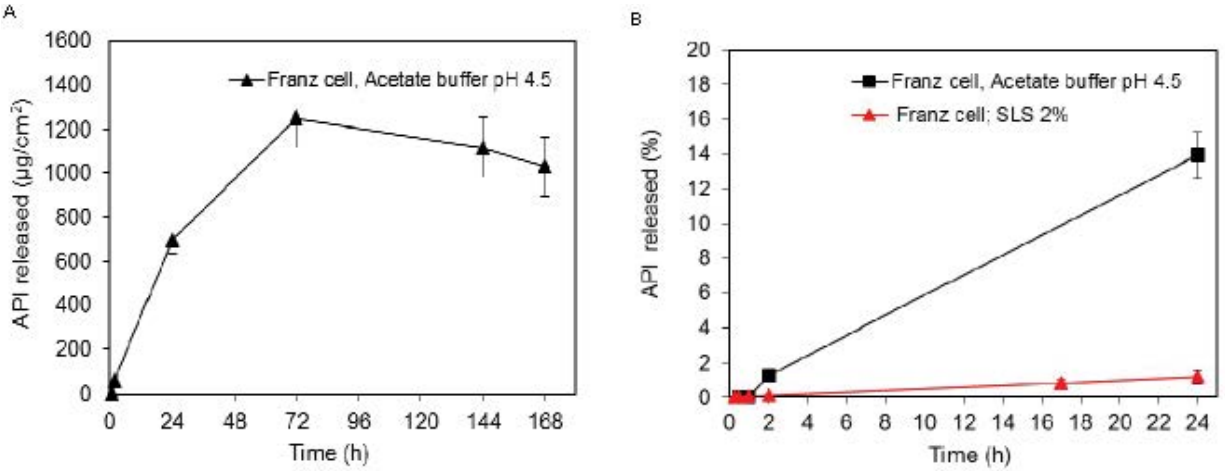

Figure 6: TNP-470 permeability results through the pig skin. **A:** TNP-470 solution release amount through the pig skin during the diffusion test in buffer acetate pH-4.5 medium, in µg/cm²; **B:** TNP-470 solution release amount through the pig skin during the diffusion test in buffer acetate pH-4.5 (stabilizing medium) compared to SLS 2% (destabilizing medium), in % from the initial content of TNP-470.

developed a simple analytical HPLC method for assay, dissolution and impurity studies of TNP-470. The TNP-470 peak parameters, symmetry factor and number of theoretical plates, conformed a common USP requirements (Figure 1A). According to the calibration curve linearity factor, R^2=0.9995, the developed method was linear for a wide range of TNP-470 concentrations (Figure 1B). The repeatability test, the RSD for 5 injections was found less than 2% which also conformed to common USP system suitability test requirements. Additionally, the method was found robust for a wide range of chromatographic parameters: injection volume, mobile phase, flow rate and different types of column, which enables its wide and universal application.

In this study, we investigated the stability and degradation of TNP-470 in 10 different mediums. The specific list of mediums was selected both for analytical purposes, to find suitable dissolution medium for *in vitro* skin penetration studies and for future formulation development, to find stabilizing and destabilizing factors for TNP-470. According to TNP-470 stability results we can sort the mediums to two main categories, stabilizing and destabilizing mediums (Figures 2 and 3). The first group of destabilizing mediums, which included SLS 2%, SLS 4%, Tween 80 4%, acetic acid 0.1M and citric acid solution 0.5%, characterized by intensive decomposition of TNP-470 followed by degradation product peak formation which was detected at relative retention time RRT-1.5 related to TNP-470 (Figure 2).

The second group of stabilizing mediums, which included MCT 4%, ethanol 30%, buffer acetate pH-4.5 ethanolic solution 70%, EDTA 2%, acetate buffer pH-4.5, characterized by moderate degradation of TNP-470 followed by insignificant degradant peak formation (Figure 3). It is known that TNP-470 is stable in organic solvents and undergoes rapid hydrolysis in the presence of water [26,27,33] which explains its rapid degradation in aqueous solution of SLS 2%, SLS 4% and Tween 80 4% and relative stability in ethanol 30%. The stability of TNP-470 in MCT could be explain by solubility of highly lipophilic drug TNP-470 in MCT, lipid compound and it is supposed that water could not so easily access the TNP-470 surrounded by fat and oil molecules. Our stability results of TNP-470 in MCT 4% emulsion supports previous microspheres formulation development containing TNP-470 with MCT performed by Kakinoki et al. [34].

Relative stability of TNP-470 in buffer acetate pH-4.5, EDTA 2% and buffer acetate pH-4.5 ethanolic solution 70% led us to believe that we found stabilizing agent of TNP-470 acetate ion, CH3COO−, which presented in all 3 solutions. Unfortunately, this hypothesis disproved by rapid degradation of TNP-470 in acetic acid 0.1M, pH-2.8. This fact led us to understanding that the main stabilizing factors of TNP-470 in aqueous solutions is pH. Our findings correlated to previous study performed by Figg et al. suggested that TNP-470 would be most stable in a slightly acidic medium, pH 4-5 [27]. Although the stability of TNP-470 was almost equivalent in MCT 4%, buffer acetate pH-4.5 and buffer acetate pH-4.5 ethanolic solution 70%, we selected buffer acetate pH-4.5 as dissolution medium for *in vitro* skin permeation studies because it is common buffer solution accepted by US pharmacopeia and most approximate to physiological environment conditions.

We performed TNP-470 *in vitro* skin permeation study using Franz cell method in selected dissolution medium, stabilizing buffer acetate pH-4.5 and compared the results to the destabilizing medium, SLS 2%. Skin's protective function provides an outstanding transdermal barrier which limits the penetration of drugs through the skin and usually most transdermal patches after removal contain at least 95% of the total amount of drug initially in the patch, which means that only 5% from the initial amount permeate the skin [19,20]. In light of this facts, the TNP-470 permeability results through the pig skin, 1250 µg/cm² after 72 hours which is 25% from the initial amount (Figure 6A), demonstrate excellent delivery of TNP-470 via transdermal route. The great difference between permeability results in two different dissolution mediums, stabilizing buffer acetate pH-4.5 and destabilizing SLS 2%, highlights the significance of TNP-470 stability studies and the importance of appropriate dissolution medium selection. In the acetate buffer pH-4.5 the permeated amount of TNP-470 after 24 hours was 14% compared to only 1% in SLS 2% (Figure 6B). It is obvious that the negligible amount of TNP-470 detected in SLS 2% was associated with degradability of TNP-470 in this medium and well correlated to stability studies we performed where destabilizing mediums group which included SLS 2% characterized by intensive decomposition of TNP-470.

Conclusion

This study demonstrates a simple reliable method for skin permeation studies of TNP-470, a potent anti-angiogenic drug, using Franz diffusion cells and HPLC detection methods. Our results introduce TNP-470 excellent candidature to transdermal drug delivery, which can offer a novel and promising clinical usage for potent anti-angiogenic drug to improve its poor bioavailability and safety profile, caused by peak concentration of the drug in plasma. Additionally, our study provides a broad data of TNP-470 stability in different mediums, which can lead to stable and improved formulation development in future. Taking together our findings can lead to the development of slow release transdermal delivery system, which may be used as a long-term maintenance therapy for angiogenesis-dependent diseases such as cancer, psoriasis, rheumatoid arthritis and age-related macular degeneration.

Acknowledgement

This research was partially supported by Israel Science Foundation (ISF), no. 0394883.

References

1. Folkman J (2007) Angiogenesis: an organizing principle for drug discovery. Nat Rev Drug Discov 6: 273-286.
2. Miller JW, Adamis AP, Shima DT, D'Amore PA, Moulton RS, et al. (1994) Vascular endothelial growth factor/vascular permeability factor is temporally and spatially correlated with ocular angiogenesis in a primate model. Am J Pathol 145: 574-584.
3. Folkman J (1997) Angiogenesis and angiogenesis inhibition: An overview. Exs 79: 1-8.
4. Ingber D, Fujita T, Kishimoto S, Sudo K, Kanamaru T, et al. (1990) Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. Nature 348: 555-557.
5. Figg WD, Pluda JM, Lush RM, Saville MW, Wyvill K, et al. (1997) The pharmacokinetics of TNP-470, a new angiogenesis inhibitor. Pharmacotherapy 17: 91-97.
6. Chun E, Han CK, Yoon JH, Sim TB, Kim YK, et al. (2005) Novel inhibitors targeted to methionine aminopeptidase 2 (MetAP2) strongly inhibit the growth of cancers in xenografted nude model. Int J Cancer 114: 124-130.
7. Griffith EC, Su Z, Niwayama S, Ramsay CA, Chang YH, et al. (1998) Molecular recognition of angiogenesis inhibitors fumagillin and ovalicin by methionine aminopeptidase 2. Proc Natl Acad Sci USA 95: 15183-15188.
8. Bhargava P, Marshall J, Rizvi N, Dahut W, Yoe J, et al. (1999) A phase I and pharmacokinetic study of TNP-470 administered weekly to patients with advanced cancer. Clin Cancer Res 5: 1989-1995.
9. Herbst R, Madden T, Tran H, Blumenschein GJ, Meyers C, et al. (2002) Safety and pharmacokinetic effects of TNP-470, an angiogenesis inhibitor, combined with paclitaxel in patients with solid tumors: evidence for activity in non-small-cell lung cancer. J Clin Oncol 20: 4440-4447.
10. Kruger E, Figg WD (2000) TNP-470: an angiogenesis inhibitor in clinical development for cancer. Expert Opin Investig Drugs 9: 1383-1396.
11. Kudelka A, Levy T, Verschraegen C, Edwards C, Piamsomboon S, et al. (1997) A phase I study of TNP-470 administered to patients with advanced squamous cell cancer of the cervix. Clin Cancer Res 3: 1501-1505.
12. Kudelka A, Verschraegen C, Loyer E (1998) Complete remission of metastatic cervical cancer with the angiogenesis inhibitor TNP-470. N Engl J Med 338: 991-992.
13. Logothetis C, Wu K, Finn L, Daliani D, Figg W, et al. (2001) Phase I trial of the angiogenesis inhibitor TNP-470 for progressive androgen-independent prostate cancer. Clin Cancer Res 7: 1198-1203.
14. Moore JD, Dezube BJ, Gill P, Zhou XJ, Acosta EP, et al. (2000) Phase I dose escalation pharmacokinetics of O-(chloroacetylcarbamoyl) fumagillol (TNP-470) and its metabolites in AIDS patients with Kaposi's sarcoma. Cancer Chemother Pharmacol 46: 173-179.
15. Stadler W, Kuzel T, Shapiro C, Sosman J, Clark J, et al. (1999) Multi-institutional study of the angiogenesis inhibitor TNP-470 in metastatic renal carcinoma. J Clin Oncol 17: 2541-2545.
16. Tran H, Blumenschein GJ, Lu C, Meyers C, Papadimitrakopoulou V, et al. (2004) Clinical and pharmacokinetic study of TNP-470, an angiogenesis inhibitor, in combination with paclitaxel and carboplatin in patients with solid tumors. Cancer Chemother Pharmacol 54: 308-314.
17. Kruger EA, Figg WD (2000) TNP-470: an angiogenesis inhibitor in clinical development for cancer. Expert Opin Investig Drugs 9: 1383-1396.
18. Benny O, Fainaru O, Adini A, Cassiola F, Bazinet L, et al. (2008) An orally delivered small-molecule formulation with antiangiogenic and anticancer activity. Nature Biotechnology 26: 799-807.
19. Schoellhammer CM, Blankschtein D, Langer R (2014) Skin permeabilization for transdermal drug delivery: recent advances and future prospects. Expert Opin Drug Deliv 11: 393-407.
20. Gupta H, Babu RJ (2013) Transdermal delivery: product and patent update. Recent Pat Drug Deliv Formul 7: 184-205.
21. McLafferty E, Hendry C, Alistair F (2012) The integumentary system: anatomy, physiology and function of skin. Nurs Stand 27: 35-42.
22. Ita KB (2015) Chemical penetration enhancers for transdermal drug delivery-success and challenge. Curr Drug Deliv.
23. Jain A, Jain P, Kurmi J, Jain D, Jain R, et al. (2014) Novel strategies for effective transdermal drug delivery: a review. Crit Rev Ther Drug Carrier Syst 31: 219-272.
24. Lehman PA, Raney SG, Franz TJ (2011) Percutaneous absorption in man: in vitro-in vivo correlation. Skin Pharmacol Physiol 24: 224-230.
25. Bartosova L, Bajgar J (2012) Transdermal drug delivery in vitro using diffusion cells. Curr Med Chem 19: 4671-4677.
26. Whalen CT, Hanson GD, Putzer KJ, Mayer MD, Mulford DJ (2002) Assay of TNP-470 and its two major metabolites in human plasma by high-performance liquid chromatography-mass spectrometry. J Chromatogr Sci 40: 214-218.
27. Figg WD, Yeh HJ, Thibault A, Pluda JM, Itoh F, et al. (1994) Assay of the antiangiogenic compound TNP-470, and one of its metabolites, AGM-1883, by reversed-phase high-performance liquid chromatography in plasma. J Chromatogr 652: 187-194.
28. Ong VS, Stamm GE, Menacherry S, Chu S (1998) Quantitation of TNP-470 and its metabolites in human plasma: sample handling, assay performance and stability. J Chromatogr B Biomed Sci Appl 710: 173-182.
29. Folkman J (1996) Fighting cancer by attacking its blood supply. Scientific American 275: 150.
30. Folkman J (1996) Tumor angiogenesis and tissue factor. Nature Medicine 2: 167-168.
31. Folkman J (1995) Angiogenesis inhibitors generated by tumors. Molecular Medicine 1: 120-122.
32. Wiedersberg S, Guy R (2014) Transdermal drug delivery: 30+ years of war and still fighting. Journal of Controlled Release 190: 150-156.
33. Ong V, Stamm G, Menacherry S, Chu S (1998) Quantitation of TNP-470 and its metabolites in human plasma: sample handling, assay performance and stability. Journal of Chromatography B 710: 173-182.
34. Kakinoki S, Yasuda C, Kaetsu I, Uchida K, Yukutake K, et al. (2003) Preparation of poly-lactic acid microspheres containing the angiogenesis inhibitor TNP-470 with medium-chain triglyceride and the in vitro evaluation of release profiles. Eur J Pharm Biopharm 55: 155-160.