Topical delivery of clofazimine, artemisone and decoquinate utilizing vesicles as carrier system

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If we knew what we were doing, it would not be called research, would it?

-Albert Einstein

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ABSTRACT

Artemisone, clofazimine and decoquinate are part of the MALTBRedox MRC South African University Flagship Projects, which focus on oxidant-redox drug combinations for the treatment of tuberculosis and a few other diseases. These active pharmaceutical ingredients (APIs) were chosen as a possible treatment of cutaneous tuberculosis (CTB), an uncommon and undefined disease that is often misdiagnosed (Abdelmalek *et al.*, 2013; Baig *et al.*, 2014; Fader *et al.*, 2010). Currently CTB is only treated with regular oral anti-tuberculous medication, with occasional invasive procedures such as skin grafts (Yates, 2010).

Artemisone, clofazimine and decoquinate have a log P of 2.49, 7.7 and 7.8, respectively (Biamonte *et al.*, 2013; Dunay *et al.*, 2009; Nagelschmitz *et al.*, 2008; Steyn *et al.*, 2011). A high log P-value indicates that the API is highly lipophilic and therefore a delivery system, namely vesicles, was chosen to improve skin permeation. Many vesicles are currently being investigated all over the world as carriers for APIs in topical delivery, though for this study liposomes, niosomes and transferosomes were selected.

Dispersions containing a single API, a combination of all three APIs, as well as no API, were prepared for all three types of vesicles. Characterisation of dispersions containing 0.2%, 0.4% and 1% API was performed. Isothermal calorimetry indicated that no incompatibility occurred in the 1% API combination dispersions, except the niosome dispersion, which indicated a probable incompatibility. Encapsulation efficiency was above 85% for all 1% API dispersions. The empty vesicles depicted an average size of 154 nm, 167.5 nm and 106.3 nm for liposomes, niosomes and transferosomes, respectively. Vesicle sizes increased with increase in API concentration, whereas stability decreased. Clofazimine was found to have the most significant impact on vesicle size and stability when added as 1%, increasing the average niosome size to 2 461 nm. Viscosity was below 2 mPa.s for all 1% API dispersions, ensuring even spreadability when applied to the skin. The pH of all the dispersions were between 5–6, thus limiting skin irritation.

In vitro transdermal diffusion studies were conducted on black skin, using dispersions containing 1% of all three APIs. No APIs could be detected in the receptor phase. Artemisone was not detected in the skin by means of HPLC analysis, which might be due to the fact that the concentration was below the limit of detection (LOD). The LOD for artemisone was determined at 4.42 µg/ml, whereas it was 0.042 µg/ml for clofazimine and 0.703 µg/ml for decoquinate. Higher API concentrations were present in the stratum corneum-epidermis (SCE), compared to in the epidermis-dermis (ED) for all the dispersions. Transferosomes delivered the highest

concentration clofazimine into the SCE and ED, as well as the highest concentration decoquinate into the ED. The highest concentration decoquinate in the SCE, however, was obtained by the niosome dispersion.

Efficacy against tuberculosis of the APIs (1%) encapsulated in vesicles was tested on strain H37Rv. All dispersions were found to be effective to some degree against the tuberculosis strain tested, with clofazimine in niosomes being the most effective with 52% growth inhibition. The least effective was decoquinate in niosomes, with only 8% inhibition. The combination dispersions delivered inhibitions of 42%, 38% and 12% for liposomes, niosomes and transferosomes, respectively. Surprisingly, it was found that the vesicle dispersions containing no APIs also presented some efficacy against the tuberculosis strain tested.

New knowledge contributed to pharmaceutics by this study includes encapsulating the three APIs in liposomes, niosomes and transferosomes and successfully delivering them into the skin as proved by transdermal diffusion studies. Developing an HPLC method for the concurrent analysis of the three APIs and determining the activity of the vesicle dispersion against the specific tuberculosis strain tested also contributed new knowledge. Results indicated that decoquinate, an API never before considered for tuberculosis, does have anti-tuberculous activity. No significant increase in efficacy against the tuberculosis strain was noted when combining the three APIs in a vesicle dispersion, compared to when the APIs were incorporated separately into the vesicles, though the blank vesicles had surprisingly high activity against the specific tuberculosis strain tested.

Keywords: Clofazimine, artemisone, decoquinate, liposomes, niosomes, transferosomes, transdermal

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UITTREKSEL

Artemisoon, klofasimien en dekokwinaat is deel van die MALTBRedox MRC Suid-Afrikaanse Universiteit Flagship Projekte wat fokus op oksidasie-reduksie geneesmiddelkombinasies vir die behandeling van tuberkulose en ’n paar ander siektes. Hierdie geneesmiddels is gekies vir moontlike behandeling van kutaneuse tuberkulose (KTB), ’n ongewone en ongedefinieerde siekte wat dikwels verkeerd gediagnoseer word (Abdelmalek *et al.*, 2013; Baig *et al.*, 2014; Fader *et al.*, 2010). Tans word KTB slegs behandel met gewone orale anti-tuberkulose-medisyne, en soms met indringende prosedures soos veloorplantings (Yates, 210).

Artemisoon, klofasimien en dekokwinaat besit ’n log P van 2.49, 7.7 en 7.8, onderskeidelik (Biamonte *et al.*, 2013; Dunay *et al.*, 2009; Nagelschmitz *et al.*, 2008; Steyn *et al.*, 2011). ‘n Hoë log P dui op ’n sterk lipofiliese geneesmiddel en om hierdie rede is ‘n afleweringsisteem, naamlik vesikels, gekies om veldeurlaatbaarheid te verbeter. Baie vesikels word tans reg oor die wêreld ondersoek as draers van geneesmiddels vir topikale aflewering, maar vir hierdie studie is liposome, niosome en transferosome geselekteer.

Dispersies met ’n enkele geneesmiddel, ’n kombinasie van al drie geneesmiddels, sowel as geen geneesmiddel, is voorberei vir al drie tipes vesikels. Karakterisering van dispersies wat 0.2%, 0.4% en 1% geneesmiddel bevat, is uitgevoer. Isotermiese kalorimetrie-resultate het aangetoon dat geen onverenigbaarhede voorkom in die 1% geneemiddeldispersie nie. Resultate verkry vanaf die niosoomdispersie het egter op ’n moontlikheid van onverenigbaarheid gedui. Enkapsuleringeffektiwiteit was bo 85% vir alle 1% geneesmiddeldispersies. Die leë vesikels het ’n gemiddelde grootte van 154 nm, 167.5 nm en 106.3 nm gehad vir liposome, niosome en transferosomes, onderskeidelik. Vesikelgrootte het toegeneem met ’n toename in geneesmiddelkonsentrasie, terwyl stabiliteit afgeneem het. Dit is gevind dat klofasimien die grootste impak gehad het op vesikelgrootte en stabiliteit wanneer dit bygevoeg is in ’n 1% konsentrasie, met ’n gemiddelde vesikelvergroting tot 2 461 nm. Viskositeit was onder 2 mPa.s vir alle 1% geneesmiddeldispersies, wat eweredige spreibaarheid sal verseker tydens aanwending op die vel. Die pH van al die dispersies was tussen 5–6, wat vel-irritasie beperk.

In vitro transdermale-afleweringstudies is uitgevoer op swart vel, deur van dispersies gebruik te maak wat 1% van al drie geneesmiddels bevat. Geen geneesmiddel is waargeneem in die reseptorfase nie. Artemisoon kon nie in die vel opgespoor word met behulp van die HPLC-metode nie, wat moontlik verduidelik kan word deur die feit dat die konsentrasie onder die opsporingslimiet was. Die opsporingslimiet van artemisoon is bepaal as 4.42 µg/ml, terwyl dit

0.042 µg/ml vir klofasimien en 0.703 µg/ml vir dekokwinaat is. Hoër konsentrasies van die geneesmiddels was wel teenwoordig in die stratum korneum-epidermis (SKE) in vergelyking met die epidermis-dermis (ED) vir alle dispersies. Transferosome het die hoogste konsentrasie klofasimien afgelewer in die SKE en ED, sowel as die hoogste konsentrasie dekokwinaat in die ED. Die hoogste konsentrasie dekokwinaat in die SKE is egter verkry deur die niosoomdispersie.

Effektiwiteit van die geneesmiddels (1%) ingesluit in vesikels is getoets teen die spesifieke bakteriële stam van tuberkulose teen die H37RV variasie. Daar is gevind dat al die dispersies effektiwiteit toon, hoewel in 'n klein mate; met klofasimien in niosome die effektiefste met 52% groei-onderdrukking. Die laagste effektiwiteit teen die spesifieke tuberkulose-stam is getoon deur dekokwinaat in niosome met 8% onderdrukking. Die kombinasie-dispersies het onderdrukkings van 42%, 38% en 12% gelewer vir liposome, niosome en transferosomes, onderskeidelik. Verbasend is daar gevind dat die vesikeldispersies wat geen geneesmiddels bevat het nie, ook 'n mate van effektiwiteit getoon het.

Nuwe kennis wat bydra tot Farmaseutika deur hierdie studie, sluit in die enkapsulering van die drie geneesmiddels in liposome, niosome en transferosome, asook die suksesvolle aflewering daarvan in die vel soos bepaal deur transdermale afleweringsstudies. Ontwikkeling van 'n HPLC-metode vir die gesamentlike analise van die drie geneesmiddels, asook die getoetste aktiwiteit van die vesikeldispersies teen die spesifieke tuberkulose-stam, dra ook by tot nuwe kennis. Resultate het aangedui dat dekokwinaat, 'n geneesmiddel wat nooit voorheen oorweeg is teen tuberkulose nie, wel anti-tuberkulose-aktiwiteit besit. Geen merkwaardige toename in effektiwiteit teen tuberkulose is waargeneem wanneer die drie geneesmiddels gekombineer is in 'n vesikeldispersie, teenoor wanneer die geneesmiddels apart ingesluit is in die vesikels nie, alhoewel die blanko-vesikels verbasend hoë aktiwiteit teen die spesifieke tuberkulose-stam getoon het.

Sleutelwoorde: Klofasimien, artemisoon, dekokwinaat, liposome, niosome, transferosome, transdermaal

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