentrations, different concentrations of the drugs were prepared in phosphate buffer (pH 7.4), absorbances were noted at their respective λ_{max}, and standard calibration curves were plotted for all the four drugs (Table 1).

2.4. Equilibrium dialysis method

Equilibrium dialysis is one of the methods used for the determination of protein binding and this method is used to study the complexation between BSA and the drug. If binding occurs, the drug concentration in the sac containing the protein is greater at equilibrium than the concentration of drug in the vessel outside the sac. At regular intervals, samples were withdrawn and analyzed to obtain the concentration of free and complexed drug.

2.5. Study of protein binding of individual drugs

Protein binding of all the four drugs was determined by equilibrium dialysis method. For this, 25 ml of 1×10^{-4} M concentrations of all the four drug solutions were prepared. 25 ml of 1×10^{-4} M bovine serum albumin (BSA) solution was taken in glass tube attached to semi permeable membrane (Sigma dialysis sacs, 21 mm diameter, and 30 cm length). The dialysis membranes were previously activated by immersing it in warm water for 30 min. These tubes were then immersed in beakers with 25 ml of phosphate buffer containing fixed concentration of drug solutions (1×10^{-4} M). Immediately at zero time, 1 ml of the solution was pipetted out from the beaker and it was replaced with 1 ml of phosphate buffer of pH 7.4. Readings were taken at various time intervals by UV spectrophotometer at respective λ_{max} of all the four drugs till the absorbance values were constant.

2.6. Displacement interaction studies

2.6.1. In vitro interaction between retinoic acid isomers and telmisartan using BSA

2.6.1.1. Study of effect of telmisartan on in vitro protein binding of retinoic acid isomers. To study the effect of telmisartan on *in vitro* protein binding of retinoic acid isomers, 25 ml of 1×10^{-4} M bovine serum albumin solution (BSA) was taken in each of 4 cylindrical glass tubes attached to semi permeable membrane (Sigma dialysis sacs, 21 mm diameter, 30 cm length). The dialysis membranes were previously activated by immersing it in warm water for 30 min. These tubes were then immersed in beakers with 25 ml of phosphate buffer containing fixed concentration of isotretinoin (1×10^{-4} M)/tretinoin (1×10^{-4} M). Telmisartan was added in increasing concentrations into four beakers containing isotretinoin/tretinoin solution to give a final ratio (BSA:isotretinoin/tretinoin:telmisartan, 1:1:1, 1:1:2, 1:1:3, 1:1:4). This system was maintained at room temperature for 6 h. After 6 h, 1 ml of the solutions were withdrawn from the beaker, and diluted to 10 ml using potassium dihydrogen phosphate buffer and the responses of free drugs were measured at a wavelength of 351 nm and 353 nm for isotretinoin and tretinoin, respectively. The concentrations of the drugs were determined from the calibration graph.

2.6.1.2. Study of effect of retinoic acid isomers on in vitro protein binding of telmisartan. To study the effect of retinoic acid isomers on *in vitro* protein binding of telmisartan, 25 ml of 1×10^{-4} M bovine serum albumin solution (BSA) was taken in each of 4 cylindrical glass tubes attached to semi permeable membrane (Sigma dialysis sacs, 21 mm diameter, 30 cm length). The dialysis-membranes were previously activated by immersing it in warm water for 30 min. These tubes were then immersed in beakers with 25 ml of phosphate buffer containing fixed concentrations of telmisartan (1×10^{-4} M). Isotretinoin and tretinoin were added in increasing concentration into four beakers containing telmisartan solutions to give a final ratio (BSA:telmisartan:isotretinoin/tretinoin, 1:1:1, 1:1:2, 1:1:3, 1:1:4). This system was maintained at room temperature for 6 h. After 6 h, 1 ml of the solutions were withdrawn from the beaker, diluted to 10 ml using potassium dihydrogen phosphate buffer and the responses of free drugs were measured at a wavelength of 239 nm. The concentrations of the drugs were determined from the calibration graph.

2.6.2. In vitro interaction between retinoic acid isomers and amlodipine using BSA

2.6.2.1. Study of effect of amlodipine on in vitro protein binding of retinoic acid isomers. To study the effect of amlodipine on *in vitro* protein binding of retinoic acid isomers, 25 ml of 1×10^{-4} M bovine serum albumin solution (BSA) was taken in each of 4 cylindrical glass tubes attached to semi permeable membrane (Sigma dialysis sacs, 21 mm diameter, 30 cm length). The dialysis membranes were previously activated by immersing it in warm water for 30 min. These tubes were then immersed in beakers with 25 ml of phosphate buffer containing fixed concentrations of isotretinoin (1×10^{-4} M)/tretinoin (1×10^{-4} M). Amlodipine was added in increasing concentration into four beakers containing isotretinoin/tretinoin solutions to give a final ratio (BSA:isotretinoin/tretinoin:amlodipine, 1:1:1, 1:1:2, 1:1:3, 1:1:4). This system was maintained at room temperature for 6 h. After 6 h, 1 ml of the solution were withdrawn from the beakers, diluted to 10 ml using potassium dihydrogen phosphate buffer and the responses of free drug were measured at a the selected wavelengths of 351 nm and 353 nm for isotretinoin and tretinoin, respectively. The concentrations of the drugs were determined from the calibration graph.

2.6.2.2. Study of effect of retinoic acid isomers on in vitro protein binding of amlodipine. To study the effect of retinoic acid isomers on *in vitro* protein binding of retinoic acid isomers, 25 ml of 1×10^{-4} M bovine serum albumin solution (BSA) was taken in each of 4 cylindrical glass tubes attached to semi permeable membrane (Sigma

Table 2
Percentage protein binding and % of free concentration of drugs.

Drug	% Protein binding	% Free drug concentration
Isotretinoin	99.1	2.1
Tretinoin	97.7	2.3
Amlodipine	98.8	1.2
Telmisartan	95.4	4.6

dialysis sacs, 21 mm diameter, 30 cm length). The dialysis membranes were previously activated by immersing it in warm water for 30 min. These tubes were then immersed in beakers with 25 ml of phosphate buffer containing fixed concentrations of amlodipine (1×10^{-4} M). Isotretinoin/tretinoin were added in increasing concentrations into four beakers containing amlodipine solution to give a final ratio (BSA:amlodipine:isotretinoin/tretinoin, 1:1:1, 1:1:2, 1:1:3, 1:1:4). This system was maintained at room temperature for 6 h. After 6 h, 1 ml of the solutions were withdrawn from the beaker, diluted to 10 ml using potassium dihydrogen phosphate buffer and the responses of free drug were measured at a selected wavelength of 228 nm. The concentrations of the drugs were determined from the calibration graph.

3. Results

3.1. In vitro protein binding study

The concentration of the drugs was determined from the calibration graph. The % of protein binding and free fraction of individual drugs were calculated and it was found that among the isomers, the % of protein binding of isotretinoin was more than that of tretinoin. Among the antihypertensive drugs, % of protein binding of amlodipine was found to be more than that of telmisartan (Table 2).

3.2. Displacement interaction studies

3.2.1. Effect of telmisartan on in vitro protein binding of isotretinoin and tretinoin

Telmisartan showed more interaction with tretinoin when compared to isotretinoin. The free fraction of tretinoin increased from 2.3% to 4.7% when the ratio of telmisartan to BSA was increased from 1 to 4. For isotretinoin, the % of free fraction increased from 2.1% to 3.7%. Hence, increase in free drug concentration was found more incase of tretinoin due to greater displacement by telmisartan (Table 3 and Fig. 1).

3.2.2. Effect of retinoic acid isomers on in vitro protein binding of telmisartan

In the reverse experiment, the free fraction of telmisartan was more in the presence of tretinoin than isotretinoin. In the presence of tretinoin, the free fraction of telmisartan increased from 4.6% to 5.6% and with isotretinoin, the % free fraction increased from 4.6% to 5.1%. Hence, tretinoin displaces telmisartan more than that of isotretinoin (Table 4 and Fig. 2).

Table 3
Effect of telmisartan on *in vitro* protein binding of retinoic acid isomers.

BSA:ISO/ TRET:telmisartan	% Protein binding		% Free drug concentration	
	ISO	TRET	ISO	TRET
1:1:1	97.7	97.3	2.3	2.7
1:1:2	97.1	96.9	2.8	3.1
1:1:3	96.8	95.9	3.2	4.1
1:1:4	96.3	95.3	3.7	4.7

ISO: isotretinoin; TRET: tretinoin.

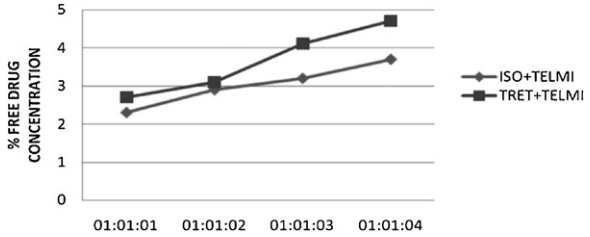

Fig. 1. Effect of telmisartan on *in vitro* protein binding of retinoic acid isomers.

Table 4
Effect of retinoic acid isomers on *in vitro* protein binding of telmisartan.

BSA:TEL:ISO/ TRET	% Protein binding of telmisartan		% Free drug concentration telmisartan	
	With isotretinoin	With tretinoin	With isotretinon	With tretinoin
1:1:1	97	96 8	3	3 2
1:1:2	96 8	96 2	3 2	3 8
1:1:3	95 9	95 2	4 1	4 8
1:1:4	94 9	94 4	5 1	5 6

ISO: isotretinoin; TRET: tretinoin; TEL: telmisartan.

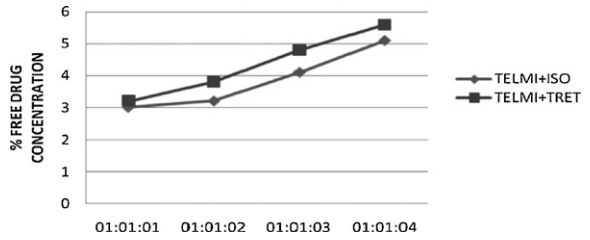

Fig. 2. Effect of retinoic acid isomers on *in vitro* protein binding of telmisartan.

3.2.3. Effect of amlodipine on in vitro protein binding of isotretinoin and tretinoin

It was found that the free drug concentration of tretinoin was more than that of isotretinoin, which shows that amlodipine has a significant interaction with tretinoin when compared to isotretinoin. When the amount of amlodipine was increased, the free fraction of tretinoin increased from 2.3% to 7.3% and for isotretinoin, free fraction increased from 2.1% to 6% (Table 5 and Fig. 3).

3.2.4. Effect of retinoic acid isomers on in vitro protein binding of amlodipine

In the reverse experiment, the free fraction of amlodipine was increased from 1.2% to 7.3% with tretinoin and from 1.2% to 10.2% with isotretinoin. Hence, tretinoin displaces amlodipine more than that of isotretinoin (Table 6 and Fig. 4).

Table 5
Effect of amlodipine on *in vitro* protein binding of retinoic acid isomers.

BSA:ISO/ TRET: AML	% Protein binding		% Free drug concentration	
	ISO	TRET	ISO	TRET
1:1:1	96.8	95.6	3.2	4.4
1:1:2	96.2	94.9	3.8	5.1
1:1:3	95.2	93.6	4.8	6.4
1:1:4	94	92.7	6	7.3

ISO: isotretinoin; TRET: tretinoin; AML: amlodipine.

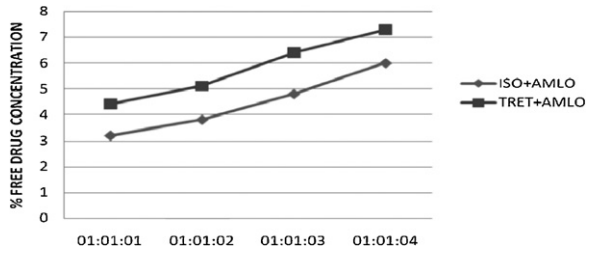

Fig. 3. Effect of amlodipine on *in vitro* protein binding of retinoic acid isomers.

Table 6
Effect of retinoic acid isomers on *in vitro* protein binding of amlodipine.

BSA:AML:ISO/TRET	% Protein binding of amlodipine		% Free drug concentration (amlodipine)	
	With isotretinoin	With tretinoin	With isotretinoin	With tretinoin
1:1:1	95.6	93.6	4.4	6.4
1:1:2	94.9	91.9	5.1	8.1
1:1:3	93.6	91.2	6.4	8.8
1:1:4	92.7	89.8	7.3	10.2

ISO: isotretinoin; TRET: tretinoin; AML: amlodipine.

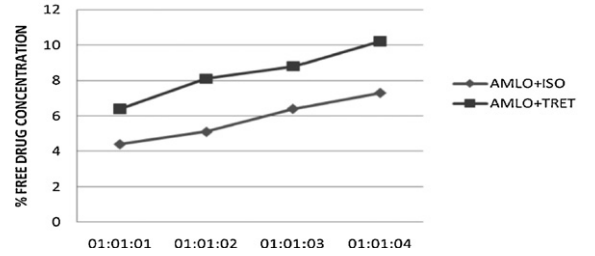

Fig. 4. Effect of retinoic acid isomers on *in vitro* protein binding of amlodipine.

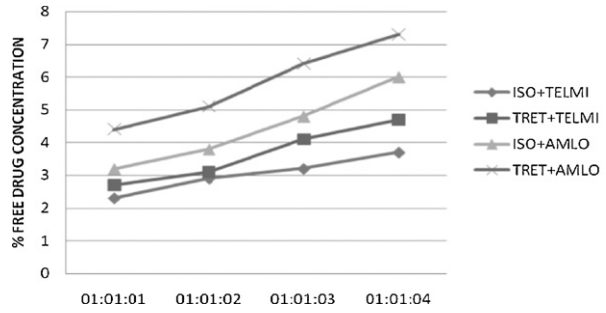

Fig. 5. Effect of telmisartan and amlodipine on *in vitro* protein binding of retinoic acid isomers.

4. Discussion

4.1. Effect of telmisartan and amlodipine on in vitro protein binding of retinoic acid isomers

Comparison of the effect of antihypertensive drugs (telmisartan and amlodipine) on *in vitro* protein binding of retinoic acid isomers was carried out (Fig. 5). In the presence of amlodipine, the free drug concentration of isotretinoin increased from 2.1% to 6% and free drug concentration of tretinoin increased from 2.3% to 7.3%. In the presence of amlodipine, the free drug concentration of tretinoin was 1% higher than that of isotretinoin. Similar effects were seen for a combination of isotretinoin + telmisartan and tretinoin + telmisartan. In the presence of telmisartan, free drug concentration of isotretinoin increased from 2.1% to 3.7% and for tretinoin, free drug concentration increased from 2. 3% to 4.7%. In the presence of telmisartan, the free drug concentration of tretinoin was 1.2% higher than that of isotretinoin. It is evident that major side effects of tretinoin are teratogenicity, pseudotumour cerebri, peripheral edema, photosensitivity etc. Therefore, from the study it may be concluded that when concomitant therapy of any one of the retinoic acid isomer is advised along with telmisartan or amlodipine, a combination of antihypertensive drug with isotretinoin is more preferred than tretinoin.

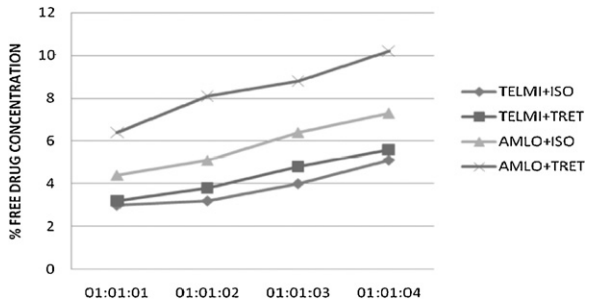

Fig. 6. Effect of retinoic acid isomers on *in vitro* protein binding of amlodipine and telmisartan.

4.2. Effect of retinoic acid isomers on in vitro protein binding of amlodipine and telmisartan

Here a comparison of effect of isomers on the free drug concentration of amlodipine and telmisartan were studied (Fig. 6). The study showed that the free drug concentration amlodipine increased from 1.2% to 7.3% in the presence of isotretinoin and 1.2–10.2% in the presence of tretinoin. The free drug concentration of telmisartan increased from 4.6% to 5.1% and 4.6% to 5.6% in the presence of isotretinoin and tretinoin, respectively. From this data it is evident that the interaction between amlodipine and tretinoin or isotretinoin is predominant when compared to that of telmisartan.

5. Conclusion

It may be concluded that telmisartan is the preferred choice among the antihypertensive drugs studied for concomitant therapy with retinoic acid isomers if advised. Among the isomers, isotretinoin may be the preferred over tretinoin.

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