3. Wakelin, L.P.G., **Med. Res. Rev.,** 1986, 28, 1568.
4. Denny, W.A. **Anticancer Drug Des.,** 1989, 4, 241.
5. Esnault, C., Roques, P.B., Sablon, J.A. and Le Pecq, J.B., **Cancer Res.,** 1984, 44, 4355.
6. Gamage, S.A., Spicer, J.A., Atwell, G.J., Finlay, G.J., Baguley, B.C. and Denny, W.A., **J. Med. Chem.,** 1999, 42, 2383.
7. Srikanth, K., Kumar, A., Goswami, D., De, A.U. and Jha, T., **Indian J. Biochem. Biophy.,** 2001, 38, 120.
8. Roy, K., Pal, D.K., Ghosh, C.C., De, A.U. and Sengupta, C., **Indian J. Chem.,** 2001, 40B, 209.
9. Shah, M.D., Desai, N.C., Awasthi, K.K. and Saxena, A.K., **Indian J. Chem.,** 2001, 40B, 201.

Intestinal Permeation Mediated Absorption Interactions between Atenolol and Furosemide

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Peroral multi-drug administration is often associated with severe drug-drug interactions. These interactions can occur either in the gut lumen, at absorptive site or after absorption. Present work was undertaken to study the interaction between atenolol and furosemide at the absorption site. Alteration in the intestinal permeability was studied using rat single-pass intestinal perfusion technique, to assess the changes on administration of single and combination drugs. Permeability coefficient (P_{eff}) of atenolol reduced to a statistically significant level ($P<0.05$) when co-perfused with furosemide, with P_{eff} values (x10^{-4} cm/sec) of 0.0686±0.0433 and 0.0154±0.0326 for drug perfused individually and in combination, respectively, indicating the possibility of drug-drug interaction occurring at the absorption site.

Oral route of drug administration is the most preferred one, both by physicians and patients, due to ease of delivery and better patient compliance[1], and the treatment may be accomplished using single drug or a combination of two or more drugs. The latter may be either fixed dose combinations or concomitant administration of individual dosage units. This multi-drug administration can be a potential cause for a variety of drug-drug interactions, the consequence of which can be significant, especially in the case of narrow therapeutic index drugs. Most of the drug interactions have been studied at the pharmacokinetic and pharmacodynamic levels[2-4]. However, drug-drug interactions may also occur in the (i) pre-absorption stage due to changes in stability, dissolution characteristics, and/or (ii) absorption stage due to altered intestinal permeability induced by biochemical or physiological changes[4], leading to altered bioavailability.

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The present work aims to identify absorption interactions between atenolol (ATN) and furosemide (FRD) using *in situ* rat single-pass intestinal perfusion (SPIP) model. The combination of ATN, a β blocker, and FRD, a diuretic, was selected as it is one of the most widely prescribed combinations in cardiovascular disorders. Both these drugs are poorly permeable from gastrointestinal tract[5], and hence, their oral absorption would be more critically affected by any changes in the intestinal permeability. The permeability coefficient (P_{eff}) values of ATN and FRD, when perfused alone and in combination, were calculated after suitable correction for water flux, using gravimetric method after density correction for exiting intestinal perfusate.

The permission for the present study was obtained from Institutional Animal Ethics Committee (approval number IAEC-01-079). The composition of perfusion solution was similar to that already reported[6], with drug concentrations being 0.83 mM (for ATN) and 0.2 mM (for FRD), both in single and combination drug study[7]. The pH and osmolality of perfusion solution was maintained at 6.5±0.02 and 290±10 mOsmol/kg, respectively. The methodology followed was similar to that already reported[6], with the following salient features: intestinal portion used – jejunum; flow rate of drug perfusion solution – 0.2 ml/min using peristaltic pump (P-1, Pharmacia Biotech, Kwai Chung NT, Hong Kong); frequency of sample collection – 15 min intervals for duration of 90 min in preweighed 5 ml glass vials. Length of intestinal segment studied was measured at the end of each experiment, and its radius was taken to be 0.18 cm[8]. The density of collected samples was determined using calibrated autopipette (Finnpipette, Labsystems, Helsinki, Finland) and electronic weighing balance (AG285, Mettler Toledo, Greifensee, Switzerland). All the samples were analyzed for drug content using validated HPLC method of analysis. The HPLC system (Shimadzu Corporation, Kyoto, Japan) consisted of SCL-10A system controller, LC-10AT pump, FCV-10AL flow control valve, DGU-14A degasser, SPD-10A UV/Vis detector, SIL-10AD autoinjector, CTO-10AS column oven, with Class VP software. The HPLC method of analysis comprised of Lichrospher 100 RP-18e column, 250x4, 5 μm (Merck, Darmstadt, Germany); acetonitrile:50 mM pH 6.5 phosphate buffer (20:80) as mobile phase at a flow rate of 0.7 ml/min; detection wavelength of 276 nm for both ATN and FRD; and an injection volume of 50 μl. Phenol red was detected at 434 nm using acetonitrile:20 mM pH 6.5 phosphate buffer (20:80) as mobile phase at a flow rate of 0.8 ml/min, and an injection volume of 20 μl.

The P_{eff} values for drugs were calculated from steady-state concentrations in the exiting intestinal perfusate, as determined by steady-state recovery of phenol red (30 min after the start of experiment), using parallel tube model[8], after applying suitable correction for water flux by gravimetric method after density correction for exiting intestinal perfusate. The latter involves determination of density of the "entering" and 'exiting" intestinal perfusate, and applying that to calculate the water flux. This differs from the currently prevalent practice of assuming the density of "entering" and "exiting" intestinal perfusate as 1.0 g/ml[9]. The "density corrected" gravimetric method was found to be more accurate for studying subtle changes in intestinal permeability of the drugs (unpublished data). $P_{eff}=[-Q_{in}.\ln(C_{out}/C_{in})]/A$, where, Q_{in} is the flow rate (ml/min) of entering perfusion solution, C_{in} and C_{out} are the drug concentrations (μg/ml) in entering and exiting perfusion solutions, and A is the surface area (cm^2) of the intestinal segment.

The HPLC methods of drug analysis passed all the validation parameters studied. For ATN, linearity was established in the range of 60-300 μg/ml (r^2=0.9984), method being 101.5 % accurate with 0.12 % RSD for precision. For FRD, linearity was established in the range of 20-100 μg/ml (r^2=0.9991), method being 97.9 % accurate with 0.30 % RSD for precision. For drugs in combination, linearity was established with r^2=0.9995 and 0.9997, accuracy being 102.3 % and 100.1 %, and 0.39 % RSD and 0.45 % RSD for precision for ATN and FRD, respectively. For phenol red, linearity was established in the range of 15-75 μg/ml (r^2=0.9998), method being 102.0 % accurate with 0.39 % RSD for precision.

The solution state stability for combination of ATN and FRD at a concentration of 0.83 mM (221.1 μg/ml) and 0.2 mM (66.2 μg/ml), respectively, in the perfusate at 37° for 3 h was confirmed with the recovery values of 100 % for ATN and 100.6 % for FRD, at the end of the experiment.

The P_{eff} values of ATN and FRD, when perfused alone and in combination, are presented in fig. 1. The mean P_{eff} ($x10^{-4}$ cm/sec) of ATN, when perfused alone and in combination with FRD, was found to be 0.0686±0.0433 and 0.0154±0.0326 (statistically significant, $P<0.05$ in student's

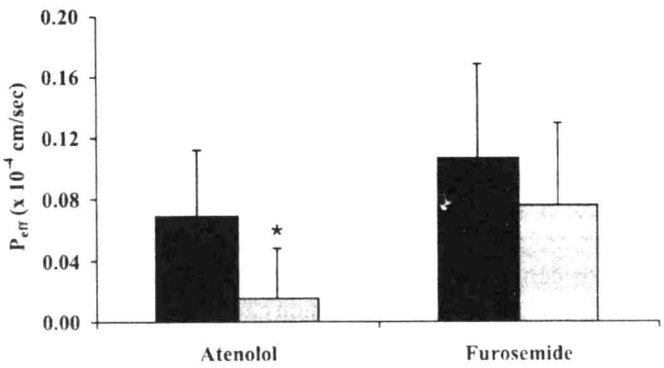

Fig. 1: Permeability coefficients of drugs perfused through jejunum.

Permeability coefficients of ATN and FRD when perfused alone (■) and in combination (▨). Data is expressed as mean±S.D. for drugs perfused alone (n=8) and in combination (n=6). Statistically significant difference in P_{eff} was observed at $P<0.05$.

t-test), respectively, and that of FRD, when perfused alone and in combination with ATN was 0.1060±0.0620 and 0.0758±0.0531 (statistically insignificant, P>0.05 in student's t-test), respectively.

The possible contribution of (i) dissolution and/or (ii) stability, towards altered intestinal permeability was excluded by (i) using drugs in solubilized form and (ii) by performing stability studies, respectively. FRD is absorbed by paracellular transport[10] and is reported to be a substrate for intestinal efflux transporter systems[11]. However, the contribution of well characterized efflux transporters, i.e. P-glycoprotein, and multidrug-associated proteins – MRP1 and MRP2 could not be established for FRD[11]. The P_{eff} of FRD is not affected by co-administration of ATN, indicating the latter's inertness towards carrier-mediated efflux processes involved in the transport of FRD. An earlier study had reported inhibitory effect of ATN on efflux of celiprolol[12], thus indicating towards different efflux transporters for FRD and celiprolol. ATN is a hydrophilic molecule, passively absorbed by paracellular pathway, and is used as a marker for paracellular transport[10,13]. A significant reduction in the P_{eff} of ATN during co-administration with FRD points towards interaction at the paracellular route, as both the drugs are primarily absorbed through this route. This study established the usefulness of rat SPIP technique as a quick tool for studying absorption level interactions between concomitantly administered drugs. However, it does not deemphasize the potential of *in vivo* studies in establishing clinically significant drug-drug and drug-food interactions.

REFERENCES

1. Franklin, M.R., In; Gennaro, A.R., Eds., Remington: The Science and Practice of Pharmacy, 19th Edn., Vol. I, Mack Publishing Company, Easton, PA, 1995, 697.
2. Welling, P.G., **Clin. Pharmacokinet.**, 1984, 9, 404.
3. Mehra, B.K., Eds., In; Drug Interactions, Basic and Business Publications, Mumbai, India, 1982, 41.
4. Fleisher, D., Li, C., Zhou, Y., Pao, L.H. and Karim, A., **Clin. Pharmacokinet.**, 1999, 36, 233.
5. Lobenberg, R. and Amidon, G.L., **Eur. J. Pharm. Sci.**, 2000, 50, 3.
6. Fagerholm, U., Johansson, M. and Lennernas, H., **Pharm. Res.**, 1996, 13, 1336.
7. Fagerholm, U., Lindahl, A. and Lennernas, H., **J. Pharm. Pharmacol.**, 1997, 49, 687.
8. Komiya, I., Park, J.Y., Kamani, A., Ho, N.F.H. and Higuchi, W.I., **Int. J. Pharm.** 1980, 4, 249.
9. Sutton, S.C. and Rinaldi, M.T S., **AAPS Pharm. Sci.** 2001, 3, 1.
10. Hilgendorf, C., Spahn-Langguth, H., Regardh, C.G., Lipka, E., Amidon, G.L. and Langguth, P., **J. Pharm. Sci.**, 2000, 89, 63.
11. Flanagan, S., Cummins, C., Susanto, M., Liu, X., Takahashi, L. and Benet, L., **Pharmacology**, 2002, 64, 126.
12. Karlsson, J., Kuo, S., Ziemniak, J. and Artursson, P., **Brit. J. Pharmacol.**, 1993, 110, 1009.
13. Collett, A., Sims, E., Walker, D., He, Y., Ayrton, J., Rowland, M. and Warhurst, G., **Pharm. Res.** 1996, 13, 216.