In-vitro red blood cell partitioning of doxycycline

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ABSTRACT

Objective: In-vitro red blood cell (RBC) partitioning of doxycycline was studied to determine whether doxycycline penetrates RBC and its concentration was assayed keeping in view its high lipophilicity.

Materials and Methods: Standardization of doxycycline was performed in whole blood and plasma of cattle by microbiological assay using Bacillus subtilis ATCC 6633 as indicator organism. Actual concentration of the drug was obtained by comparing zone inhibition with standard graph and the extent of partitioning was mathematically calculated.

Results: The R^2 value of standard graph for doxycycline was 0.9934 and 0.9727 for plasma and whole blood, respectively. Overall, RBC partitioning of doxycycline was found to be 18.40 ± 1.70%.

Conclusions: Overall RBC partitioning of doxycycline indicated low penetration into RBC. Plasma is the fluid suggested for pharmacokinetic evaluation of doxycycline.

KEY WORDS: Doxycycline, partitioning, red blood cell
of inhibition were measured using zone reader scale (Himedia Ltd.) and the mean zone size was recorded. Standard graph of concentration (X-axis) versus mean zone size (diameter in mm - Y-axis) was plotted for whole blood and plasma [Figure 1].

Red blood cell partitioning

To study RBC partitioning, serial dilutions (10, 5, 2.5 µg/ml) of doxycycline were performed in whole blood (6 ml) of which PCV was measured after collection and incubated for 24 hours at 37°C allowing sufficient time for drug to penetrate RBCs. After incubation, plasma and RBCs were separated by centrifugation of four ml whole blood at 1200 rpm for 10 minutes. About 100 µl of remaining whole blood (after incubation), plasma, RBC pack and standard dilutions (to ensure performance of the set-up) each were added in punched wells in triplicates and a zone of inhibition was observed after 12 hours. The concentration of drug was estimated with the help of a standard graph and average from triplicate was drawn. The procedure was repeated three times to eliminate errors.

Extent of partitioning

Concentration estimated with the help of standard graph was considered as observed and based on which concentration in plasma and RBC was calculated using its PCV. Percentage of RBC penetration was calculated based upon this value.

\[
\text{Observed concentration in RBC} = \frac{\text{Observed concentration}}{100} \times \text{RBC} \times \text{PCV} \%
\]

\[
\text{Calculated concentration in Plasma} = \frac{\text{Observed concentration}}{100} \times \text{Plasma} \times \text{Plasma} \%
\]

Further extent of partitioning of doxycycline was calculated by using following formula: [8]

\[
K_{e/p} = \frac{\text{Concentration of Doxycycline in RBC}}{\text{Concentration of Doxycycline in plasma}}
\]

\[
K_{b/p} = \frac{\text{Concentration of Doxycycline in Whole Blood}}{\text{Concentration of Doxycycline in plasma}}
\]

Where, \(K_{e/p}\): Erythrocyte to plasma concentration ratio; \(K_{b/p}\): Whole blood-to-plasma concentration ratio

Results

R² value of standard graph for doxycycline was 0.9934 and 0.9727 for plasma and whole blood, respectively. PCV of whole blood was 43%. Microbiological assay plate showing zones of inhibition for whole blood, plasma and RBC pack is shown in Figure 2. Overall, RBC partitioning of doxycycline was found to be 18.40 ± 1.70% indicating moderate penetration into RBC. In-vitro concentrations of doxycycline (µg/ml) in whole blood, plasma and RBCs following addition of different known concentration are depicted in Table 1. \(K_{e/p}\), \(K_{b/p}\) values and RBC partitioning of doxycycline at different known concentrations is depicted in Table 2.

Discussion

Doxycycline is a drug having high lipophilicity and also high plasma protein binding. So in such a contrasting situation it would be interesting to study what course doxycycline will follow in-vitro so as to predict its behavior in-vivo.
This study was done with the hypothesis that doxycycline might be entering RBCs owing to its high lipophilicity and may help in arresting the development of intraerythrocytic stages of protozoan parasite, so as to eliminate infected stages of protozoa in RBCs responsible for the spread of disease to healthy population via different intermediate hosts.

Doxycycline is reported to have 92.3 ± 0.8% of protein binding. Other tetracycline have no comparable plasma protein binding (Oxytetracycline - 18 to 22%, Chlortetracycline - 47 to 51% and Tetracycline - 31 to 41%)[10] and lipophilicity as doxycycline; therefore RBC partitioning, although low or moderate, cannot be compared with other co group members. However, most of the concentration remains in plasma, hence; the study suggests that plasma is the biological fluid to be collected for assay of the drug. However, in case of drugs with high plasma protein binding, consideration should be given to penetration into RBC as this may alter in-vivo behavior of drug. It was reported that for drugs with $K_{o,p}$ or $K_{b,p}$ larger than two in human subjects, measuring the concentration in whole blood or erythrocyte rather than plasma increases the sensitivity of an assay.[1] Considering this, 18.40 ± 1.70% partitioning obtained in the study is low with respect to its $K_{o,p}$ and $K_{b,p}$ values.

Further, RBC partitioning depends upon factors such as chemical nature of the drug, temperature, pH etc. RBCs may metabolize some of the drugs with the help of the enzymes present in it.[1] When considering assaying concentrations of drugs in whole blood, possible degradation by enzymes located in the RBCs must be excluded.[1] However, it was difficult to trace any reference whether doxycycline is metabolized by RBCs or not. The study revealed penetration into RBC but the method of uptake into RBC is yet to be explored. Further research may throw light on this aspect of the RBC. Hinderling reported that most of the drugs enter RBC by passive diffusion but lipophilicity was the single most important factor determining the extent of partitioning.

In future, in-vitro erythrocytic protozan culture can be prepared and the effect of treatment of various anti-haemoprotozoan drugs on erythrocytic stages could be revealed. At the same time various drugs having anti-haemoprotozoan activity can be assayed with such culture especially antimalarial drugs. Also, species variation in RBC partitioning of doxycycline can be assayed so as to use it in treatment against erythrocytic stages of haemoprotozoan infections. This will help consider RBC as a compartment in studying kinetic behavior of the drug.

This study confirms the use of plasma as a milieu for pharmacokinetic analysis of doxycycline and focuses on further need of research in RBC partitioning so as to reaffirm pharmacokinetic calculations.

References

Annexure

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Calculated concentration in Plasma = \[
\frac{\text{Observed concentration in plasma} \times \text{Plasma %}}{100}\]

Calculated concentration in RBC = \[
\frac{\text{Observed concentration in plasma} \times \text{PCV %}}{100}\]