Bioavailability and In-vivo Transdermal Delivery of Haloperidol
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Abstract:
Background: Sustained blood level with effective therapeutic blood level in psychotic patients in the range of usual therapeutic dose is favorable.
Objectives: To investigate where this sustained and effective therapeutic blood level and improve in bioavailability could be achieved by using haloperidol/transdermal gel formulation.
Materials and Methods: In-vivo transdermal delivery of haloperidol was studied in rabbits comparing transdermal gel formulation containing 1, 8-cineole as penetration enhancer and oral tablet. Concentrations of haloperidol in plasma were measured by reverse phase HPLC. The pharmacokinetic parameters generated from this study were evaluated statistically using one-way analysis of variance (ANOVA).
Result: The results showed that transdermal gel formulation increased rate and extent of absorption and improve bioavailability of haloperidol. The plasma concentrations of haloperidol were declined in biexponential fashion where the area under the curves and absorption rate $C_{\text{max}}$/AUC elimination rate constant $K_{\text{el}}$, $T_{\text{max}}$, mean residence time (MRT), mean absorption time (MAT), and total clearance (CL_{total}) were significantly different $p < 0.05$, but volume of distribution ($V_d$) did not differ significantly $p >0.05$. The absolute bioavailability from the oral tablet, and the transdermal formulation was 38% and 57% respectively and highly significant $P < 0.01$.
Conclusion: This study suggest that it is possible to achieve significant sustained therapeutic blood levels for longer time and also suggest that further human investigations of the transdermal dosage are warranted.
Key words: Haloperidol/Transdermal gel formulation, Oral tablet, Rabbits, Bioavailability, Pharmacokinetic.

Haloperidol is a neuroleptic butyrophenone antipsychotic used in the treatment of schizophrenia acute and chronic psychotic syndromes, hyperactive conditions in children, abnormal movements and confused states, aggressive behavior in elderly, hyperactivity, psychomotor agitation and restlessness. The drug has also been used for schizoaffective disorders, mania, paranoid, and tourettes syndrome\(^1\). This drug is rapidly and almost completely absorbed when taken orally, (60-70%). But the oral bioavailability is about 59% due to extensive first-pass metabolism in liver\(^2,3\).

Transdermal delivery of drug remains an important alternative route for drug administration to overcome its poor oral bioavailability. It maintains a sustained blood levels for longer period of time. Over the last years transdermal preparations have been developed to be administered via this route. Success has been achieved in the administration of some drugs\(^4-7\). The rate of release of drug from these transdermal delivery systems has determined the successfullness of the performance of the dosage form. The purpose of this study is to assess the pharmacokinetic characteristic of haloperidol delivered transdermally via a transdermal gel matrix formulation containing enhancer in vivo using rabbits and compare that with a commercial oral tablet and intravenous injection of haloperidol.

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Materials and Methods

Materials
Haloperidol and hydroxypropylmethyl cellulose (Sigma Chem. Co., St. Louis, MO, U. S. A.), propylene glycol, 1,8 cineole, glacial acetic acid, acetonitrile and methanol (BDH Chemicals Ltd., Poole, UK), Sodium hydroxide (E. Merck AG, Dermstadt, Germany), (Fluka Ag, Buchs SG, Switzerland), Potassium dihydrogen phosphate, disodium hydrogen phosphate, phosphoric acid, ethanol and triethanolamine (Riedel-De-Haen AG, Seelze, Hannover, Germany) and methyl paraben (Winlab Ltd, Maidenhead, Berkshire, U. K). Acetonitrile and Methanol were HPLC grade. All chemicals were used as received.

HPLC Analysis of haloperidol
The concentrations of haloperidol samples were determined using an HPLC system equipped with binary pump model LC-10 AD and the variable wavelength ultraviolet detector SPD-10A set at 247 nm. (Shimadzu Corporation, Kyoto, Japan), and automatic injector (Waters associates Bedford). A stainless steel adsorbasphere phenyl column (4.6 mm id X 150 mm length) packed with 5 µm particle size ultrasphere (Alltech Associates Inc., IL, U.S.A.) was used as analytical column at 30 °C using column oven. The mobile phase consisted of acetonitrile: methanol: 0.05 M phosphate buffer (25: 25: 50% v/v) in addition to 1.0 ml triethylamine adjusted to pH 7.10 with Phosphoric acid (0.25 ml). The mobile phase was degassed by passing it through 0.22-µm membrane filter type GV (Millipore, Bedford, MA U.S.A.) and pumped isocratically at a flow rate of 1.2 ml/minute. The chart speed was 0.30 cm/minutes. Phase was pumped at flow rate of 1.5 ml /minutes. A volume of 50µl was injected for all samples.

Preparation of haloperidol gel formulations
Fifty milligrams of haloperidol were dissolved in a heated aliquot of propylene glycol (about 3 g), containing 20 mg of methyl paraben. Then 1.2 g of HPMC was dispersed in a warm aliquot of propylene glycol and left in freezer for 1 hour at –20 °C, after the gel was formed, 1,8-cineole-propylene glycol solution was added drop wise to the gel with continuous stirring (0.1gm 1,8-cineole). The pH of the formula was adjusted to 7.1 by adding few drops of sodium hydroxide, and the final weight was adjusted to 10 g with propylene glycol containing 10% w/w of 1,8-cineole.

In-vivo Formulation Studies
In order to gain a full insight into the transdermal absorption of haloperidol, in-vivo studies in human subject has to be performed. However, human studies are costly and time consuming due to the necessary safeguards, including the approval of clinical protocols for human use. Therefore, experimental animals are widely used as models for in-vivo percutaneous drug absorption despite of the fact that animal models differ significantly from man in features which affect percutaneous absorption such as density of hair follicles and sweat glands, thickness and nature of stratum cornium, and papillary blood supply. Investigators usually utilized rabbits as model for cutaneous absorption studies. Rabbits are easily handled concerning housing, feeding, supply and collecting blood samples. Thus, rabbits have been chosen for the in-vivo studies in this investigation 8, 9.

Intravenous administration
The intravenous solution was prepared by dissolving 50 mg of haloperidol in 20-ml volumetric flask containing 5mls ethanol and 5 ml propylene glycol the volume was completed to 20 ml by isotonic normal saline solution. This procedure was carried out in a laminar flow hood under aseptic technique. The final solution contains 2.5 mg/ml of haloperidol.

Five white male New Zealand rabbits weighing 3.5-4.5 kg were utilized in the study. The animals were fasted for 18 hours before and during the experiment. After immobilization of the rabbits in a rabbit restrainer, the hair was removed from the ears with an animal clipper. One ml of haloperidol solution previously prepared containing 2.5 mg/ml was administered slowly through the marginal vein. Before the drug administration
each rabbit was injected with 3000 units of heparin to avoid cannular occlusion. Blood samples, 1.5 ml each, were collected in 5ml heparinized evacuated blood collection tubes via iv-catheter (18G X 1¼ inch) inserted into the central artery, at 0, 0.08, 0.16, 0.33, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0 and 10.0 hours post injection. Blood samples were centrifuged immediately for 10 minutes at 4000 rpm and plasma were separated by using Pasteur pipettes and transferred to Bijue bottles and stored at -20 °C before analysis. 0.25 ml plasma was transferred to eppendorf tubes (2-ml micro-centrifuge tube). 0.25 ml of plasma was taken and analyzed for haloperidol concentrations using the HPLC method described above.

**Transdermal application of the gel formulation**

The same rabbits receiving the I. V. were used for determination of the plasma level-time profiles following transdermal administration dose after washout period of one month prior to the application of the dose. The hair was removed from the back of the rabbits (28.28cm^2) with animal clipper 24 hours before application of the gel. The skin was examined with a high power magnifying lens after shaving. The animal in which the skin barrier was disrupted or has scars or skin irritations was excluded from the study. The rabbits were immobilized in a restraining box during the entire experiments to prevent the animal from removing the gel. An area of 5.033 cm^2 was marked with a marker pen on the shaved area. A dose of 7.5 mg of haloperidol was spread uniformly over the designated area of each rabbit. Blood samples 2.0 ml each were collected from the central artery via a catheter (18 x 1¼ inch) at the following time intervals 0, 1, 2, 3, 4, 6, 8, 10, 12, 24, and 30 hours post application and processed for the assay of haloperidol as described before.

**Oral Administration**

The same 5 rabbits, which received the intravenous dose, were used after 1-month washout and rest period for the oral study. The animals were fasted for 18 hours before and during the experiment but were allowed to water. Before the animals were immobilized in a rabbit restrainer the tablet 5 mg was administered by pushing the tablet after depressing the tongue (forced swallowing) followed by 8-10 ml water via a gastric tube. After receiving the dose the animals were immobilized in restrainers during sampling time. The central artery of the right ear was cannulated with i.v. catheter (18G X 1¼ inch) for blood sampling. Blood samples, 1.5-2.0 ml each, were collected just prior to haloperidol administration and at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10 and 12.0 hours post drug administration. The plasma was then separated after centrifugation, collected and stored frozen at -20 °C pending analysis.

Before administration of the dose each rabbit was injected with 3000 units of heparin via marginal vein to avoid cannular occlusion at the sampling time. Blood samples, 1.5 ml each, were collected in 5ml heparinized evacuated blood collection tubes via iv-catheter (18G X 1¼ inch) inserted into the central artery. Blood samples were centrifuged immediately for 10 minutes at 4000 rpm and plasma were separated by using Pasteur pipettes and transferred to Bijue bottles and stored at –20 °C before analysis. 0.25 ml plasma was transferred to eppendorf tubes (2-ml-micro centrifuge tube) which were centrifuged at 13000 rpm for 5 minutes after addition of acetonitrile using microcentrifuge to precipitate the proteins. 200-250 µl was then injected into a 50 µl loop, into the chromatogram and assayed for haloperidol.

**Pharmacokinetic and statistical analysis**

**Pharmacokinetic parameters of haloperidol**

The plasma concentrations following intravenous administration of the drug were analyzed by a linear two compartment pharmacokinetic model using PKA analyst (Micro, Math Salt Lake city UT). The plasma concentration of haloperidol (Cp) as a function of time (t) was described by the following bi-exponential equation fitted in a two-compartment model.\[Cp = Ae^{-at} + Be^{-bt}\]
Where: Cp is the drug concentration in the blood. A, B, are the exponential multipliers α, β denote hybrid rate constants in the compartment 1 and 2 respectively. The relevant pharmacokinetic parameters such as distribution half-life (t1/2α) the terminal half-life (t1/2β) the area under the plasma-concentration time curve (AUC0-∞), the area under the first moment curve (AUMC0-∞), and the mean residence time of the drug in the body (MRT) were calculated using the following equations:

\[
    t_{1/2\alpha} = \frac{0.693}{\alpha} \\
    t_{1/2\beta} = \frac{0.693}{\beta} \\
    AUC_{0-\infty} = \frac{A}{\alpha} + \frac{B}{\beta} \\
    AUMC_{0-\infty} = \left(\frac{A}{\alpha^2} + \frac{B}{\beta^2}\right) \\
    MRT = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}}
\]

The pharmacokinetic parameters for haloperidol following oral and transdermal administration were determined from the plasma concentration time data. The maximum plasma concentration (Cmax) and its corresponding time (Tmax) were obtained directly from the plasma concentration-time data. The area under the plasma concentration-time curve up to the last measurable plasma concentration (AUC0-t) and the area under the first moment curve (AUMC0-t) were estimated by linear trapezoidal rule and extrapolated to infinity using the following equations:

\[
    AUC_{0-\infty} = AUC_{0-t} + C_p t/K_{el} \\
    AUMC_{0-\infty} = AUMC_{0-t} + C_p t/K_{el} + C_p t^2/K_{el}^2
\]

Where:

- Cp is the last measurable concentration at time t.
- \( K_{el} \) is the terminal elimination rate constant calculated by the technique of the least-squares regression analysis.

The elimination half-life (t1/2) was calculated from the equation:

\[
    t_{1/2} = \frac{0.693}{K_{el}}
\]

The mean residence time of the drug in the body (MRT) was calculated using the following equation 10:

\[
    MRT = \frac{AUMC}{AUC}
\]

The mean absorption time (MAT) and the absolute bioavailability (F) of the oral tablet and the transdermal gel formulation were calculated using the following equations 11:

a. After oral administration:

\[
    MAT = MRT_{Po} - MRT_{IV} \\
    F_{(oral)} = \frac{AUC_{0-\infty (oral)} X Dose_{(iv)}}{AUC_{0-\infty (iv)} X Dose_{(oral)}}
\]

b. After transdermal application:

\[
    MAT = MRT_{TS} - MRT_{IV} \\
    F_{(TS)} = \frac{AUC_{0-\infty (TS) X Dose_{(iv)}}}{AUC_{0-\infty (iv)} X Dose_{(TS)}}
\]

Where:

- MRTpo is the mean residence time after oral administration, MRTIV is the mean residence time after intravenous administration and MRTTS is the mean residence time after transdermal administration.

**Statistical analysis**

For comparative bioavailability study, plasma data were collected and the pharmacokinetic parameters, Cmax, Tmax, t1/2, Cmax/Auc and AUC, and AUMC were evaluated.

Two compartmental pharmacokinetic model analysis were employed to estimate the pharmacokinetic parameters of intravenous dose, while pharmacokinetic parameters of oral and transdermal dosage were estimated using non-compartmental model.

The pharmacokinetic parameters of haloperidol calculated following oral and transdermal administration were evaluated statistically using one-way ANOVA. Differences between two related parameters were considered statistically significant for p ≤ 0.05.

**Results**

**In-vivo formulation studies**

**Intravenous administration**

After intravenous bolus administration the plasma level concentrations time-curves of haloperidol versus time for individual rabbits and the mean plasma level of the 5 rabbits were shown in figure 1. Haloperidol was eliminated from the blood in biphasic pattern. A rabid and large decline in plasma curve was observed during the α-phase with average plasma concentration declined from 206.9 ng/ml after 10 minutes to 74.56 ng/ml after 45 minutes.
And then declined slowly during the beta phase after 4 hours in 5 animals the plasma concentration ranged between 21.5-33.5ng/ml and then declined slowly till reached 6.60ng/ml in 10 hours. Based on this data the deposition of haloperidol following intravenous dose could be adequately described by first order compartment model with first order rates in rabbits.

The mean pharmacokinetic parameters $\alpha$, $\beta$, $\beta$-half-lives, $\text{AUC}_{0-\infty}$, $\text{AUMC}_{0-\infty}$ mean residence time (MRT), total clearance and steady state volume of distribution were summarized in table 1.

Table: 1 Mean pharmacokinetic parameters of haloperidol and the absolute bioavailability after oral and transdermal administration to five rabbits and the results of statistical analysis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Transdermal</th>
<th>Oral</th>
<th>P -Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F$ (%)*</td>
<td>57.86 ± 8.74</td>
<td>38.17 ± 10.73</td>
<td>≤ 0.05 (S)</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}$ (ng.hr/ml)</td>
<td>1773.53 ± 570.19</td>
<td>474.28 ± 111.97</td>
<td>≤ 0.05 (S)</td>
</tr>
<tr>
<td>$\text{C}_{\text{max}}$ (ng/ml)</td>
<td>77.9 ± 4.50</td>
<td>92.26 ± 10.19</td>
<td>≤ 0.05 (S)</td>
</tr>
<tr>
<td>$\text{T}_{\text{max}}$ (hr)</td>
<td>3.60 ± 0.89</td>
<td>1.60 ± 0.22</td>
<td>≤ 0.05 (S)</td>
</tr>
<tr>
<td>$\text{K}_{\text{el}}$ (1/hr)</td>
<td>0.082 ± 0.05</td>
<td>0.18 ± 0.03</td>
<td>≤ 0.05 (S)</td>
</tr>
<tr>
<td>$t_{1/2}$ (hr)</td>
<td>10.91 ± 4.16</td>
<td>3.56 ± 0.4</td>
<td>≤ 0.05 (S)</td>
</tr>
<tr>
<td>$\text{C}<em>{\text{max}}/\text{AUC}</em>{0-\infty}$</td>
<td>0.047 ± 0.013</td>
<td>0.18 ± 0.02</td>
<td>≤ 0.05 (S)</td>
</tr>
<tr>
<td>$\text{AUMC}_{0-\infty}$ (ng.hr$^2$/ml)</td>
<td>33583.17 ± 20450.97</td>
<td>3054.20 ± 519.26</td>
<td>≤ 0.05 (S)</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>19.55 ± 3.98</td>
<td>6.23 ± 0.48</td>
<td>≤ 0.05 (S)</td>
</tr>
<tr>
<td>$\text{CL}_{\text{total}}$ (ml/min/kg)</td>
<td>20.13 ± 6.17</td>
<td>57.70 ± 0.55</td>
<td>≤ 0.05 (S)</td>
</tr>
<tr>
<td>$V_d$ (ml/Kg)</td>
<td>393.76 ± 129.44</td>
<td>359.70 ± 71.10</td>
<td>&gt; 0.05 (NS)</td>
</tr>
</tbody>
</table>
**Oral tablet administration:**

The data after this dose were represented in figures 2, the mean of the plasma concentration of 5 rabbits clearly showed a rapid declining phase for most of the animals.

![Oral Tablet](image-url)

**Figure: 2 Average plasma concentration-time profiles after application of oral and transdermal dosages of haloperidol to 5 rabbits**

The absorption of haloperidol was rapid with peak plasma concentration of 89.42 ng/ml observed after mean of 1.5 hour post dosing. The pharmacokinetic parameters calculated after this dose were presented in table 2.

**Table: 2 Mean pharmacokinetic parameters of Haloperidol after intravenous administration of 2.5mg dose.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean* + SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{el}$</td>
<td>1.19 0.18</td>
</tr>
<tr>
<td>$T_{1/2\alpha}$</td>
<td>0.63 0.47</td>
</tr>
<tr>
<td>$T_{1/2\beta}$</td>
<td>5.48 2.53</td>
</tr>
<tr>
<td>$AUC_{0\rightarrow\infty}$</td>
<td>432.40 42.76</td>
</tr>
<tr>
<td>$AUMC_{0\rightarrow\infty}$</td>
<td>2460.09 1256.73</td>
</tr>
<tr>
<td>MRT</td>
<td>5.51 2.34</td>
</tr>
<tr>
<td>$AUC_{0\rightarrow t}$</td>
<td>373.95 56.81</td>
</tr>
<tr>
<td>$AUMC_{0\rightarrow t}$</td>
<td>905.72 44.23</td>
</tr>
</tbody>
</table>

*Mean of 5 rabbits

**Transdermal administration**

The data of average plasma concentrations following oral tablets and after transdermal administration of 7.5 mg gel formulation of haloperidol containing 1,8-cineole as an enhancer were shown in figure 2. After application of trasdermal dose the plasma haloperidol concentration rose slowly to reach a peak after 2-4 hours with mean of 3 hours then declined very slowly and sustained at steady state in all animals for 10-12 hours. Then the drug concentration remained constant at steady state pattern till the end of experiment. The pharmacokinetic generated from this data after the application of transdermal gel formulation were illustrated in table 2.

The mean pharmacokinetic parameters of haloperidol and the absolute bioavailability after oral and transdermal administrations to five rabbits and the results of Statistical Analysis were summarized in table 2.

**Discussion**

**Intravenous administration**

The plasma concentration of haloperidol was known to follow two compartments distribution pattern with linear pharmacokinetics. The plasma concentration could be observed as follows; $C = 30.5 \ e^{-4.8t} + 7.2e^{-0.17t}$

The terminal half life was 4.20 hours which is close to that shown in rabbits after administration of the oral and application of transdermal dosage forms of haloperidol. Haloperidol could be measured in all samples, and the concentration of haloperidol was close to the limit of detection at the last sampling time, (12 hours for oral and 30 hours for transdermal samples).

Although subject to some individual variation, the plasma concentration curves of haloperidol for the two preparations oral and transdermal, in all animals had the same general appearance (Figure 2)

After application of both oral tablet and transdermal formulation the peak plasma concentration of haloperidol tablet was faster, reaching a peak plasma concentration in 1.6 hours, than that observed for the transdermal
formulation which reached the peak in 3.60 hours. This is due to higher rate of absorption of the tablet than that of the transdermal as the last preparation needs to cross the barrier property of the skin, and the tablet becomes immediately available for absorption. In addition the plasma levels of haloperidol were detectable for 30 hours for the transdermal formulation and this may indicate maintenance of haloperidol plasma levels for longer time.

Since the area under plasma concentration is the measure of the amount of the drug absorbed into the circulation\(^{12}\) (extent of absorption), the areas AUC\(_{0-\infty}\) and AUC\(_{0-t}\) were calculated for the two preparations (Table 2). These data in table 2 clearly showed that the mean AUC\(_{0-\infty}\) after transdermal preparation was significantly higher than that obtained after tablet dosage form (p < 0.05). The increase in the amount of drug absorbed was thus associated with the increase of peak blood levels; this was further confirmed by linear regression analysis showing a good correlation between percentages of drug absorbed, \(C_{\text{max}}\) and AUCs.

The absolute bioavailability of the transdermal dosage form [57.8\%] was significantly higher than the oral dosage form level [38.1\%] (P < 0.05). This may be due to the fact that transdermal administration could at least partially avoid the liver first pass effect. The relative bioavailability of transdermal to the oral tablet was 150\%. The transdermal formulation was also characterized by an increase of elimination half-life and decrease in elimination rate constant and a decrease in absorption rate compared to the oral tablet (Table 2). This data clearly shows a flip-flop phenomenon, which is well documented in the literature\(^{13}\). This phenomenon is often associated with sustained release formulation where absorption of drug from the formulation associated with apparent increase in elimination half-life and decreases in elimination rate constant and therefore, over estimation of rate constant may lead to over estimation of area under the curve. Comparing the peak plasma concentration of haloperidol after oral and transdermal, which were 92.26 and 77.9 ng/ml respectively, shows statistical difference between them (p < 0.05).

Comparing the \(T_{\text{max}}\) for the two preparations indicated that a shift in time for the maximum blood concentration 1.60 hours for oral tablet and 4.60 hours for transdermal formulation. The \(T_{\text{max}}\) is one of the parameters taken for the measure of the drug absorption rate and it gives a good indication of sustained formulation.

Another indication of prolonged drug delivery of haloperidol transdermal formulation is higher mean residence time (MRT) and slower absorption rate \(C_{\text{max}}/\text{AUC}_{0-\infty}\). The ratio of \(C_{\text{max}}/\text{AUC}\) was shown to be a good parameter and preferred to \(C_{\text{max}}\) for evaluation rates of absorption of single dose prolonged preparations\(^{14, 15}\). Moreover the, the ratio tends to have smaller variations and strongly recommended for assessing the equivalence of absorption rates\(^{16}\). The MRT was significantly higher after transdermal application and absorption rate was significantly lower for transdermal preparation. The MRT and MAT were shown to be good parameters for describing the pharmacokinetic behavior of drug in the body after oral and sustained preparations\(^{17, 10}\). The MRT and MAT were dramatically increased after application of transdermal formulation compared to the oral tablet. The delayed in absorption of the drug [longer \(T_{\text{max}}\)] following the transdermal application could explain the increase of the MRT obtained after application of transdermal application of haloperidol dosage compared to that of the oral tablet. The volume of distribution and systemic clearance were higher after application of transdermal application preparations indicating that the increase in the MRT is due to delayed absorption of haloperidol.
Conclusion

In conclusion, the transdermal haloperidol gel formulation produced acceptable sustained plasma drug concentration for longer time with an increase of extent of absorption and longer residence time of drug in a constant therapeutic blood level without the danger of dose dumping effect seen in the oral tablet. The study revealed that haloperidol/ hydroxypropyl methyl cellulose gel formulation containing 1,8-cineole exhibited acceptable gel consistency required for transdermal formulation and was readily spread when applied to the skin and remained on the skin to provide continuous drug absorption. This formulation could be used for further human clinical studies to produce effective and clinically acceptable transdermal formulation.

Acknowledgement

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References