A drug-in-adhesive anti-onychomycotic nail patch: influence of drug and adhesive nature on drug release, ungual permeation, *in vivo* residence in human and anti-fungal efficacy

K Rizi[1], K Xu[1], T Begum[1], J Faull[2], S Bhakta[2], S Murdan[1,*]

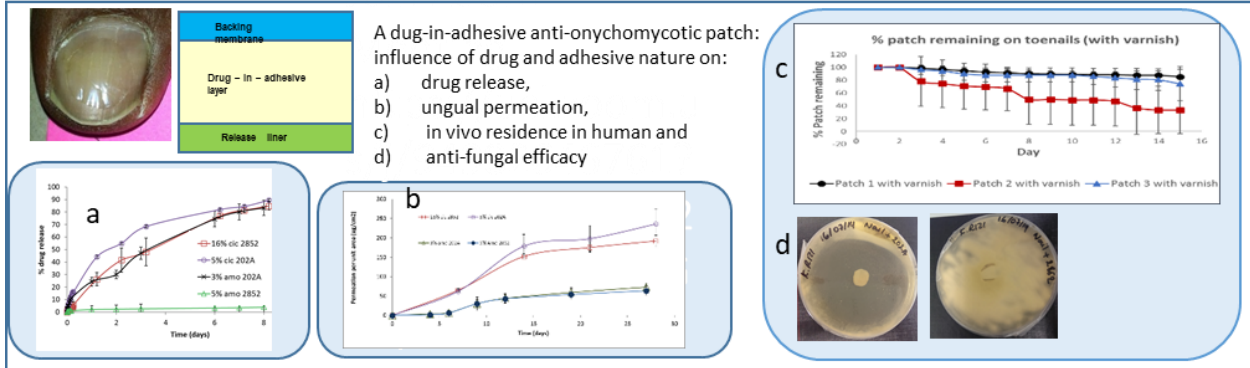[1]Department of Pharmaceutics, UCL School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, UK

[2]Department of Biological Sciences, Institute of Structural and Molecular Biology, Birkbeck, University of London, Malet Street, London, WC1E 7HX, UK.

* Corresponding author
Email address: s.murdan@ucl.ac.uk

Graphical abstract

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a)   drug release,
b)   ungual permeation,
c)   in vivo residence in human and
d)   anti-fungal efficacy

Abstract

A nail patch is an attractive option for the topical treatment of onychomycosis, although no product is commercially available. We previously identified optimal nail patch formulations for two anti-onychomycotic drugs, based on their properties, as well as those of the other patch components. In this paper, our aim was to further investigate the potential of the patch formulations as topical nail medicines, in particular, whether the drug-in-adhesive patches release drug which then permeates into and through the nail plate and show anti-fungal efficacy, and whether and to what extent they remain adhered to the human nail plate in vivo when tested over 2 week durations. In addition, the influence of the drug (amorolfine HCl, ciclopirox olamine) and PSA (Duro-Tak 2852 or Duro-Tak 202A) on these parameters was determined. We found that both the nature of the drug and of the PSA influenced in vitro drug release. The nature of the drug, but not that of the PSA, influenced ungual drug permeation through human nail clippings, with considerably greater (almost double) permeation for ciclopirox olamine, the smaller and less lipophilic molecule. In vivo residence, tested with 3 out of the 4 patches, excluding the patch where ciclopirox olamine degraded with time, showed greater residence on toenails compared to fingernails reflecting their far lesser exposure to environmental stresses during daily activities. In vivo residence was enhanced when the patch was cut to the shape of the nail, was applied at bedtime, and when a clear colourless nail varnish was applied on top of the patch to 'seal' it into place on the nail. Comparison of the patches indicated greater residence of Duro-Tak 202A containing patches over those containing Duro-Tak 2852. Amorolfine HCl in Duro-Tak 202A based patch also showed antifungal efficacy in contrast to Duro-Tak 2852-based patch, and is particularly promising for further development as a potential toenail medicine, remaining almost fully adhered to toenails for at least two weeks.

1. Introduction

The patch is a convenient drug delivery system and is used for a range of indications, including hormone replacement therapy, attention deficit hyperactivity disorder, pain relief and nicotine replacement therapy. Commercially available patches are intended for application to the skin, for local and/or systemic drug administration, and have been marketed for decades. More recently, patches have been investigated for application to the nail plate, for the local treatment of nail diseases. For example, we reported a systematic approach to the formulation of nail patches (Rizi, Mohammed et al. 2018). Myoung and Choi (2003) and Ahn et al. (2013) reported the influence of patch components on ungual (i.e. of the nail) drug permeation *in vitro* (Myoung and Choi 2003, Ahn, Lee et al. 2013). *In vivo*, patches have been used to evaluate photodynamic therapy (Donnelly, McCarron et al. 2005), and iontophoretic drug delivery (Amichai, Nitzan et al. 2010), as well as enable passive drug delivery in humans (Susilo, Korting et al. 2006).

So far, nail patches have been investigated for the treatment of onychomycosis (fungal infections of the nail), which accounts for half of all nail disorders (Rich and Scher 2003), and affects 14-18 % of the general population, 20% of people aged over 60 years, up to 50% of people aged over 70 years, and up to one-third of diabetics. Despite the high incidence and significant effects of the infection (such as pain, depression and many lost working days (Lubeck, Patrick et al. 1993)), current therapies are not ideal. Oral drugs cause adverse effects, such as gastro-intestinal disturbances and liver toxicity, while topically applied medicines (in the form of drug solutions and lacquers) are not very effective (tabled in (Murdan 2016)). For example, cure rates are under 20% for the two newest medicines (Jublia® and Kerydin®) which were approved by the FDA in 2014. Commercialisation of these two medicines despite their low successes highlights the largely unmet need for effective topical anti-onychomycotic medicines, and the need for new therapeutic approaches.

A nail patch is an attractive option for the treatment of onychomycosis and several patents have been granted. Local drug administration minimises the incidence of drug-related side effects and drug interactions. The patch can be formulated to contain sufficient drug to reduce the frequency of drug administration to, for example, weekly, which could enhance patient compliance. By acting as a visual reminder, the patch can also enhance patient's adherence to their medication regime. An ideal nail patch should be aesthetically acceptable, easy to apply, remain adhered to the nail plate for the intended duration, and be easy to remove cleanly when desired. Most importantly, the nail patch should release the loaded drug, which would then permeate into and through the nail plate, reach the nail bed, and effect its action against the fungi.

We recently reported a systematic approach to the formulation of a simple drug-in-adhesive ungual patch (Rizi, Mohammed et al. 2018). The patch, prepared by solvent-casting, consisted of a drug-loaded pressure sensitive adhesive (PSA) sandwiched between an occlusive backing membrane and a release liner. The prepared patches were between 380 and 480 µm thick, had good uniformity of thickness and of drug content, and showed minimal drug crystallisation during six months of storage. Drug stability in the patch upon storage and the in vitro patch adhesion to the nail depended on the nature of the drug, the PSA and the backing membrane. Having identified the optimal formulations based on the drugs' and patch components' pharmaceutical and mechanical properties, including in vitro adhesion (Rizi, Mohammed et al. 2018), the aim of the work reported in this manuscript was to investigate further whether the patch formulations can become topical nail medicines, in particular, whether they release drug which then permeates into and then through the nail plate and show anti-fungal efficacy, and whether they adhere to the human nail plate in vivo. In addition, the influence of the drug and PSA nature on these was determined.

2. Materials and methods

2.1 Materials

Amorolfine HCl (Ranbaxy Research Laboratories, India) and ciclopirox olamine (Zhejiang Huadee Chemicals, China) were used as model antifungal drugs. Amorolfine HCl and ciclopirox olamine were both included in order to assess the effects of the nature of the drug on patch properties. The Pressure Sensitive Adhesives, Duro-Tak 87-2852 and Duro-Tak 87-202A (henceforth referred to as Duro-Tak 2852 and Duro-Tak 202A, respectively) were kindly provided by Henkel Ltd, UK, as solutions of the polymer in organic solvents. The backing membrane Scotchpak 9757 and release liner 3M Scotchpak 9744 were purchased from 3M, UK. Trifluric acid (TFA), phosphoric acid, triethylamine, acetonitrile, sodium octane sulfonate and methanol were purchased from Sigma-Aldrich (UK) and were of HPLC grade. Sabouraud's dextrose agar (SDA) and Sabouraud's dextrose broth (SDB) were purchased from Sigma-Aldrich, UK. Human nail clippings (used in permeation experiments) were obtained from volunteers, while cadaver nail plates (used in anti-fungal efficacy assays) were purchased from Cambridge Bioscience Ltd, UK.

2.2 Preparation of drug-loaded nail patches

The purchased PSA solutions were diluted with dichloromethane (to aid drug dissolution) to obtain a PSA concentration of 15% w/w. Amorolfine HCl and ciclopirox olamine were then added at their saturation solubilities in each PSA, i.e. ciclopirox olamine at 5% w/w in Duro-Tak 202A and at 16% w/w in Duro-Tak 2852; and amorolfine HCl at 3% w/w in Duro-Tak 202A and at 5% w/w in Duro-Tak 2852. The drug-PSA mixtures were then cast onto the backing membrane (PET side, i.e. the outside of the roll) over an area of 37.5 cm^2 (7.5 cm x 5 cm) whose edges had been pre-defined by affixing microscope slides with double sided tape. The solvent was allowed to evaporate for 24 hours. Subsequently, the release liner was placed on the PSA surface to create a backing membrane-PSA-release liner sandwich.

2.3 Drug release from ungual patches

Release experiments were performed over a duration of eight days using Franz-type diffusion cells, where the surface area for drug release was 3.14 cm^2. The patches were applied onto

nitrocellulose membranes, which were then sandwiched between the donor and receiver compartments of a Franz diffusion cell, with the patch on the donor side. Subsequently, receptor fluid (phosphate buffer pH 5 for amorolfine HCl or water for ciclopirox olamine) was added to the receptor compartment ensuring that no air bubbles were introduced. The diffusion cells were placed on a magnetic stirrer immersed in a water bath at 37±1° C, in order to maintain the membrane surface temperature at 32±1° C. Samples of the receptor fluid (1ml) were collected periodically and replaced with fresh pre-heated receptor phase at each sampling point. Sink conditions were maintained, taking into account that the solubility of amorolfine HCl in the receptor fluid was 7.9 ± 0.3 mg/ml and that of ciclopirox olamine was 0.4 ± 0.05 mg/ml (Hossin 2015).   The receptor samples were analysed by HPLC (as described in Section 2.5.) to determine the amount of drug released, and the cumulative % drug release over time was plotted. All release studies were performed in triplicate.

2.4 Measurement of ungual drug permeation from patches

Healthy nail clippings were obtained from volunteers (ethics approval, REC/B/10/01, School of Pharmacy), washed with distilled water and excess debris was carefully removed using a spatula without damaging the nail plate. The clippings were then allowed to air-dry for at least 30 minutes and equilibrate at room temperature for 20 minutes prior to use. Permeation experiments were performed using modified Franz-type diffusion cells (receptor volume 0.9 ml, surface area 0.025 cm^2). Nail clippings (mean thickness= 76 +/- 14 um) cut into size (diameter 0.3 cm; surface area of 0.07 cm^2) and with a patch applied to the dorsal surface (area of 0.07 cm^2) were mounted into the Franz cell with the patch facing the donor compartment and the ventral nail surface facing the receptor compartment. The receptor phase was phosphate buffer pH 5 for amorolfine HCl or water for ciclopirox olamine to ensure the stability of the drug over the testing period compartment and was placed in the donor compartment. The diffusion cell was assembled and left to stir on a magnetic stirrer placed in a water bath at 37°C. Samples of the receptor phase (100 µl) were taken periodically over 30 days, replaced with fresh pre-heated media and analysed by HPLC (as per section 2.5) to determine the amount of drug

permeated across the nail over time. Sink conditions were maintained throughout. Each experiment was repeated six times.

2.5 Measurement of drug concentrations by HPLC

HPLC was conducted using a 1260 Infinity Agilent HPLC system using a Luna C18 column (150 x 4.6 mm, 5 µm). For amorolfine HCl, a flow rate of 1 ml/min and an injection volume of 20 µl were used with UV detection at 220 nm respectively. The mobile phase for amorolfine HCl consisted of 0.1% TFA in water and acetonitrile at a ratio of 55:45 v/v and resulted in the elution of the drug after 4.8 min. For ciclopirox olamine, a mobile phase of 5mM sodium octane sulfonate in water and acetonitrile (50:50 v/v) was employed using a flow rate of 2 ml/min, a UV detection of 267 nm and an injection volume of 40 µl. This resulted in the elution of ciclopirox olamine at 3.1 min. The HPLC methods were validated for linearity, accuracy, precision (intra-day and inter-day) and limits of detection and quantification.

2.6 Measurement of antifungal activity of the patches

The dermatophyte *T. rubrum* was chosen as it is the most common causative organism of onychomycosis, and the ability of the patch to inhibit fungal growth was measured. The classical disc diffusion method was adapted, where the disc was replaced by a circular piece of cadaver nail onto which a patch had been applied, taking care to leave a patch-free margin at the nail edge, such that the drug would have to be permeate into and through the nail plate to reach the agar gel. This method was used as there is currently no standard in vitro method to assess the anti-fungal efficacy of anti-onychomycotic formulations.

Stock inoculum suspensions of *T. rubrum* (CBS 118892) were prepared from 14-day old cultures grown on Sabouraud's dextrose agar (SDA) slants at 25°C. The fungal colonies were covered with 2 ml of Sabouraud's dextrose broth (SDB), and suspensions were obtained by gently probing the surface with the tip of a Pasteur pipette, generating a mixture of conidial and hyphal fragments. The obtained suspensions were filtered through four layers of muslin, which retained hyphal fragments and permitted the passage of dermatophyte microconidia. The

number of colony forming units (CFU) per ml of suspension was determined to be 46×10^4 spores/ml using the plate dilution assays. 100 µl of the inoculum suspension was then swabbed onto SDA petri dishes (9 cm diameter).

Autoclaved and hydrated cadaver nail plates (thickness 94 ± 9 µm) were cut into circles (1.2 cm diameter) using a sharp-edged stainless steel tube, air-dried and equilibrated to room temperature. A smaller circular (diameter of 1 cm) drug-containing patch was then placed on the dorsal surface of the nail plate, keeping a patch-free zone at the edge of the nail. The nail plates were then placed in the centre of *T. rubrum* inoculated SDA plates, with the ventral nail surface contacting the agar gel. The set-up was incubated at 32°C for 7 days, and the diameter of the zone of inhibition (ZoI) around the nail plate (if any) was determined using Image J software (version 1.48, from the National Institutes of Health, Bethesda, Maryland, USA). The negative and positive control experiments were conducted similarly, except for the use of a drug-free patch and of Curanail® (commercially-available lacquer containing amorolfine HCl) on the nail plates respectively.   Experiments were performed in triplicates.

2.7 Measurement of patch adhesion in volunteers

Following ethics approval (PID: 4359/001), five male and nine female participants aged 20-41 years with healthy nails and no skin conditions were recruited and informed consent were obtained. Participants were asked to remove any nail polish or artificial nails and to wash and dry the hands and feet before patch application. The in vivo study was conducted in 2 parts as follows:

Part A – to identify the optimal conditions for patch application.

 Five trials of patch application to the nails were investigated consecutively as the limitations of each method became apparent:

- 1st trial: square patch of 0.5 cm x 0.5 cm
- 2nd trial: circular patch of 0.8 diameter

- 3rd trial: patch was cut to the shape of each nail. The patches were cut into squares large enough to cover the entire nail plate, applied to the nail plate, and then the excess patch from around the nail plate was carefully cut off using nail scissors
- 4th trial: as for the 3rd trial, but the patch was applied at bedtime
- 5th trial: as for the 4th trial, followed by the application of a clear colourless nail varnish (supplied to volunteers) over the entire patch.

A patch formulation was applied to all ten fingernails and both big toenails of volunteers, and an average patch adhesion was calculated. Subsequently, the procedure for repeated for the 2nd trial, then the 3rd trial and so on.   Participants carried out daily activities as normal and the extent to which the patches remained on the nails was observed daily at approximately the same time for 2 weeks. Photographs of the nails were taken daily by participants and sent to the researcher via e-mail or a mobile messaging application. The researcher then estimated the percentage of nail patch that remained adhered on each of the fingernail and toenail, by visually dividing the nail plate into quadrants which were then further divided into sub-quadrants (Fig. 1 below) and calculating the average.
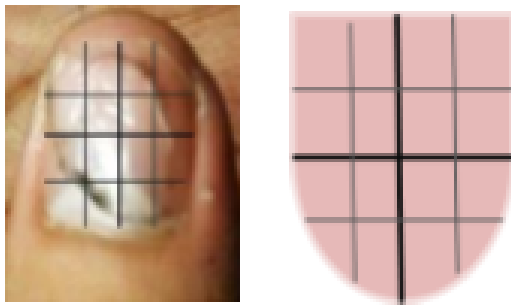

Figure 1: Visualisation of nail plate into quadrants, then sub-quadrants to measure % patch remaining adhered with time.

Part B – to identify the influence of patch formulation if any.

Three patches (ciclopirox olamine in Duro-Tak 202A, amorolfine HCl in Duro-Tak 2852 and amorolfine HCl in Duro-Tak 202A) were used and applied to volunteer fingernails and toenails

as per trials 4 and 5 above. Ciclopirox olamine in Duro-Tak 2852 was not used as ciclopirox olamine had been found to be unstable upon patch storage over many months (Rizi, Mohammed et al. 2018).

2.8 Statistical analysis

Statistical calculations were conducted using IBM SPSS 27 software. Data are expressed as mean ± standard deviation (SD) and the number of replicates for each experiment is shown in the Figure legends. Statistical significance was assessed using the Student's t-test to compare 2 groups and one way analysis of variance (ANOVA) and repeated measures ANOVA followed by Tukey for multiple group comparisons. In all cases, $p<0.05$ denotes significance.

3. Results

The prepared patches were simple drug-in-adhesive systems, consisting of a drug-loaded PSA (Duro-Tak 2852 or Duro-Tak 202A), sandwiched between a release liner (Scotchpak 9744) and an occlusive backing membrane (Scotchpak 9757). Amorolfine HCl and ciclopirox olamine (Figure 2, Table 1) were used as the model drugs, given that their formulations were the most effective topical preparations for many years, prior to the FDA-approval of Kerydin® and Jublia® in 2014. Drug loading in the patches is shown in Table 2; at these concentrations, the drugs were just below their crystallisation threshold and so were fully dissolved in the PSA (Rizi, Mohammed et al. 2018). Duro-Tak 2852 and Duro-Tak 202A (properties shown in Table 3) had been selected as the PSAs, due to their higher solvency for the two drugs compared to other PSAs tested (Rizi, Mohammed et al. 2018). Drug release, ungual permeation, in vivo adhesion and anti-fungal efficacy of the patches are reported below.
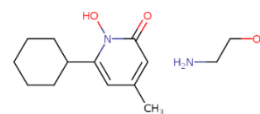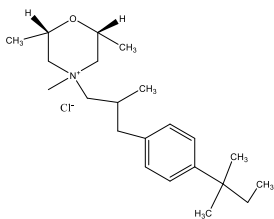
Ciclopirox olamine

Amorolfine HCl

Figure 2. Chemical structures of amorolfine HCl and ciclopirox olamine.

Table 1: Selected properties of the model drugs used.   [x]Chemistry Dashboard
https://comptox.epa.gov/dashboard); [+]DRUGBANK Online;
*https://www.fishersci.com/shop/products/amorolfine-hydrochloride-98-thermo-scientific/AC458752500; **http://www.chemspider.com/Chemical-Structure.35605.html?rid=2396e11a-3186-44a5-bae9-b62503c89528&page_num=0;

Drug	Amorolfine HCl	Ciclopirox olamine
Molecular mass[x]	354 Da	268 Da
Molar volume[x]	343 cm^3	174 cm^3
Log Partition Coefficient[+]	5.44; 5.62 [+]	2.15; 2.22 [+]
pKa[+]	8.49 (Strongest Basic) [+]	6.84 (Strongest Acidic) [+]
Melting Point*, **	221.0°C to 223.0°C*	143°C**

Table 2: The loading of the drugs in the four patches prepared.

PSA in patch	Drug loading (% w/w) in prepared patches	Drug concentration at which drug solubility in PSA is exceeded and crystals start to appear (% w/w)
Duro-Tak 2852	16% ciclopirox olamine	17
	5% amorolfine HCl	7
Duro-Tak 202A	5% ciclopirox olamine	6
	3% amorolfine HCl	4

Table 3: Properties of the two PSAs used in the patches. Surface energy data was from Rizi et al. 2018, Hansen Solubility Parameters values were from Hossin et al. 2016, while the other properties were from the material data sheets.

PSA Name	Chemical Nature	Functional group	viscosity (mPas)	Tg (°C)	Surface energy [mJ/m^2]	Hansen Solubility Parameters (MPa$^{\frac{1}{2}}$)		
						δ_D	δ_P	δ_H
Duro-Tak 202A	Acrylic	-OH	1700	-53	34.1	17.2 ±0.3	8.7 ±0.5	6.5 ±0.3
Duro-Tak 2852	Acrylic	-COOH	2500	-26	29.8	16.4 ±0.2	5.6 ±0.3	6.9 ±0.2

3.1 Drug release from patches

Drug release profiles from the four patches are shown in Figure 3. It can be seen that drug release was almost complete from 3 patches and almost negligible from one patch over the 8-day experiment. The fastest release was from the ciclopirox olamine-loaded Duro-Tak 202A, followed by ciclopirox olamine-loaded Duro-Tak 2852 and amorolfine HCl-loaded Duro-Tak 202 A. Meanwhile no drug was released from the amorolfine HCl-loaded Duro-Tak 2852.
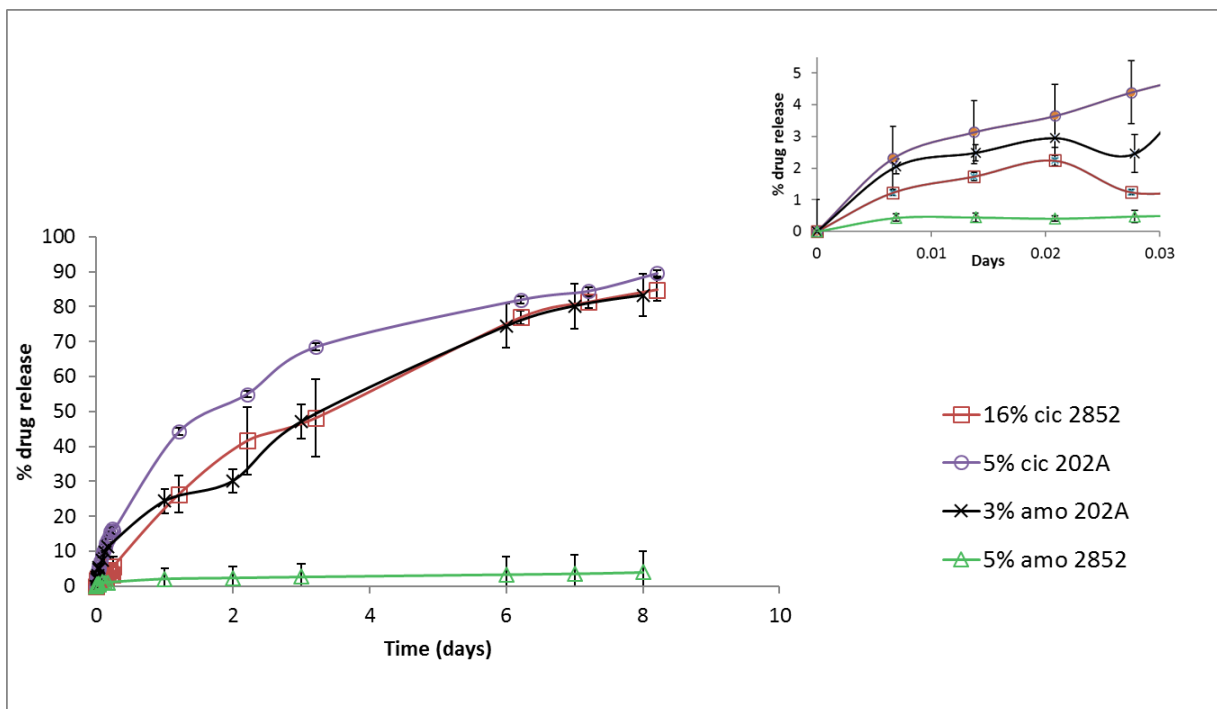
Figure 3: Drug release from a patch containing the drug dissolved in Duro-Tak 202A PSA or Duro-Tak 2852 PSA. Ciclopirox olamine in Duro-Tak 2852 PSA (□); Ciclopirox olamine in Duro-Tak 202A PSA (○), Amorolfine HCl in Duro-Tak 202A (x), Amorolfine HCl in Duro-Tak 2852 PSA (Δ). The inset shows release at the earliest time points. Ciclopirox olamine was loaded at 5% in the Duro-Tak 202A and at 16% in the Duro-Tak 2852. Amorolfine HCl was loaded at 3% in the Duro-Tak 202A and at 5% in the Duro-Tak 2852. Means and SD (error bars) are shown. N=3.

3.2 Ungual drug permeation from patches

Ungual drug permeation profiles from the four patches are shown in Figure 4. Ungual permeation of both drugs, from both PSAs was achieved. When the two drugs are compared, we observe greater amounts of ciclopirox olamine (almost double) permeating through the nail plates compared to amorolfine HCl. When the two PSAs are compared for each drug, a negligible influence of PSA nature is found (repeated measures ANOVA, $p > 0.05$).
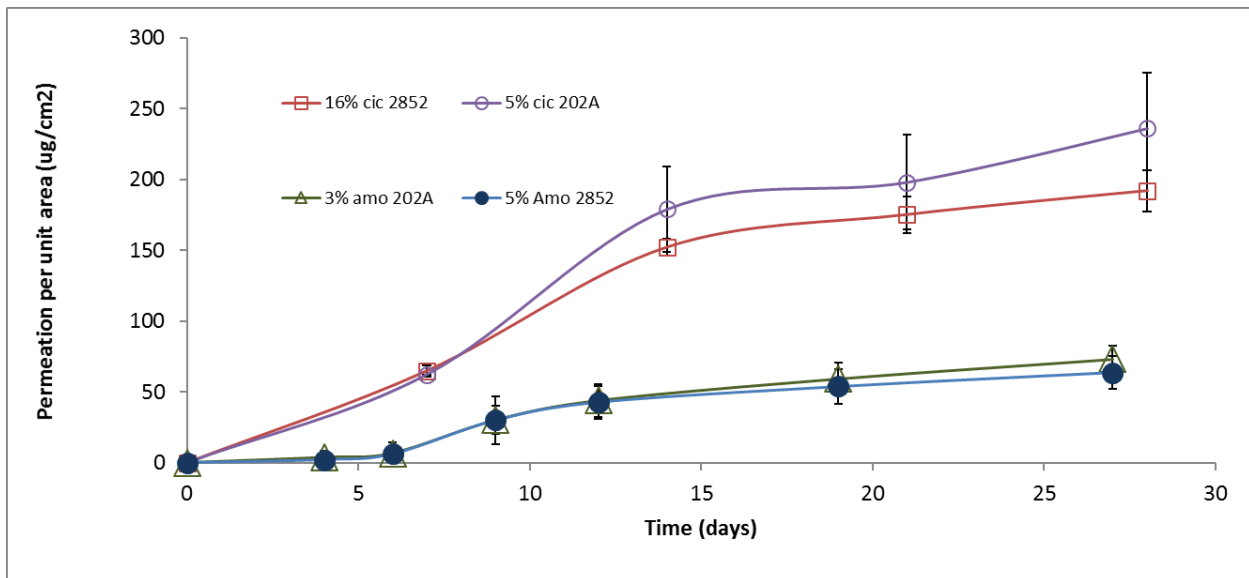
Figure 4: Drug (amorolfine HCl or ciclopirox olamine) permeation from a patch containing the drug dissolved in Duro-Tak 202A PSA or Duro-Tak 2852 PSA, through nail plates. Ciclopirox olamine in Duro-Tak 2852 PSA (□); Ciclopirox olamine in Duro-Tak 202A PSA (o), Amorolfine HCl in Duro-Tak 202A (Δ), Amorolfine HCl in Duro-Tak 2852 PSA (●). Ciclopirox olamine was loaded at 5% in the Duro-Tak 202A and at 16% in the Duro-Tak 2852. Amorolfine HCl was loaded at 3% in the Duro-Tak 202A and at 5% in the Duro-Tak 2852. Means and SD (error bars) are shown. N=6.

3.3 *In vivo* adhesion of patches to nail plate in volunteers

In vivo patch adhesion to finger and toe nails over a period of two weeks was tested in 5 sequential experiments as we sought to improve patch residence from trial 1 (square shaped patch) to trial 2 (circular patch) to trial 3 (patch cut to nail shape) to trial 4 (patch cut to nail shape and applied at bedtime) to trial 5 (patch cut to nail shape, applied at bedtime and covered by a layer of clear nail varnish) as shown in Figure 5. Due to the negligible influences of the left versus right hands, left versus right foot and hand/foot digit on in vivo adhesion, the data was averaged for the 10 fingernails and two big toenails. For both fingernails and toenails, in vivo residence improved when a square-shaped patch was replaced by a circular patch, and was further improved when the patch was cut to the shape of the nail, applied at bedtime and when a nail varnish was applied on top of the patch (Figure 5).
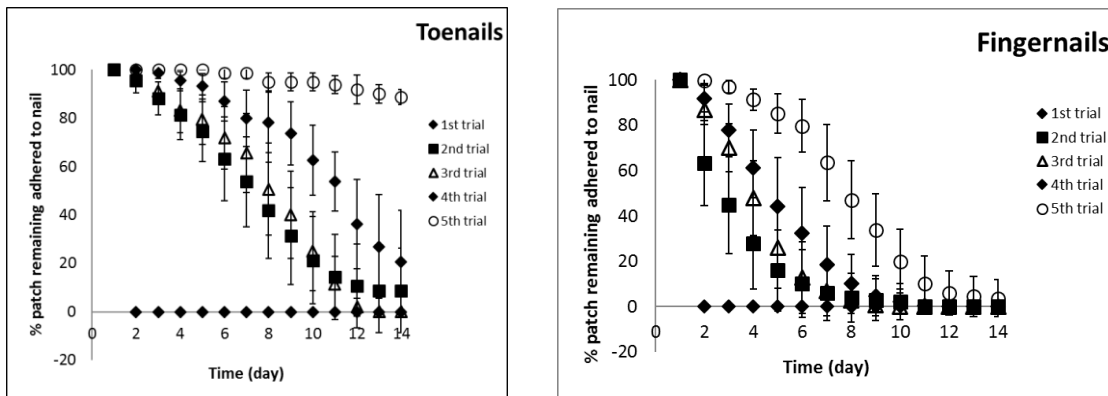
Figure 5: *In vivo* residence of patches on the toenails and fingernails in volunteers; means and standard deviations are shown. 1st trial was with a square patch; 2nd trial with a circular patch; 3rd trial was with a patch cut to the shape of the nail; 4th trial was like the 3rd trial but the patch was applied at bedtime; 5th trial was like the 4th trial, followed by application of a colourless nail varnish over the patch.

When the influence of the nature of the patch was investigated using patches cut to the shape of the nail, applied at bedtime, followed by an application of nail varnish, or not, there were significant differences among the 3 patches (Figure 6), both when nail varnish was applied on top of the patch and when nail varnish was not applied (repeated measures ANOVA, $p \leq 0.01$). On fingernails, Patch 1 (3% amorolfine HCl in Duro-Tak 202A) had a greater ($p < 0.01$) in vivo residence than Patch 3 (5% ciclopirox olamine in Duro-Tak 202A) in the absence of applied nail varnish, while when nail varnish was applied, Patch 1 had a greater residence than both Patches 2 (5% amorolfine HCl in Duro-Tak 2852) and 3 ($p < 0.0005$). On toenails, Patch 1 had a greater residence than Patch 2 in the absence or the presence of nail varnish ($p \leq 0.01$), but had a similar residence to Patch 3 in the absence ($p = 0.052$) or the presence of nail varnish ($p > 0.05$). Overall Patch 1 seems to the best with Patch 2 the worst, in terms of in vivo residence.
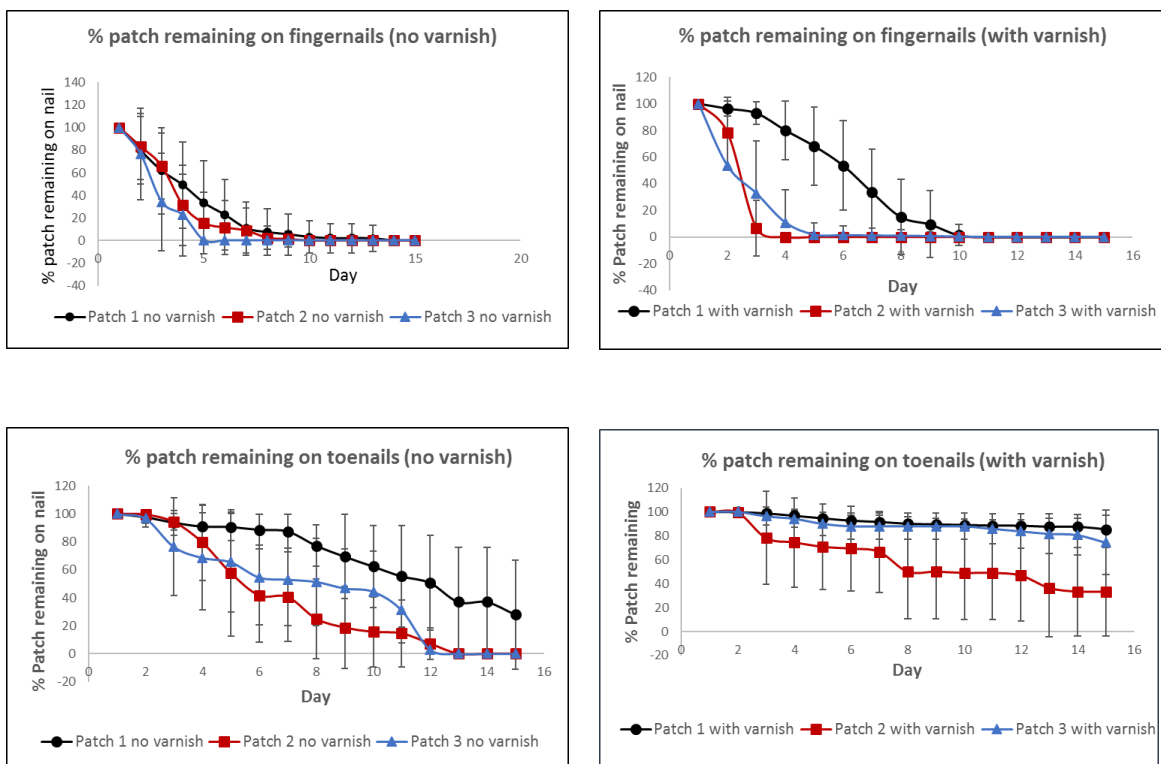
Figure 6: *In vivo* residence of patches on the fingernails and toenails in 14 volunteers. The patch was cut to the shape of the nail and applied at bedtime, followed (or not) by the application of a clear, colourless nail varnish over the patch. Patch 1 is 3% amorolfine HCl in Duro-Tak 202A; Patch 2 is 5% amorolfine HCl in Duro-Tak 2852; Patch 3 is 5% ciclopirox olamine in Duro-Tak 202A. Means (n=20-40 for fingernails: 4-8 for toenails) and standard deviations are shown.

3.4 Anti-fungal efficacy of patches applied to nail plate

The anti-fungal efficacy of nail patches was only investigated for amorolfine HCl due to the instability of ciclopirox olamine in Duro-Tak 2852 PSA based patch as mentioned above (Rizi, Mohammed et al. 2018), and is shown in Figure 7. It can be seen that the amorolfine HCl-loaded Duro-Tak 202A patch inhibited the growth of *Trichophyton rubrum* (the most common onychomycosis causative organism (Ghannoum, Hajjeh et al. 2000)), to a similar extent to the commercially-available amorolfine HCl containing Curanail lacquer (used as a positive control), such that no fungal growth occurred in the petri dish. In contrast, the Duro-Tak 2852 was ineffective at inhibiting fungal growth.
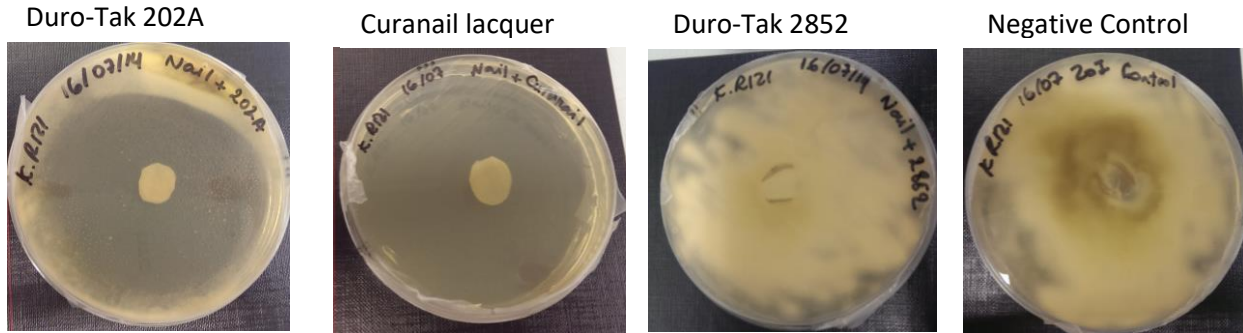
Figure 7: Representative photographs of petri dishes showing inhibition of fungal growth by amorolfine-loaded Duro-Tak 202A patch (similar to the commercially-available amorolfine-loaded Curanail nail lacquer). In contrast amorolfine-loaded Duro-Tak 2852 did not inhibit fungal growth (and was similar to the negative control). N=3

Discussion

Drug release profiles (Figure 3) indicate a considerable influence of the PSA and of the drug. Amorolfine HCl was released from Duro-Tak 202A, but not from a Duro-Tak 2852 patch, where the drug crystallised when the amorolfine-loaded Duro-Tak 2852-based patch was immersed in an aqueous medium, shown by light microscopy of the patches. Duro-Tak 202A also released ciclopirox olamine to a greater extent compared to Duro-Tak 2852, especially at the earlier time points, until day 6, when drug release became similar from the 2 PSA-based patches. Greater release from Duro-Tak 202A compared to Duro-Tak 2852 can be explained by the PSAs' mechanical properties. The two PSAs have very different stress-strain curves with Duro-Tak 2852 showing a considerably greater modulus while Duro-Tak 202A is much more flexible (Rizi, Mohammed et al. 2018). Greater polymer chain flexibility enables higher drug release, and has also been reported for other patches (Li, Quan et al. 2017)

When the influence of drug nature was considered by examining drug release from Duro-Tak 202A based patch, ciclopirox olamine was found to be released at a faster rate, especially at the earlier timepoints. This was despite the fact that the two drugs had similar thermodynamic activities, i.e. the drugs were at almost saturation solubility in the patches. The lower release of

amorolfine HCl is most likely due to its greater molar volume (343 vs 174 cm^3 i.e. almost twice that of ciclopirox olamine), drug diffusion coefficient in a polymer being known to be inversely related to its molar volume.

Ungual drug permeation profiles (Figure 4) might have been expected to reflect the respective drug release profiles (Figure 3). However this was not observed. The most surprising observation was ungual permeation of amorolfine HCl from Duro-Tak 2852 based patch, despite the fact that release experiments showed no drug release from this patch. This cautions us against using release experiments to screen formulations for inclusion/exclusion in further development work as they do not accurately predict ungual permeation. As stated in Section 3.1, the lack of amorolfine HCl release from Duro-Tak 2852 was due to drug crystallisation when the patch was in contact with aqueous medium during release experiments. It appears that such drug crystallisation did not occur during ungual permeation experiments, and dissolved drug was able to be released from the patch and permeate into and through the nail plate. The major difference between release and permeation experimental setups was patch application to a nitrocellulose membrane in release experiments, and to nail plates in permeation experiments. The nitrocellulose membrane is not a barrier to water and the patch would have been in direct contact with the aqueous receptor phase during release experiments, which led to crystallisation of amorolfine HCl in the Duro-Tak 2852 patch. In contrast, the nail plate is a barrier, and although it absorbs water and would have been hydrated during permeation experiments, the patch would not have been in direct contact with bulk water. This might explain why amorolfine HCl did not crystallise from Duro-Tak 2852 patch during permeation experiments, and was therefore available for permeation.

When the two drugs are compared, we observe greater amounts of ciclopirox olamine permeating through the nail plates compared to amorolfine HCl (Figure 4), which is most likely to be due to its smaller size (268 vs 354 Da) and lower lipophilicity (log P of 2.1 vs 5.3), ungual permeation being known to be inversely related to molecular size and lipophilicity of permeants (Mertin and Lippold 1997) (Mertin and Lippold 1997) (Kobayashi, Komatsu et al. 2004). The

much higher loading of ciclopirox olamine in the PSAs, which was 16% versus 5% (i.e. 0.06 mole versus 0.01 mole per 100g) in Duro-Tak 2852 and 5% versus 3% (i.e. 0.02 mole versus 0.01 mole per 100g) in Duro-Tak 202A could also have played a part, presenting a higher concentration gradient for drug release from the patch, ungual permeation and diffusion through the nail. However, as the patches were all at just below 100% thermodynamic activities, the differential drug loading may have played a less important role. Similarly, given that both ciclopirox and amorolfine have a high affinity for the nail plate and bind to great extents to keratin (Tatsumi, Yokoo et al. 2002, Sugiura, Sugimoto et al. 2014, Hossin, Rizi et al. 2016), drug binding to the nail plate is not expected to influence the greater permeation of ciclopirox seen in Figure 4.

When the two PSAs are compared, a negligible influence of PSA nature is found. Similar permeation profiles of ciclopirox olamine from Duro-Tak 2852 and Duro-Tak 202A despite a difference in drug release (Figures 3-4) shows that drug permeation through the nail plate was being controlled by the nail plate itself, rather than by the drug released from the patch. This is not surprising since the nail plate is known to be extremely poorly permeable to topically-applied drugs, such that only a few percent of applied drugs permeate into and through it (Murdan 2002, Murdan 2008) (vanHoogdalem, vandenHoven et al. 1997).

In vivo **ungual adhesion of patches** (Figures 5-6) showed that, although the patch did not remain adhered at 100% throughout the 2-week study, the patches did adhere to nail plates for several days, as we had previously predicted (Rizi, Mohammed et al. 2018), based on their lower surface energies (~30 mJ/m^2) compared to that of the human nail plate (34.1 ± 5.5 mJ/m^2; (Murdan, Poojary et al. 2012), reflecting the need for the same or lower surface energy of the adhesive compared to that of the substrate for good intrinsic adhesion, (Venkatraman and Gale 1998).

The extent of patch adhesion was clearly dependent on the patch shape, timing of patch application, whether the applied patch was 'sealed' by a layer of nail varnish and whether the

patch was applied to finger or toe nails. Square-shaped patches (in trial 1) were rapidly removed as their four corners caught on materials that were handled or rubbed against socks/shoes. Circular patches (in trial 2) did not have corners, so had a longer residence than the square patches. However their edge still provided a route of entry for extraneous materials from the environment, and the dirt/fluff collected led to the patch peeling off the nail with time. Patches that were cut to the nail shape (in trial 3) had their edges closer to the nail folds, and thus were less susceptible to catching on material being handled or to rubbing against socks and bedding, which increased their residence compared to the circular patches. Application of patches at bedtime (in trial 4) reduced the immediate patch exposure to environmental material/socks/shoes, and enabled the patch adhesive to bond more effectively to the nail, increasing the patch residence. Finally, application of a nail lacquer on top of the patch (in trial 5) further protected the patch edges, so that trial 5 showed the longest patch residence.

Longer patch adhesion on toe nails compared to fingernails (Figures 5-6) reflect the much greater interaction of the hands with the environment and exposure to water e.g. during washing, which would negatively affect patch residence.   When the influence of the patch nature on in vivo residence was considered, differences among the three patches were small in magnitude albeit statistically significant. Patch 1 (3% amorolfine HCl in Duro-Tak 202A) seems to be the best, followed by Patch 3 (5% ciclopirox olamine in Duro-Tak 202A) with Patch 2 (5% amorolfine HCl in Duro-Tak 2852) being the worst (Figure 6). This was somewhat surprising. In vitro measurements showing higher tack strength of Duro-Tak 2852 compared to Duro-Tak 202A (Rizi, Mohammed et al. 2018) had indicated higher forces would be needed to remove the Duro-Tak 2852-based patch. The mismatch shows that other factors, such as the greater tack work of adhesion of Duro-Tak 202A (Rizi, Mohammed et al. 2018) was more important for patch adhesion as the Duro-Tak 202A adhesive deformed to a greater extent with lesser loss of contact with the nail when the patch was worn in practice and exposed to various mechanical stresses.

Ideally, patch adhesion should be 100% for the desired duration, i.e. until the wearer peels off the patch. In these experiments, for the best patch, 92% adhesion on toenails was achieved on Day 7, dropping to 85% on Day 14 for the optimal application. Thus, it may be possible to wear these patches, with weekly or fortnightly replacement, for toenail onychomycosis, which is more common than fingernail onychomycosis (Midgley, Moore et al. 1994). For fingernail infections, these patches are less acceptable and need further optimisation to increase their adhesion.

Anti-fungal efficacy of patches applied to nail plate. A clear dependence of the PSA nature was seen, with amorolfine HCl- loaded Duro-Tak 202A patch inhibiting fungal growth in contrast to the Duro-Tak 2852 patch which was ineffective (Fig. 7). Failure of Duro-Tak 2852 patch indicates that insufficient drug moved out of the patch into the nail, then out of the nail and into the agar gel. It is unclear why this should be so, given that the ungual permeation experiments showed almost identical permeation profiles from the two patches (Figure 4). Once the experiment is set up, i.e. patch is applied on nail which is then placed on agar gel in the anti-fungal efficacy experiment, or on the aqueous receptor phase in the permeation experiment, the hydration level of the nail plate is expected to equilibrate with that of the agar gel and with the aqueous receptor phase respectively. It is possible that the nail plate becomes less hydrated when placed on the agar gel, compared to the liquid aqueous receptor phase, and that the lower nail plate hydration could be resulting in lesser drug permeation through the nail plate and into the agar gel; nail plate hydration being known to positively influence ungual drug permeation (Gunt and Kasting 2007). However a lower nail plate hydration, if this happened, would be expected for both patches which comprise the same occlusive backing membrane, and does not explain the extreme difference in anti-fungal activities of the patches. The lack of anti-fungal efficacy of amorolfine-loaded Duro-Tak 2852 reflects the negligible drug release seen in Figure 3, which was related to drug crystallisation in the patch. It is possible that drug crystallisation also occurred in the anti-fungal efficacy experiments, although this was difficult to confirm microscopically following the experiments.

Conclusion

With the ultimate goal of developing ungual patches for the topical treatment of onychomycosis, we previously formulated ungual patch formulations, following the identification of the appropriate PSAs, backing membranes and release liner and optimisation of patch preparation. In this paper, we have further investigated drug release, ungual drug permeation, anti-fungal efficacy, and in vivo adhesion to the human nail plate, and any influence of drug and PSA nature. As expected the nature of the drug and of the PSA influenced these parameters. However, the influences did not always align. For example, in vitro anti-fungal efficacy could have been predicted by the release studies, but not by the ungual permeation experiments, while ungual permeation could not have been predicted by the release experiments. Of the three patches tested for in vivo residence on the nail, one was clearly a more adhesive formulation, and stayed almost fully adhered to toenails for one-two weeks, showing promise for further development.

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