Exp Dermatol 2000: 9: 165–169
Printed in Denmark · All rights reserved

EXPERIMENTAL DERMATOLOGY
ISSN 0906-6705

Viewpoint

The 500 Dalton rule for the skin penetration of chemical compounds and drugs

Bos JD, Meinardi MMHM. The 500 Dalton rule for the skin penetration of chemical compounds and drugs.
Exp Dermatol 2000: 9: 165–169. © Munksgaard, 2000

Abstract: Human skin has unique properties of which functioning as a physicochemical barrier is one of the most apparent. The human integument is able to resist the penetration of many molecules. However, especially smaller molecules can surpass transcutaneously. They are able to go by the corneal layer, which is thought to form the main deterrent. We argue that the molecular weight (MW) of a compound must be under 500 Dalton to allow skin absorption. Larger molecules cannot pass the corneal layer. Arguments for this "500 Dalton rule" are; 1) virtually all common contact allergens are under 500 Dalton, larger molecules are not known as contact sensitizers. They cannot penetrate and thus cannot act as allergens in man; 2) the most commonly used pharmacological agents applied in topical dermatotherapy are all under 500 Dalton; 3) all known topical drugs used in transdermal drug-delivery systems are under 500 Dalton. In addition, clinical experience with topical agents such as cyclosporine, tacrolimus and ascomycins gives further arguments for the reality of the 500 Dalton rule. For pharmaceutical development purposes, it seems logical to restrict the development of new innovative compounds to a MW of under 500 Dalton, when topical dermatological therapy or percutaneous systemic therapy or vaccination is the objective.

Jan D. Bos and Marcus M. H. M. Meinardi

Department of Dermatology, Academic Medical Center, University of Amsterdam, The Netherlands

Key words: drug design – skin – topical drugs – transdermal

Jan D. Bos, Department of Dermatology A0-235, Academic Medical Center, University of Amsterdam, P.O. Box 22700, 1100 DE Amsterdam, The Netherlands
Tel.: +31 20 566 2587. Fax: +31 20 696 0076
e-mail: j.d.bos@amc.uva.nl

Accepted for publication 27 October 1999

Introduction

Human skin has many functions and its most apparent is that of a defense organ, both physical and biological (1). Penetration from outside into the body of any compound is primarily prevented by the corneal layer of the epidermis. This outer layer is just a few micrometers thick, but effectively forms a barrier that is indeed preserving life. Although absorption is not only dependent on penetration, but also on other variables such as skin metabolism, insufficient release from the carrier, partitioning in an unwanted reservoir, without penetration nothing happens. It is important to realize that the human skin has unique properties in this respect and that penetration studies performed in animal models are of limited use for our understanding of the human skin barrier.

Essentially, the corneal layer consists of apoptotic keratinocytes that have transformed themselves into keratin-rich, lipoprotein-containing envelopes and lipid bilayers with hydrophilic regions in between. Most medicaments will pass the epidermal barrier through the intercellular route. As a consequence of its hydrophobic nature, the stratum corneum barrier will allow the penetration of lipid soluble molecules more readily than water-soluble compounds. Strong lipophilic compounds will however be hampered by the hydrophilic regions in the bilayer. Water-soluble molecules may penetrate through an alternative way, the openings of sweat glands and hair follicles. The total surface of these openings amounts to 0.1% of the total skin surface area, making it probably not significant.

The only way to circumvent the properties of the corneal layer is by disrupting it, for example with ultrasound, a method also known as phonopheresis (2), or with high-voltage electrical pulsing, also known as electroporation (3, 4). Alternative methods such as stripping the corneal layer using adhesive tape have also been advocated but are not reliable. The use of skin penetration enhancers such as dimethylsulphoxide or carriers such as liposomes have never been confirmed to make a difference. Iontopheresis (5) using low-voltage has been developed for increasing the flux of particular

Table 1. Molecular weight of compounds included in the ICDRG European Patch Testing Standard Series

Compound	Test concentration	Molecular weight (Dalton)
Potassium Di**chromate**	0.5% pet	294
Neomycin Sulphate	20% pet	**712**
neomycin (dimer of neamine **322**)		**614**
Thiuram Mix	1% pet	
Dipentamethylenethiuram Disulphide	0.25% pet	318
Tetraethylthiuram Disulphide	0.25% pet	295
Tetramethylthiuram Disulphide (TMTD)	0.25% pet	240
Tetramethylthiuram Monosulphide (TMTM)	0.25% pet	208
p-Phenylenediamine	1% pet	108
Cobalt Chloride (6H2O)	1% pet	130
Benzocaine	5% pet	165
Formaldehyde in water	1% aqua	30
Colophony (90% abeitic acid)	20% pet	302
Clioquinol	5% pet	306
Pinus (**Balsam of Peru**) (60% cinnamein)	25% pet	148
IPPD	0.1% pet	216
Lanolin (**Wool Alcohols**)	30% pet	
Mercapto Mix	1% pet	
Dibenzothiazyl Disulphide	0.333% pet	332
Morpholinylmercaptobenzothiazole	0.333% pet	252
N-Cyclohexylbenzothiazyl-Sulphenamide	0.333% pet	308
Epoxy Resin	1% pet	
Paraben Mix	16% pet	
Butylparaben (Butyl Parahydroxybenzoate)	4% pet	194
Ethylparaben (Ethyl Parahydroxybenzoate)	4% pet	166
Methylparaben (Methyl Parahydroxybenzoate)	4% pet	152
Propylparaben (Propyl Parahydroxybenzoate)	4% pet	180
Paratertiarybutyl Phenol Formaldehyde Resin	1% pet	
Fragrance Mix	8% pet	
alpha-Amyl-Cinnamaldehyde	1% pet	202
Cinnamaldehyde	1% pet	132
Cinnamyl Alcohol	1% pet	134
Eugenol	1% pet	164
Geraniol	1% pet	154
Hydroxycitronellal	1% pet	154
Isoeugenol	1% pet	164
Oak Moss Absolute	1% pet	
Quaternium-15	1% pet	201
Nickel Sulphate, 6H2O	5% pet	155
Methyl(chloro)isothiazolinone (**Kathon CG**)	0.01% aqua	184
Mercaptobenzothiazole	2% pet	167
Sesquiterpene Lactone Mix (allantolactone)	0.1% pet	232
Primin	0.01% pet	209
Wood Tar Mix	12% pet	
Methyldibromoglutaronitrile (**Euxyl K400**)	0.5% pet	266
1,2 Benzisothiazolin-3-On (**BIT**)	0.1% pet	151
Tixocortol-21-Pivalate	1% pet	463
Budesonide	1% pet	431
4,4'-**Diaminodiphenylmethane**	0.5% pet	198

(pet=petrolatum.)

compounds, but only of low MW. Thus, when large molecules have to be absorbed after topical application, only phonopheresis and electroporation are available, but these techniques are far from practical and not suitable for routine use.

In a recent review, it was stated that "optimal absorption will occur for molecules that are small, have low melting points . . . and have few pendant groups capable of H-bonding" (6). But what is small? The subject of this contribution is the upper molecular weight (MW) limit for chemical compounds and drugs enabling absorption through the human skin barrier. An answer to this question is of use for those developing epicutaneous application of compounds to the human skin for destinations varying from topical to systemic treatment to vaccination. We have therefore looked at the MW of common contact allergens and commonly used topical drugs. We propose the 500 Dalton rule, which says that with a MW increasing over 500 Dalton, absorption of molecules through normal human skin rapidly declines.

Dermato-allergology: chemicals causing allergic contact dermatitis are under 712 Dalton

The variety of chemicals that are known to lead to allergic contact dermatitis in persons exposed to them forms a true encyclopedia of modern society. Thousands of molecules have been described to be associated with the induction and maintenance of allergic contact dermatitis in a limited or more extensive number of persons. The intrinsic sensitizing capacity of molecules is varying widely. Some compounds rarely lead to clinical contact dermatitis. Others are virtually sensitizing any person whose skin is exposed to it. The routine patch test series, advised by the International Contact Dermatitis Research Group (ICDRG), is used for the diagnosis of contact allergy, and it is composed of the most common sensitizing agents known to mankind.

Taking this series of single chemical compounds as well as mixtures of sensitizing agents together, a look at their MW might give us a clue to what size allows penetration through the human skin barrier. A molecule that comes into contact with human skin, but that cannot penetrate the skin barrier in sufficient quantities, will not be a sensitizing agent. The MWs of the ICDRG patch test series compounds were identified using the Merck Index or using software (the Molecular Weight Calculator for Windows 95 – version 4.1 by Matthew Monroe – http://www.unc.edu/~monroen/). They are summarized in Table 1.

Often, the test allergens are mixes and where possible, the MW of individual compounds in these mixes is given (thiuram mix, mercapto mix, paraben mix, fragrance mix). In other instances, the main constituents of a given crude extract is taken (colophony=90% abietic acid: Balsam of Peru=60% cinnamein; sesquiterpene lactone mix= allantolactone). MWs could not be identified for mixes such as lanolin (wool alcohols), epoxy resin,

paratertiarybutyl phenol formaldehyde resin, oak moss absolute, and wood tar mix.

The most common allergens have a MW under 500 Dalton (Table 1). The only exception to the 500 Dalton rule is neomycin sulphate, which has a MW of 712 Dalton. However, this molecule is a dimer of two neamine molecules, each having a MW of 322 Dalton, and it may well be that it is the monomer that sensitizes. All other components of the ICDRG patch test series are under 500 Dalton, with tixocortol-21-pivalate and budesonide being the largest having MWs of 463 and 431 Dalton respectively.

Topical immunosuppressants: cyclosporin's 1202 Dalton is too large

Late in the 1980s, cyclosporin (MW 1202 Dalton) was introduced as a systemic agent for the treatment of dermatological disease, especially psoriasis (7). It soon became known that the drug was highly effective in skin diseases known to respond favorably to topical or systemic corticosteroids. It also was immediately conceived that topical treatment with this new class of cyclic immunosuppressants might potentially revolutionize topical dermatological therapy. However, the topical use of cyclosporin was found to be ineffective in psoriasis as well as in atopic dermatitis and allergic contact dermatitis (8). When injected intralesionally however, cyclosporin is effective in psoriasis (9, 10). Thus, a MW of 1202 Dalton apparently prohibits sufficient skin penetration.

Subsequently, the focus was redirected at other inflammatory cytokine inhibitors, such as tacrolimus (822 Dalton) and the ascomycin derivative SDZ ASM 981 (811 Dalton). Tacrolimus had been found to be effective as a systemic therapy in psoriasis (11). There is one abstract indicating its efficacy in psoriasis when applied under occlusion (12). A controlled study could not detect efficacy in an open application approach (13). Ascomycin derivatives have also been tested for possible topical efficacy. After a first encouraging study in which the ascomycin derivative SDZ 281–240 was found to be effective in psoriasis when used under occlusion (Finn chamber) (14), SDZ ASM 981 was also found to be effective in psoriasis when applied topically under occlusion (15). No reports have been published confirming efficacy in psoriasis using non-occluded applications.

Ascomycin derivative SDZ ASM 981 (16) and tacrolimus (17) do however work when topically applied in atopic dermatitis. It seems that atopic dermatitis patients are the exception to the rule and can, as a result of a somewhat defective barrier, absorb molecules of slightly over 800 Dalton, such as tacrolimus and ascomycin.

Thus, with the exception of atopic dermatitis where 1202 Dalton is too large a molecule to be topically effective, but where 822 Dalton molecules are absorbed, the human skin does not allow penetration of molecules around 800 Dalton, fitting with the 500 Dalton rule.

Molecular weight of the most commonly used topical drugs in dermatotherapy

In dermatological therapy, a wide variety of different active molecules is available for topical treatment of individual skin lesions. A list of the most commonly used topical drugs is presented in Table 2. Their molecular weights were identified using the Chemfinder Webserver (http://chemfinder.camsoft.com). Again, the most commonly used and effective topical drugs in dermatotherapy all have a molecular weight under 500 Dalton, the only exceptions being fusidic acid which is slightly larger with 517 Dalton, and ketoconazole which has a MW of 531 Dalton.

Table 2. List of the world's most commonly used topical drugs and their molecular weight

Compound	Molecular weight (Dalton)
Topical antifungals:	
Ketoconazole	531
Clotrimazole	345
Terbinafine	291
Miconazole	416
Topical corticosteroids	
Hydrocortisone acetate	404.5
Bethamethasone valerate	477
Diflucortolone valerate	394
Clobetasol propionate	467
Mometasone fuorate	?
Topical anti-infectives	
Fucidic acid	517
Gentamycin	478
Acyclovir	225

Table 3. List of drugs available in transdermal drug-delivery systems (Langer 1998)

Compound	Molecular weight (Dalton)
Scopolamine	305
Nitroglycerine	227
Nicotine	162
Clonidine	230
Fentanyl	336
Oestradiol	272
Testosterone	288

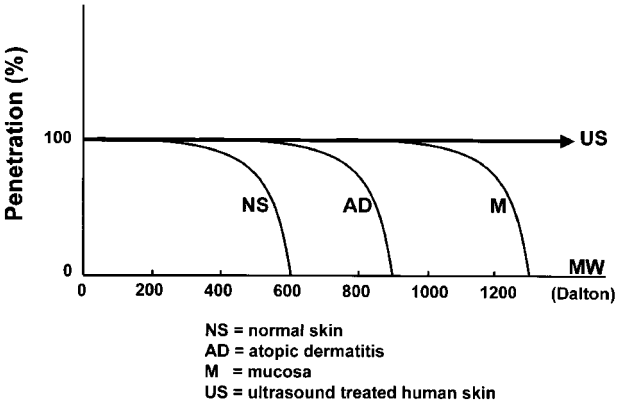

Figure 1. Estimated penetration barrier characteristics for normal human skin, atopic dermatitis skin, mucosa, and phonopheretically disrupted skin.

Molecular weight of drugs used in transdermal drug-delivery systems

Certain drugs for systemic use are delivered through the skin for reasons varying from avoiding the liver (destroying drugs when taken orally) to enabling sustained release. Patches with transdermal drug delivery systems are available for at least 7 different drugs (18). In Table 3, these compounds and their molecular weights are summarized, using the same MW finding strategy as for the topical drugs described earlier. As may be seen from the Table, MWs of drugs used in transdermal drug-delivery systems are all well under 500 Dalton, in fact they are all smaller than 350 Dalton.

Arguments brought forward against the 500 Dalton rule

In discussions of the upper limit of skin absorption, several arguments might be brought forward against the suggested 500 Dalton rule. The first argument is that it is not a penetration problem but a formulation challenge. That is, with the use of the appropriate pharmaceutical formulation, the drug will be absorbed, taking into account its lipophilic or hydrophilic character, adjusting the vehicle in such a way that the drug prefers to leave it and go into the integument. However, authors are not aware of any formulation that contains a drug with a MW well over 500 Dalton, that is clinically effective in any skin condition. Penetration enhancers then would solve the problem, but again, there is no proof for this hypothesis. In fact, the only way described thus far to overcome the epidermal barrier is by exposing skin to phonopheresis or electroporation, as described earlier. These disrupt the corneal layer and allow subsequent penetration of very large proteins. From a practical point of view however, these approaches are not feasible.

A completely different type of argument is the existence of latex allergy. Contact urticaria and contact dermatitis may indeed occur after skin exposure to latex, which is generally believed to consist of high MW molecules. Immediate type reactions have been described and are believed to be IgE mediated. Allergic contact dermatitis has also been reported and is believed to be T-cell mediated. However, it is also known that IgE molecules as well as T-cell receptors do not recognize large proteins, but only peptide epitopes, generally being 6–8 amino acid derivatives. It is unacceptable to believe that latex allergy sufferers have greatly diminished skin barriers allowing large proteins (over 50,000 Dalton) to penetrate. More acceptable is the explanation that latex proteins are degraded by proteases present on the skin surface, allowing smaller peptides derived from it to penetrate and to lead to allergic reactions. Alternatively, latex might naturally contain small molecules that are the immunogenic compounds.

The same might be said about the atopy patch test, where equally large proteins are used epicutaneously to detect allergy, and the superantigen skin challenge, where bacterial superantigens are used for topical provocation of skin lesions. These must most probably be first degraded into smaller molecules to enable penetration and subsequent binding to the molecules that can attach to them, or already contain these smaller breakdown products.

Finally, some investigators believe that larger molecules do indeed penetrate the skin but are quickly metabolized, making them clinically ineffective (19). However, the same mechanism would then apply to smaller molecules, that are metabolized as well, and would not be effective either.

Concluding remarks

The human skin is indeed an effective barrier but it cannot prevent smaller molecules to enter. In Fig. 1, the estimated penetration barrier characteristics for normal human skin, atopic dermatitis skin, mucosa, and phonopheretically disrupted skin are indicated. Somewhere around 500 Dalton is the start of a rapid decline in skin absorption due to molecular size. The barrier is formed by the corneal layer since when absent, such as in mucous membranes, larger molecules may penetrate and thus be effective. Topical treatment of mucosal lichen planus with cyclosporin (1202 Dalton) is an example (20), although this is not without controversy (21). Atopic dermatitis forms the exception to the 500

Dalton rule, since it can be managed by topical application of tacrolimus and ascomycin derivatives (822 and 811 Dalton respectively).

For pharmaceutical development purposes, it seems logical to restrict the development of new innovative compounds to a MW of under 500 Dalton, when topical dermatological therapy or percutaneous systemic therapy or vaccination is the objective. We therefore propose the 500 Dalton rule for the skin penetration of chemical compounds and drugs. We believe that in drug development, a maximum molecular weight of 500 Dalton should be adhered to, before considering its further development for topical therapy or transcutaneous vaccination in man.

Acknowledgement

We wish to thank Ramzi Tubbeh of Novartis Pharma (Basel) for his help in collecting data for the list of most commonly used drugs in dermatology.

References

1. Bos J D, Das P K, Kapsenberg M L. The Skin Immune System (SIS). In: Bos J D, ed., Skin Immune System (SIS): Cutaneous Immunology and Clinical Immunodermatology, 2nd edn., Boca Raton: CRC Press, 1997: 9–16.
2. Mitragotri S, Blankschtein D, Langer R. Ultrasound-mediated transdermal protein delivery. Science 1995: 269: 850–853.
3. Prausnitz M R, Bose V G, Langer R, Weaver J C. Electroporation of mammalian skin: a new mechanism to enhance transdermal drug delivery. Proc Natl Acad Sci 1993: 90: 10504–10508.
4. Chen T, Langer R, Weaver J C. Skin electroporation causes molecule transport across the stratum corneum through localized transport regions. J Invest Dermatol (Symp Proc) 1998: 3: 159–65.
5. Merino V, Kalia Y N, Guy R H. Transdermal therapy and diagnosis by iontophoresis. Trends Biotechnol 1997: 15: 288–290.
6. Hadgraft J, Puch W J. The selection and design of topical and transdermal agents: a review. J Invest Dermatol (Symp Proc) 1998: 3: 131–135.
7. Bos J D, Meinardi M M H M, Van Joost Th, Heule F, Powles A V, Fry L. Use of cyclosporin in psoriasis. Lancet 1989: ii: 1500–1502.
8. De Rie M A, Meinardi M M H M, Bos J D. Lack of efficacy of topical cyclosporin A in atopic dermatitis and allergic contact dermatitis. Acta Derm Venereol (Stockh) 1991: 71: 452–454.
9. Powles A V, Baker B S, McFadden J, Rutman A J, Griffiths CEM, Fry L. Intralesional injection of cyclosporin in psoriasis. Lancet 1988: i: 537.
10. Burns M K, Ellis C N, Eisen D et al. Intralesional cyclosporine for psoriasis. Relationship of dose, tissue levels, and efficacy. Arch Dermatol 1992: 128: 786–790.
11. The European FK 506 Multicentre Psoriasis Study Group. Systemic tacrolimus (FK 506) is effective for the treatment of psoriasis in a double-blind, placebo-controlled study. Arch Dermatol 1996: 132: 419–423.
12. Remitz A, Reitamo S, Erkko P, Granlund H, Lauerma A. A microplaque assay-based, double-blind trial to compare the efficacy of two tacrolimus ointment formulations with two active and two negative controls in patients with chronic plaque-type psoriasis. Br J Dermatol 1996: 135: 833 (abstract).
13. Zonneveld I M, Rubins A, Jablonska S et al. Topical tacrolimus (FK506) is not effective in chronic plaque psoriasis. A pilot study. Arch Dermatol 1998: 134: 1101–1102.
14. Rappersberger K, Meingassner J G, Fialla R et al. Clearing of psoriasis by a novel immunosuppressive macrolide. J Invest Dermatol 1996: 106: 701–710.
15. Mrowietz U, Graeber M, Bräutigam M et al. The novel ascomycin derivative SDZ ASM 981 is effective for psoriasis when used topically under occlusion. Br J Dermatol 1998: 139: 992–996.
16. Van Leent E J M, Graeber M, Thurston M, Wagenaar A, Spuls Ph I, Bos J D. Topical treatment with the macrolactam SDZ ASM 981 is effective in atopic dermatitis. Arch Dermatol 1998: 134: 805–809.
17. Ruzicka Th, Bieber Th, Schöpf E et al. A short-term trial of tacrolimus ointment for atopic dermatitis. N Engl J Med 1997: 337: 816–821.
18. Langer R. Drug delivery and targeting. Nature 1998: 392 (suppl): 5–10.
19. Schulze H-J, Mahrle G, Steigleder G K. Topical cyclosporin A in psoriasis. Br J Dermatol 1990: 122: 113–114.
20. Eisen D, Ellis C N, Duell E A, Griffiths C E M, Voorhees J J. Effect of topical cyclosporin rinse on oral lichen planus. N Engl J Med 1990: 328: 290–294.
21. Levell N J, MacLeod R I, Marks J M. Lack of effect of cyclosporin mouthwash in oral lichen planus. Lancet 1991: 337: 797–780.