Effect of DLH-6 (Cystone), a Herbomineral Formulation, on the Bioavailability of Sulfamethoxazole and Trimethoprim in Rabbits

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ABSTRACT
In the present study, DLH-6 (Cystone), a herbomineral formulation that has proven activity against various urinary disorders, was investigated for its pharmacokinetic interaction with cotrimoxazole, a well-known synergistic combination of sulfamethoxazole and trimethoprim, which is commonly used for acute urinary tract infections. Sixteen rabbits were divided into 2 groups of eight animals each. Animals in the Group I were treated with cotrimoxazole (200 mg/kg body weight, p.o.) and Group II received cotrimoxazole (200 mg/kg body weight, p.o.) along with DLH-6 (Cystone) (500 mg/kg body weight, p.o.). The concentration of cotrimoxazole in plasma was estimated by high performance thin layer chromatography at 1, 2, 4, 8, 12 and 24 h after the respective drug administration. The results of the present study revealed that DLH-6 (Cystone) delayed the absorption, but significantly increased the bio-availability of sulfamethoxazole and hastened the absorption of trimethoprim without significant alteration in the bio-availability.

Key Words: DLH-6 (Cystone), Cotrimoxazole, Sulfamethoxazole, Trimethoprim, Pharmacokinetic interaction, Bio-availability.

INTRODUCTION
Sulfamethoxazole is commonly used to treat uncomplicated urinary tract infections, particularly those caused by Escherichia coli. Trimethoprim is used in the treatment, as well as prophylaxis, of urinary tract infections. Cotrimoxazole, the well-advised synergistic combination of sulfamethoxazole and trimethoprim, is preferred over the use of either drug alone, due to the increasing incidence of resistant organisms.

The bioavailability of sulfamethoxazole and trimethoprim, are 81% and 41% respectively, on oral administration. The plasma half-life of these drugs is 6 to 12 h and 8 to 11 h respectively. About 65% of sulfamethoxazole and 45% of trimethoprim are bound to plasma proteins. 15% of the total sulfamethoxazole becomes an inactive N₄-metabolite, and 80% of it is excreted through the kidney. About 10 to 20% of trimethoprim is metabolised in liver and most of the drug is excreted unchanged in the urine.

Earlier interaction studies of cotrimoxazole with other drugs like isoniazid, glipizide and glibenclamide showed no pharmacokinetic interactions. However, it displayed significant increase in plasma concentrations of mercaptopurine, lamivudine, rifampicin and methotrexate. Like other sulfonamides, sulfamethoxazole potentiates the actions of some drugs such as phenytoin, methotrexate and warfarin. Intravenous sulfamethoxazole decreases the plasma concentration of
cyclosporin. There is also a possibility of interactions with other highly protein bound drugs such as NSAIDs\textsuperscript{1}. Trimethoprim potentiates the actions of phenytoin, digoxin, procainamide, warfarin, zidovudine and lamivudine, by increasing their serum concentration. However rifampicin may decrease the plasma concentration of trimethoprim\textsuperscript{2}.

DLH-6 (Cystone) is a herbomineral formulation which contains extract of Didymocarpus pedicellata, Saxifraga ligulata, Rubia cordifolia, Cyperus scariosus, Achyranthes aspera, Onosma bracteatum and Veronica cinerea; and powder of Shilajeet and Hajrul yahood bhasma.

DLH-6 (Cystone) is an Ayurvedic formulation indicated to treat various urinary tract problems\textsuperscript{8}. It is used in urolithiasis\textsuperscript{9} due to oxalate stones, phosphate stones, uric acid and urate calculi\textsuperscript{10}, crystalluria\textsuperscript{11}. It is used as an adjuvant in urinary tract infections during pregnancy\textsuperscript{12}, nonspecific urethritis, cystitis, pyelitis, burning micturition, arthritic condition and gout. It is also used as a diuretic. It is effective against urgency incontinence of urine in women and kidney dysfunction secondary to nephritis\textsuperscript{13}. In a previous clinical study it was reported that DLH-6 (Cystone), when given along with other antibiotics to treat urinary tract infections, increased the effectiveness of the antibiotics and reduced the duration and cost of therapy\textsuperscript{14}.

Due to its diuretic action, the infective organisms that act as the nucleus for calculi formation are flushed away along with the crystals from urine. DLH-6 (Cystone) is not only effective on its own, but also has a synergistic action when used along with other antibiotics\textsuperscript{14}. In an earlier study, it was found that DLH-6 (Cystone) increased the bioavailability of norfloxacin significantly. This encouraged us to study whether DLH-6 (Cystone) has pharmacokinetic interaction with other antibiotics as well.

**EXPERIMENTAL**

**Experimental animals:**
Sixteen adult white strain male rabbits of New Zealand weighing between 1.6-2.0 kg were selected for the study. The animals were maintained in an air-conditioned room at 22 ± 2°C and 50-55% relative humidity. The animals were exposed to a 12 h light-dark cycle, fed ad libitum along with commercial pellet diet (Lipton India Ltd., Mumbai) and had free access to water. All the animals received human care according to the criteria outlined in the “Guide for the care and use of laboratory animals” prepared by the National Academy of Sciences and published by the National Institute of Health.

The animals were divided into two groups of eight animals each. A single oral dose of cotrimoxazole (containing sulfamethoxazole and trimethoprim in the ratio 4:1) at a combined dose of 200 mg/kg body weight, p.o., was used for this study. Group I received cotrimoxazole at a dose of 200 mg/kg body weight, p.o.; Group II received cotrimoxazole at a dose of 200mg/kg body weight, p.o. along with DLH-6 (Cystone) (500 mg/kg body weight, p.o.). Blood was collected from the marginal ear vein at 1, 2, 4, 12 and 24 h after drug administration. The plasma samples were separated for the analysis of sulfamethoxazole and trimethoprim.
Combined estimation of Sulfamethoxazole and Trimethoprim from plasma by HPTLC:
High performance thin layer chromatography (HPTLC) equipped with application mode (CAMAG Linomat iv), computer controlled HPTLC scanner and data station with “CAMAG CATS” software program for integration were used in this study.

The Erdman et al. method was followed for the single step extraction of sulfamethoxazole and trimethoprim in combination from the plasma with minor modifications. The Yun Keming & Li Guiming method was adopted for the TLC separation and detection of sulfamethoxazole and trimethoprim. 1.0 ml of a mixture of dichloromethane and methanol in the ratio of 75:25 was added to 1 ml of plasma and vortexed for 5 min. The mixture was centrifuged for 20 min at 7500 rpm and the organic layer was separated. The solvent was evaporated completely and the residue was dissolved in 0.2 ml of ethyl acetate. The solution (50 µl) was later applied on precoated silica gel plates and developed in the solvent system; Chloroform: Methanol: Diethylamine (45:5:1). The developed plate was scanned in UV mode at two different wavelengths; 265 nm and 350 nm, for sulfamethoxazole and trimethoprim respectively. The detection limit was about 1.0 µg for both the drugs. The recovery of sulfamethoxazole and trimethoprim were 95.2 ± 3.4% (n=8) and 96.9 ± 2.8% (n=8), respectively.

RESULTS
Table I shows the mean plasma concentration of sulfamethoxazole with and without DLH-6 (Cystone) treatment along with cotrimoxazole. After the single oral dose of cotrimoxazole, the plasma level of sulfamethoxazole at the first hour was significantly reduced followed by an increase at all time points.

As shown in Table II, the pharmacokinetic data of sulfamethoxazole with and without DLH-6 (Cystone) treatment results in an increase in AUC_{mean} of sulfamethoxazole when co-administered with DLH-6 (Cystone). However there was no change in C_{max} and T_{max} between the treated and control groups. Even though the cotrimoxazole concentration was decreased significantly in the DLH-6 (Cystone)-treated group at the first hour, later, at all the time points there was an increase in sulfamethoxazole level between the control and treated groups, which was most significant in the 8^{th} hour.

<table>
<thead>
<tr>
<th>Table I: Plasma sulfamethoxazole levels (µg/ml) at different time intervals with and without DLH-6 (Cystone) treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>Cotrimoxazole (200 mg/kg body weight)</td>
</tr>
<tr>
<td>Cotrimoxazole (200 mg/kg body weight) + DLH-6 (Cystone) (500 mg/kg body weight)</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM; (n=8). *p<0.05 as compared to cotrimoxazole alone.

<table>
<thead>
<tr>
<th>Table II: Pharmacokinetics of sulfamethoxazole after a single dose of cotrimoxazole (200 mg/kg) with and without DLH-6 (Cystone) (500 mg/kg)</th>
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</thead>
<tbody>
<tr>
<td>Parameters</td>
</tr>
<tr>
<td>C_{max} (µg/ml)</td>
</tr>
<tr>
<td>T_{max} (h)</td>
</tr>
<tr>
<td>AUC_{mean} (µg/ml/hr)</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM; (n=8). *p< 0.05 as compared to cotrimoxazole alone.
Table III shows the mean plasma concentration of trimethoprim with and without DLH-6 (Cystone) treatment along with cotrimoxazole. After a single oral dose of cotrimoxazole the plasma level of trimethoprim at first hour has been increased significantly, but there was no significant change in the bioavailability.

Table IV shows the pharmacokinetic data of trimethoprim with and without DLH-6 (Cystone) treatment. There was no significant change in $C_{\text{max}}$, $T_{\text{max}}$, $T_{1/2}$ and $AUC_{\text{mean}}$ between the control and treated groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma trimethoprim level ($\mu$g/ml)</th>
<th>Cotrimoxazole (200 mg/kg body weight)</th>
<th>Cotrimoxazole (200 mg/kg body weight + DLH-6 (Cystone) (500 mg/kg body weight))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>2 h</td>
<td>4 h</td>
</tr>
<tr>
<td>Cotrimoxazole (200 mg/kg body weight)</td>
<td>7.65 ± 0.6</td>
<td>9.49 ± 1.1</td>
<td>10.34 ± 0.46</td>
</tr>
<tr>
<td>Cotrimoxazole (200 mg/kg body weight + DLH-6 (Cystone) (500 mg/kg body weight))</td>
<td>10.79 ± 0.47*</td>
<td>11.19 ± 0.90</td>
<td>11.51 ± 0.85</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM; (n=8). *$p<0.05$ as compared to cotrimoxazole alone.

DISCUSSION AND CONCLUSION

In Figure 1, a change was seen in the absorption phase, but the elimination phase was not much altered with the DLH-6 (Cystone) treatment. Though the plasma concentration of sulfamethoxazole in the treated group was significantly lesser than that of the control group at the first hour a marked rise in the level of sulfamethoxazole was observed later. At the 4th hour $C_{\text{max}}$ markedly increased with the DLH-6 (Cystone) co-administration as compared to cotrimoxazole alone, but was not significant due to the higher SEM value (Table I). No significant change was seen in $C_{\text{max}}$, $T_{\text{max}}$, and $T_{1/2}$ but the overall $AUC_{\text{mean}}$ increased significantly. Thus, it shows that the absorption of sulfamethoxazole has been delayed, but the bioavailability of the same has been increased with the combined administration of DLH-6 (Cystone) and cotrimoxazole.

In Figure 2, a change in the absorption phase was seen, but the elimination phase was not altered much with DLH-6 (Cystone) treatment. Though the absorption of trimethoprim in the treated group was significantly greater than that of the control group at the first hour, no significant change in the
level of trimethoprim between the two groups was seen. Thus, the absorption of trimethoprim was hastened by the co-administration of DLH-6 (Cystone) with cotrimoxazole.

Thus, it can be concluded that, the co-administration of DLH-6 (Cystone) along with cotrimoxazole results in a beneficial interaction. In the first hour the maximum activity of trimethoprim and in later hours the maximum activity of sulfamethoxazole, along with the activity of DLH-6 (Cystone) could result in a better therapeutic effect on those individuals who are taking this combination therapy. However, clinical studies are warranted to conform the same.

REFERENCES

