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In vitro permeation of levothyroxine across the skin

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Abstract

The aim of this work was to investigate the in vitro transdermal permeation characteristics of sodium levothyroxine, in view of its topical application. Permeation experiments were performed in vitro, using rabbit ear skin as barrier. At the end of the experiments levothyroxine retained in the skin was extracted and quantified by HPLC. The formulations tested were solutions and a commercial cream. The use of dimethyl β-cyclodextrin as solubilizing agent increased to a significant extent levothyroxine solubility, but reduced its skin accumulation. Skin stripping before drug application produced a considerable increase in the amount retained and levothyroxine was found also in the receptor compartment. The application of the commercial cream in occlusive conditions increased to a significant extent drug retention in the skin. In conclusion, levothyroxine skin administration is promising in view of a localized effect, because it was retained in the skin. On the contrary, transdermal administration in view of systemic effect does not represent a concrete possibility.

Keywords: Levothyroxine; Skin absorption; Skin retention

1. Introduction

Levothyroxine (T4) is the main product of thyroid secretion together with triiodothyronine (T3); T3 and T4 represent the only iodine-containing hormones in vertebrates. In vivo, T4 undergoes peripheral conversion to T3, the active form of thyroid hormone.

Sodium levothyroxine, alone or in combination with liothyronine represents, at present, the preparation of choice to restore euthyroidism in hypothyroid patients (Escobar-Morreale et al., 2005). Thyroid hormones are used also topically for their activity on the skin, in particular on lipid degradation, to reduce deposits of subcutaneous adipose tissue (Pucci et al., 2000). The permeation characteristic of levothyroxine across the skin has not been studied in great detail in the literature. An in vivo study on rabbits (Arduino and Eandi, 1989), using ^{125}I T4, demonstrated that radioactivity could be found in the skin after 24 h application of a cream. Using the same radioactive marker, James and Wepierre (1974) found radioactivity also in plasma. More recent in vitro studies on a liposomal cream containing ^{125}I T4 showed that liposomes were able to deliver the hormone across the skin in vitro (20% of the amount applied crossed the skin after 24 h), although no systemic effect was observed in vivo (Santini et al., 2003).

The aim of this work was to investigate in vitro the transdermal permeation characteristics of sodium levothyroxine, in view of its topical application. An additional objective of the work was to set up and validate a new extraction procedure of T4 from the skin.

The permeation of levothyroxine into and across the skin was performed from various solutions, containing cyclodextrins or organic solvents as solubilizing agents. As reference, the commercial formulation Somatoline® was used. Rabbit ear skin was used as barrier in permeation experiments, since it has been shown to be a reasonable model for human skin in vitro in passive conditions (Artusi et al., 2004) and in the presence of iontophoresis or chemical enhancers (Nicoli et al., 2001, 2003; Hirvonen et al., 1993).

2. Materials and methods

2.1. Materials

Levothyroxine sodium pentahydrate (T4, m.w. 888.93) was obtained from Farmalabor (Milan, Italy). Dimethyl-β-

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cyclodextrin (DMβCD) was purchased from Wacker-Chemie (Burghansen, Germany). Transcutol® was a gift from Gattefossé Italia (Milan, Italy). Polyvinyl alcohol (PVA) of molecular weight of 83,400 (degree of hydrolysis 87%) was obtained from Nippon Ghosei (Osaka, Japan). Polyvinyl pyrrolidone K30 (PVP) was a gift from BASF (Ludwigshafen, Germany). The commercial formulation Somatoline® (Lot 4036, Manetti & Roberts, Florence, Italy) was purchased in a pharmacy. The composition is: levothyroxine 0.1% (w/w), escine 0.3% (w/w) as active agents, glyceryl monostearate A.E., liquid paraffin, decyl oleate, 70% sorbitol, polyacrylamide isoparaffin laureth-7, imidazolinylurea, methyl paraben, propyl paraben, citric acid, perfume, water as inactive ingredients.

All other reagents were of analytical grade.

Rabbit ears were collected immediately after sacrifice of the animals from a local slaughterhouse. The skin from the inner face was excised from the ear using a surgical blade and used immediately. The average thickness of the skin, measured with a Mitutoyo (model ID: C112BS) caliper, was 0.28 ± 0.06 mm.

2.2. Solubility determination

An excess amount of T4 (50 mg) was added to 1 ml of the appropriate solvent and left to equilibrate at room temperature under continuous stirring for 24 h. After filtration and dilution the samples were analyzed by HPLC.

2.3. Stability determination

Standard solutions of T4 in PBS solution were kept at room temperature, 37 °C and −20 °C for 24 h and then analyzed for T4 residual concentration.

2.4. In vitro diffusion experiments

Transdermal permeation of T4 was investigated at room temperature. Experiments were performed using vertical Franz type diffusion cells (DISA, Milan, Italy) with 0.6 cm^2 of diffusion area. The full thickness rabbit ear skin was mounted on the cells with the stratum corneum facing the donor compartment. The receptor compartment (4.2 ml volume) was filled with PBS (T4 solubility: 10.1 ± 0.7 μg/ml). The following formulations were used in the donor compartment:

1. Solution of T4 (0.1%, w/w) in ethanol:Transcutol®: propylene glycol:water (15:35:35:15) applied in infinite dose conditions (0.8 ml/cm^2).
2. Water saturated solutions with or without DMβCD (200 mM), applied in infinite dose conditions (0.8 ml/cm^2). In one set of experiments the skin was stripped 30 times using book tape 345 (3 M, USA) before solution application.
3. Gel (0.75%, w/w) containing PVA (12.4%, w/w), PVP (5.67%, w/w), PEG400 (3%, w/w) and sorbitol (4%, w/w) applied in infinite dose conditions (0.8 ml/cm^2).
4. Commercial formulation (Somatoline®, 0.1%, w/w) applied in infinite (0.8 ml/cm^2) or finite (2 μl/cm^2) dose condition. In one set of experiments the skin was pretreated with anodal iontophoresis, i.e. the donor compartment was filled with saline solution and the current was applied for 60 min at 0.5 mA/cm^2. Then the solution was removed and replaced by the commercial formulation.

At the end of the experiment (24 h) the receptor solution was sampled and analyzed by HPLC for the determination of T4 permeated. The donor compartment was emptied, the skin was washed with a cotton pad wetted with isopropanol, weighted and extracted (30 min at 40 °C) with 1 ml of a mixture of 10 volumes of methanolic sodium hydroxide and 90 volumes of water/acetonitrile/phosphoric acid (70:30:0.1, v/v/v). Methanolic sodium hydroxide was prepared by dissolving sodium hydroxide (400 mg) in methanol:water (50:50, v/v) (1000 ml), according to the USP 27.

2.5. Validation of T4 skin extraction

Blank skin samples, which had not previously been in contact with the drug, were used in specificity and recovery determination. Some of the blank skin tissues were submitted to the extraction procedure and the retention time of endogenous compounds extracted was compared with that of T4. For recovery determination, a known amount of T4 solution (10 μl of T4 0.1 mg/ml solution) was added to nine blank skin specimens. After 1 h of contact, the tissues were extracted and analyzed. The recovery was determined by computing the ratio of the amount of T4 extracted from spiked skin to the amount of T4 added (determined by direct injection of spiked solutions in the absence of skin).

2.6. Analytical method

The amount of T4 in the samples was quantified by high performance liquid chromatography (HPLC) using the USP 27 method. This method allows for the simultaneous determination of T4 and T3. The instrument was a Perkin-Elmer liquid chromatograph (Perkin-Elmer, Norwalk, CT, USA) which included a UV detector, set to 225 nm and an analytical Spherisorb® Cyano column, (2.1 mm × 250 mm), purchased from Waters (Millipore Corporation, Milford, USA). A mixture of water/acetonitrile/phosphoric acid (70:30:0.1, v/v/v) was used as mobile phase, at a flow rate of 0.3 ml/min. Injection volume was 20 μl. The method was validated according to the USP 27. The method resulted linear in the interval 0.09–5.0 μg/ml. The limit of detection was 0.001 μg/ml and the limit of quantification was 0.09 μg/ml.

2.7. Statistical analysis

Each experiment was replicated at least four times. The significance of the differences between values was assessed using ANOVA (KaleidaGraph® 3.6.2 software on a Macintosh PowerBook G4) followed by Bonferroni's test.

3. Results and discussion

3.1. Validation of T4 skin extraction

The extraction of T4 from the skin resulted specific and efficient, because no interference from skin components was present and the recovery was 90.9 ± 11.0%.

3.2. Levothyroxine stability

T4 aqueous solutions resulted stable for 48 h at room temperature (residual concentration 98.4 ± 18.4%), but not for 24 h at 37 °C (residual concentration 88.1 ± 12.2%) or at −20 °C (residual concentration 75.2 ± 6.7%).

3.3. Levothyroxine solubility

The solubility of levothyroxine in the various solvent is reported in Table 1. Levothyroxine is a poorly water-soluble molecule, despite the fact that it is sodium salt. In fact the solubility of T4 in pure water at room temperature resulted 0.77 mM. Since cyclodextrins are known as solubilizing agents for water insoluble compounds, we tested the solubility of T4 in the presence of 1% (w/v) of some natural and semi-synthetic cyclodextrins (dimethyl-β-cyclodextrin and α-cyclodextrin). The best result was obtained with DMβCD, which at 1% (7.6 mM) increased T4 solubility from 0.77 to 1.97 mM. The addition of increasing amounts of DMβCD increased T4 solubility to approximately 14 mM, with an almost linear trend ($R = 0.96406$), as can be seen in Fig. 1, where the phase solubility diagram obtained with DMβCD is shown. The linear increase of T4 solubility with DMβCD concentration suggests the formation of a complex between the hormone and the cyclodextrin. We then tried to estimate the stability constant of the complex, assuming a 1:1 stoichiometric ratio, according to Higuchi and Connors (1965). However, the stability constant obtained (0.085 M^{-1}) indicates a very low stability of the complex—if present (Pean et al. (1999) hypothetized that only γ-cyclodextrin can accommodate T4 inside the cavity).

The solubility behavior of T4 in the presence of sodium chloride resulted problematic. In fact, the addition of 0.9% sodium chloride to water resulted in a dramatic decrease of T4 solubility (from 0.77 to 0.013 mM), effect that was evident regardless of the other substances present such as DMβCD. For example the solubility of T4 in aqueous 7.6 mM DMβCD was 1.97 mM, but the value decreased to 0.075 mM when 0.9% of sodium chloride was present together with the cyclodextrin. This reduction of solubility is probably due to a salting out effect (Grover and Ryall, 2005), which can also explain the observed interaction of calcium supplements on T4 bioavailability.

Table 1
Levothyroxine solubility at room temperature (mean values ± S.E.M.)

Solvent	Additive	Solubility (mM)
Water	–	0.77 ± 0.44
Water	1% DMβCD	1.97 ± 0.14
Water	1% βCD	0.025 ± 0.002
Water	1% αCD	0.017 ± 0.007
Saline	–	0.013 ± 0.011
Saline	1% DMβCD	0.075 ± 0.031
PBS	–	0.011 ± 0.001

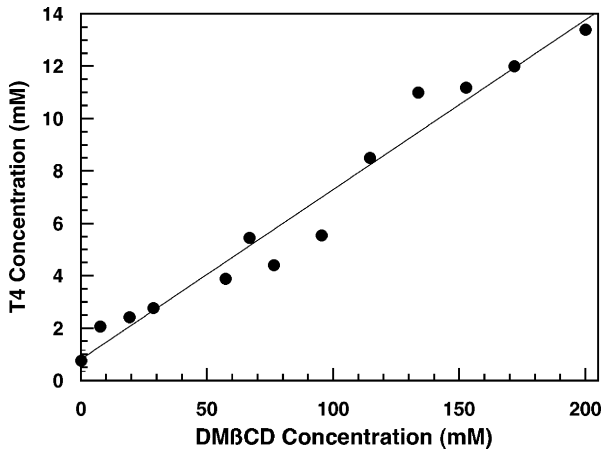

Fig. 1. Phase solubility diagram of levothyroxine in the presence of dimethylβcyclodextrin. The fitting equation resulted: T4 Conc. = 0.818 + 0.065; DMβCD Conc. ($R^2 = 0.964$).

3.4. Levothyroxine skin permeation

The permeation experiments were performed using freshly excised rabbit ear skin as barrier. Rabbit ear skin has been shown to be a reasonable model for human skin in vitro in passive conditions (Artusi et al., 2004) and during transdermal iontophoresis (Nicoli et al., 2003, 2001). Additionally, Hirvonen et al. (1993) demonstrated that the effect of enhancers, such as azone, were comparable on human and rabbit ear skin. Moreover, rabbit ear skin is readily available without the need for freezing, which is not always the case for human skin. Skin freezing can cause enzyme inactivation or cell rupture and release of intercellular enzymes responsible for drug metabolism/degradation. Preliminary experiments done on frozen skin showed that T4 was partly metabolized into the skin, giving rise to an unknown peak with a retention time shorter than T4. Thus, freshly excised rabbit ear skin was used in all the experiments performed. All experiments were performed at room temperature, due to the low stability of T4 at 37 °C.

For all the formulations tested on intact skin, T4 was never found in the receptor compartment, while it was found in the skin. Owing to the sensitivity of the analytical method, fluxes lower than 10 ng cm^{-2} h^{-1} would not be detectable.

The amounts of T4 recovered in the skin after 24 h were normalized by the weight of tissue, to calculate the concentration of the hormone in the tissue, expressed as ng of T4 per mg of tissue.

Initially, the penetration of T4 into and across the skin was studied starting from aqueous solutions and the results are reported in Fig. 2. The application of a saturated water solution of T4 to the skin produced some skin retention (187 ± 100 ng/mg), which was dramatically increased when the

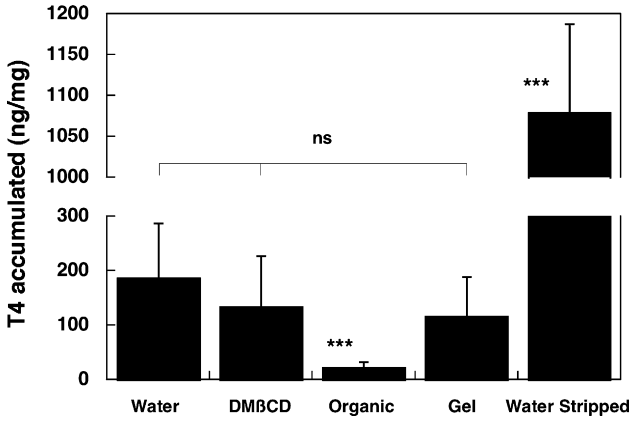

Fig. 2. Levothyroxine retained in the skin after 24 h of application of solutions of T4 in water (conc. 0.6 mg/ml), water containing DMβCD 200 mM (conc. 11.9 mg/ml), ethanol:Transcutol®:PG:water (15:35:35:15, conc. 10 mg/ml), PVA/PVA gel (conc. 75 mg/ml). The last column refers to water solution applied to stripped skin. Average values ± S.D. ***Significantly different from all other formulations ($p < 0.001$).

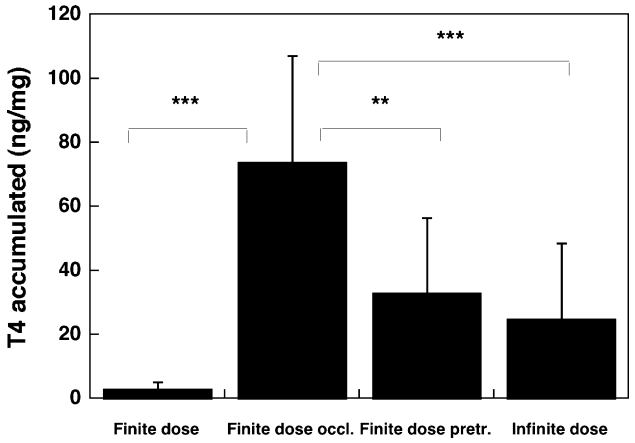

Fig. 3. Levothyroxine retained in the skin after 24 h of application of Somatoline® cream. Pretreatment refers to iontophoresis (60 min, 0.5 mA/cm^2) before formulation application. Average values ± S.D. Significantly different (***$p < 0.001$, **$p < 0.05$).

stratum corneum of the skin was removed prior to drug application (1079 ± 109 ng/mg), confirming that the rate-limiting step in T4 skin penetration is the stratum corneum. In this case, T4 was found also in the receptor compartment: after 24 h the amount permeated was 8.18 ± 4.07 μg across an area of 0.6 cm^2. This corresponds to a flux of T4 in the order of 600 ng cm^{-2} h^{-1}. Considering that the typical dose of T4 is 50–100 μg/day, the flux obtained in vitro across stripped rabbit ear skin would not be sufficient to produce a systemic effect in vivo. In fact, considering a patch area of 20 cm^2, the amount delivered over 24 h would be approximately 10 μg.

The amount of T4 recovered in the skin in the presence of 200 mM DMβCD was 134 ± 94 ng/mg, slightly lower although the difference was not statistically significant with respect to the neat water solution. Despite the large difference in drug donor concentration (0.77 mM vs. 14 mM), the amount of T4 retained in the skin was similar with and without cyclodextrin, probably because both solutions were saturated, i.e. had the same thermodynamic activity. Then, a gel containing PVA, PVP and PEG 400 was tested: also in this formulation T4 was present at saturation, namely 75 mg/ml. T4 skin retention (117 ± 72 ng/ml) was comparable to that obtained with saturated water solution with and without cyclodextrin, probably because they all were saturated solutions.

With the aim of further improving the skin retention of T4, the drug was dissolved in a mixture of organic solvents, some of which are known as penetration enhancers, such as ethanol and Transcutol®. The application of a solution made of ethanol:Transcutol®:PG:water (15:35:35:15), in which T4 was dissolved at 10 mg/ml, produced a marked reduction in T4 retention (23 ± 10 ng/mg) compared to the DMβCD solution. This marked reduction can be due to the lower thermodynamic activity of T4 in this solution, which was not counterbalanced by the enhancing effect of the organic solvents presents.

Finally, the permeation of T4 was studied starting from a commercial cream (Somatoline®), containing T4 at 10 mg/ml. The results obtained are reported in Fig. 3. The application of the cream in infinite dose conditions produced a T4 skin retention (25 ± 24 μg/mg) comparable to that obtained from the solution containing organic solvent and the same concentration of the drug (23 ± 10 ng/mg) suggesting that the type of formulation, whether it is a solution or an emulsion, has little effect on T4 skin retention.

When the cream was applied in finite dose conditions, skin retention was 3.00 ± 2.09 ng/mg, much lower compared to the infinite dose application ($p < 0.05$). The higher performance of the formulation applied in infinite dose is probably due to the occlusive effect produced by the thick layer of formulation (0.8 ml/cm^2) in infinite dose conditions. The percentage of T4 accumulated in the skin (with reference to the dose applied) was calculated only for the finite dose regimen and resulted very low, namely 0.8%. It is well known that occlusion can improve drug absorption across the skin, particularly of lipophilic permeants (Treffel et al., 1992); for this reason the commercial cream was applied in finite dose conditions with and without occlusion. Skin retention of T4 was significantly improved by occlusion ($p < 0.001$), although no skin permeation was observed. It is commonly considered that occlusion increases permeation because the stratum corneum hydrates and eventually swells in the presence of an excess of water. The percentage of T4 accumulated with the respect to the dose applied was 20.5%, in agreement with the results of Santini et al. (2003), obtained using human skin.

Finally, to overcome the stratum corneum barrier, another approach was used, i.e. the skin was pretreated with electric current (anodal iontophoresis, 0.5 mA/cm^2 for 60 min) and then the cream was applied (finite dose). The result obtained with skin iontophoretic pretreatment, which increases its passive permeability, was still an increased T4 retention, although less evident compared to occlusion ($p < 0.05$).

4. Conclusions

From the results obtained in the present work, it can be concluded that levothyroxine skin administration is promising in

view of a localized effect, because T4 was retained in the skin after topical application, in particular in occlusive conditions. The presence of DMβCD, which increased to a significant extent levothyroxine solubility, did not modify its skin accumulation. On the contrary, transdermal administration in view of systemic effect does not represent a concrete possibility, because even with the use of animal skin, no T4 crossed intact skin in significant amounts. When the skin was tape stripped prior to permeation, T4 was found in the receptor solution, although the absolute amount permeated was much lower than the dose required in man.

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References

Arduino, C., Eandi, M., 1989. Kinetics of thyroxine epicutaneous absorption. Pharmacol. Res. 21, 109–110.

Artusi, M., Nicoli, S., Colombo, P., Bettini, R., Sacchi, A., Santi, P., 2004. Effect of chemical enhancers and iontophoresis on thiocolchicoside permeation across rabbit and human skin in vitro. J. Pharm. Sci. 93, 2431–2438.

Escobar-Morreale, H.F., Botella-Carretero, J.I., Escobar Del Rey, F., Morreale De Escobar, G., 2005. Review: treatment of hypothyroidism with combinations of levothyroxine plus liothyronine. J. Clin. Endocrinol. Metab. 90, 4946–4954.

Grover, P.K., Ryall, R.L., 2005. Critical appraisal of salting-out and its implications for chemical and biological sciences. Chem. Rev. 105, 1–10.

Higuchi, T., Connors, K.A., 1965. Phase-solubility techniques. Adv. Anal. Chem. Instrum. 4, 117–212.

Hirvonen, J., Sutinen, R., Paronen, P., Urtti, A., 1993. Transdermal penetration enhancers in rabbit pinna skin: duration of action, skin irritation, and in vivo/in vitro comparison. Int. J. Pharm. 99, 253–261.

James, M., Wepierre, J., 1974. Percutaneous absorption in vivo of iodine-125-labeled thyroxine and iodine-125-labeled triiodothyronine in the rat. Ann. Pharm. Francaises 32, 633–640.

Nicoli, S., Cappellazzi, M., Colombo, P., Santi, P., 2003. Characterization of the permselective properties of rabbit skin during transdermal iontophoresis. J. Pharm. Sci. 92, 1482–1488.

Nicoli, S., Rimondi, S., Colombo, P., Santi, P., 2001. Physical and chemical enhancement of transdermal delivery of triptorelin. Pharm. Res. 18, 1634–1637.

Pean, C., Creminon, C., Grassi, J., Pradelles, P., Perly, B., Djedaini-Pilard, F., 1999. NMR investigations of the inclusion of thyroxine and derivatives in natural cyclodextrins. J. Incl. Phenom. Macro. 33, 307–319.

Pucci, E., Chiovato, L., Pinchera, A., 2000. Thyroid and lipid metabolism. Int. J. Obes. Relat. Metab. Disord. 24, S109–S112.

Santini, F., Vitti, P., Chiovato, L., Ceccarini, G., Macchia, M., Montanelli, L., Gatti, G., Rosellini, V., Mammoli, C., Martino, E., Chopra, I.J., Safer, J.D., Braverman, L.E., Pinchera, A., 2003. Role for inner ring deiodination preventing transcutaneous passage of thyroxine. J. Clin. Endocrinol. Metab. 88, 2825–2830.

Treffel, P., Muret, P., Muret-D'Aniello, P., Coumes-Marquet, S., Agache, P., 1992. Effect of occlusion on in vitro percutaneous absorption of two compounds with different physicochemical properties. Skin Pharmacol. 5, 108–113.