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Soft Drugs–XVI. Design, Evaluation and Transdermal Penetration of Novel Soft Anticholinergics Based on Methatropine

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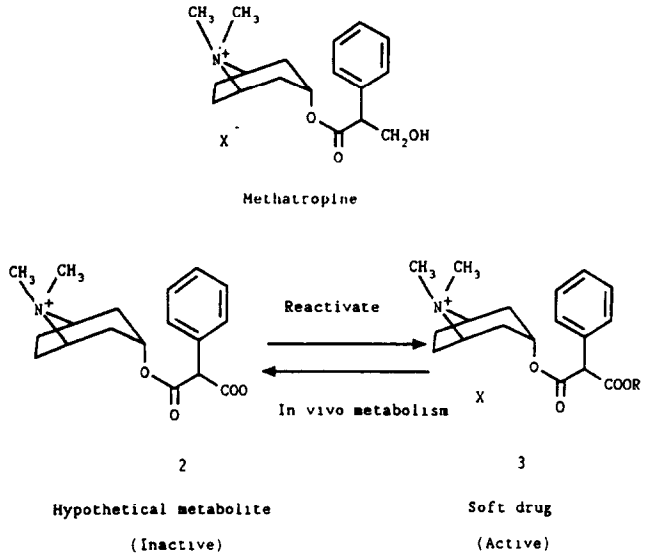
Abstract—Atropine has been reported to produce unwanted systemic side effects on topical administration into the eye. The same problem could arise when atropine is used topically as a suppressant of eccrine sweating. In this study, the principles of soft drug design were applied to methatropine. A hypothetical carboxylate metabolite of methatropine was reactivated by esterification with cyclic and alicyclic alcohols to yield a series of compounds (**3a–g**). *In vitro* evaluation by guinea pig ileum assay indicated that the compounds are potent anticholinergics and the lead carboxylate metabolite is about 60 times less potent than the most active compound of the series. The activity was found to decrease with the increasing side chain length. The *n*-octanol/water partition coefficients were found to be directly dependent on the chain length for the compounds made with straight chain alcohols. The transdermal permeability coefficients across the hairless mice skin were found to be directly dependent on the partition coefficients. The soft drugs are found to metabolize extensively during the penetration process compared to the unmetabolizable nature of methatropine. The soft drugs reported in this study will probably be able to elicit a local action at the site of application but will probably be metabolized rapidly in the systemic circulation, thereby avoiding the systemic side effects with a consequent increase in the therapeutic index.

Introduction

Atropine is an antimuscarinic alkaloid with both central and peripheral actions. Atropine and its quaternary forms, atropine methonitrate/methobromide, are used widely in therapy.[1] A number of studies carried out with anticholinergics showed their effectiveness in inhibiting the eccrine sweating,[2,3] which has been shown to be under sympathetic cholinergic control. A detailed study carried out with 95 anticholinergics showed that some of the most active antiperspirants are quaternary compounds.[4] Atropine methonitrate was found to be more effective in humans than atropine hemisulfate.

Several toxic side effects have been reported after topical administration of atropine. At least 6 deaths have been attributed to the ocular administration of atropine.[5] Exacerbation of akinetic seizures by atropine eye drops has been reported.[6] Other toxic anticholinergic side effects include psychotic and behavioral changes. Thus, when atropine was administered topically to elicit a local action it was being absorbed into the systemic circulation with consequent elicitation of side effects.

Hammer *et al.*[7] have applied the concept of soft drugs (the drugs which are inactivated in a single predictable step to a nontoxic metabolite after achieving their therapeutic role), introduced by Bodor,[8] to methatropine (**1**) by choosing a hypothetical carboxylate metabolite (**2**) of methatropine which was reactivated by esterification (Scheme I). The compounds so obtained (**3a, 3c, 3f** and **3i**) were potent anticholinergics and the hypothetical metabolite (**2**) was about 60 times less potent than the most active compound of the series.[7] They were found to metabolize in various biological media to the hypothetical metabolite (**2**) which is essentially inactive. The compounds were shown to be very short acting mydriatics on topical administration into the rabbits' eyes with possibly no systemic activity.[9] The ultrashort duration of muscarinolytic activity of compound **3a** was shown in rats against acetylcholine induced bradycardia compared to prolonged activity of methatropine.[10] Hence it was concluded that the soft drugs (**3a, 3c** and **3f**) elicit a local action at the site of application,



Scheme I. R = **3a**, ethyl; **3b**, *n*-propyl; **3c**, *n*-butyl; **3d**, *n*-pentyl; **3e**, *n*-hexyl; **3f**, *c*-hexyl; **3g**, ethyl-*c*-hexyl; **3h**, neo-pentyl; **3i**, *i*-propyl.

Keywords—Anticholinergics, atropine, soft drugs, mydriatics.

but on entering the systemic circulation undergo facile metabolism to an inactive polar metabolite which is easily eliminated. These soft drugs could have implications as topical antiperspirants. The soft drugs when applied topically onto the skin will act as antisecretory agents by inhibiting the eccrine sweating but when absorbed into the systemic circulation will undergo facile metabolism to the polar inactive metabolite, **2**, which is rapidly eliminated.

The partition coefficient between water and octanol of some compounds (e.g. steroids, alkanols) has been used to correlate their percutaneous absorption.[11] A good correlation has been reported to exist between the *in vitro* determined permeability and partition coefficients. A topically applied antiperspirant should traverse through the sweat duct to the site of action i.e., sweat gland to exert its effect. The antiperspirants that are in use today act as astringents rather than by any specific receptor mediated mechanism. Hence no detailed studies have appeared in the literature so far about the physicochemical characteristics that are ideal for an antiperspiratory agent. In the case of an antiperspirant drug, a balance should be achieved between the localization of the drug in the interior of the skin and its diffusion into the systemic circulation. There are three possible routes of transdermal penetration viz: through the hair folicle, via the sweat duct and across the stratum corneum.[12] Even though the permeability rates of polar molecules are reported to be several orders of magnitude higher through the sweat duct and hair follicles than the stratum corneum, their contribution is very insignificant since their fractional diffusional volume is low compared to stratum corneum.[13] The transdermal studies of the soft drugs synthesized in this study were conducted to determine their permeability characteristics.

The concept of soft drugs applied to methatropine[7] is extended in this study by making soft drug analogs with longer side chains, both normal and branched. The increase in the side chain length is expected to make the compounds more lipophilic and thus with an enhanced permeability through the hydrophobic barrier of the skin, the stratum corneum.

This paper describes the syntheses, determination of anticholinergic activity and partition coefficients and the *in vitro* transdermal penetration across hairless mice skin of the phenylmalonic analogs of methatropine as novel soft anticholinergics intended for potential topical therapy.

Materials and Methods

All chemicals used were reagent grade. Tropine and hexamethonium bromide were obtained from Sigma Chemical Company. Other chemicals were obtained from Aldrich Chemical Company and solvents from Fisher Scientific. All melting points were recorded using Fisher–Johns melting point apparatus and are uncorrected. NMR data were recorded with Varian T-90 NMR spectrometer and are reported in parts per million (δ) relative to tetramethylsilane. All quaternary compounds were dissolved in DMSO-d_6 and other compounds were dissolved in $CDCl_3$. The elemental analyses were carried out at Atlantic Microlab. Inc., Atlanta, GA, and are within ±0.4% of the calculated values. Thin layer chromatography was carried out using EM Science DC-plastic foil plates coated to a thickness of 0.2 mm with silica gel 60 containing Fluorescent (254) indicator. The mobile phase consisted of toluene:methanol or hexanes:acetone in various proportions. The column chromatography was performed with silica gel (70–230 mesh) with appropriate mobile phases.

All the animal studies were conducted in accordance with the guidelines set forth in the Declaration of Helsinki and The Guiding Principles in the Care and Use of Animals (DHEW Publication, NIH 80-23). Male Hartley guinea-pigs (weighing 400 g) and 4–5 weeks old female hairless mice (NIHS-bg-nu-xid) obtained from Harlan Sprague Dawley Inc., Indianapolis were used in the study.

The methods described previously for syntheses,[7] determination of anticholinergic activity by guinea pig ileum assay[7] and determination of partition coefficients[14] were followed with appropriate modifications. The HPLC analytical system described before was used for analysis and quantitation.[9]

The physical and spectral characteristics of the compounds synthesized are enumerated below:

3b. (2*R*,2*S*) (±)1-*n*-propyl 3-{3α-8,8-dimethyl-8-azabicyclo [3.2.1] oct-3-yl} 2-phenylpropanedioic acid methyl sulfate: Yield, 53%; m.p. 120 °C; ^{1}H NMR δ 0.9 (t, 3H, CH_3), 1.4–2.1 (m, 10H, bicyclic and propyl CH_2), 3.2 (m, 2H, CH–N–CH), 3.3–3.5 (3s, 9H, 2 N–CH_3 and CH_3SO_4), 4.1 (t, 2H, O–CH_2), 4.5 (s, 1H, Ar–CH), 5.1 (m, 1H, CH–O–CO) and 7.3 (m, 5H, aromatic); m^+, 360.

3d. (2*R*,2*S*) (±)1-*n*-penyl 3-{3α-8,8-dimethyl-8-azabicyclo [3.2.1] oct-3-yl} 2-phenylpropanedioic acid methyl sulfate: Yield, 49%; m.p. 126–127 °C; ^{1}H NMR δ 0.9 (t, 3H, CH_3), 1.2–2.2 (m, 14H, bicyclic and CH_2–CH_2–CH_2), 3.2 (m, 2H, CH–N–CH), 3.3–3.5 (3s, 9H, 2 N–CH_3 and CH_3SO_4), 4.0 (t, 2H, O–CH_2), 5.0 (s, 1H, Ar–CH), 5.1 (m, 1H, CH–O–CO) and 7.3 (m, 5H, aromatic); m^+, 388.

3e. (2*R*,2*S*) (±)1-*n*-hexyl 3-{3α-8,8-dimethyl-8-azabicyclo [3.2.1] oct-3-yl} 2-phenylpropanedioic acid methyl sulfate: Yield, 41%; m.p. 80 °C; ^{1}H NMR δ 0.9 (t, 3H, CH_3), 1.4–2.1 (m, 16H, bicyclic and CH_2–CH_2–CH_2–CH_2), 3.0 (m, 2H, CH–N–CH), 3.3–3.5 (3s, 9H, 2 N–CH_3 and CH_3SO_4), 4.0 (t, 2H, O–CH_2), 5.0 (s, 1H, Ar–CH), 5.1 (m, 1H, CH–O–CO) and 7.3 (m, 5H, aromatic); m^+, 402.

3g. (2*R*,2*S*) (±)1-ethyl-*c*-hexyl 3-{3α-8,8-dimethyl-8-azabicyclo [3.2.1] oct-3-yl} 2-phenylpropanedioic acid methyl sulfate: Yield, 45%; m.p. 120 °C; ^{1}H NMR δ 1.1–2.2 (m, 21H, bicyclic and CH_2-*c*-hexyl), 3.2 (m, 2H, CH–N–CH), 3.3–3.5 (3s, 9H, 2 N–CH_3 and CH_3SO_4), 4.1 (q, 2H, O–CH_2), 4.5 (s, 1H, Ar–CH), 5.1 (m, 1H, CH–O–CO) and 7.3 (m, 5H, aromatic); m^+, 428.

3h. (2*R*,2*S*) (±)1-neopentyl 3-{3α-8,8-dimethyl-8-azabicyclo [3.2.1] oct-3-yl} 2-phenylpropanedioic acid methyl sulfate: Yield, 45%; m.p. 203 °C; ^{1}H NMR δ 0.9 (s, 9H, $C(CH_3)_3$), 1.6–2.2 (m, 8H, bicyclic), 3.2 (m, 2H, CH–N–

CH), 3.3–3.5 (3s, 9H, 2 N–CH_3 and CH_3SO_4), 4.1 (s, 2H, O–CH_2), 4.5 (s, 1H, Ar–CH), 5.1 (m, 1H, CH–O–CO) and 7.3 (m, 5H, aromatic); m^+, 388.

Transdermal penetration across hairless mice skin

The mice were sacrificed by cervical dislocation and the whole dorsal skin was removed. The underlying fat and other visceral tissues were gently removed. The excised skin was fixed in the transdermal permeation system of Kresco Engineering (Palo Alto) with dorsal side facing the receptor phase. Then the receptor cell was filled 40 ml of pH 7.1 isotonic phosphate buffered saline. The whole system was gently tapped to remove air bubbles. The system was placed in an incubator at 32 °C and continuously stirred at 200 rpm. Then 1 ml of the appropriate drug solution in buffer was placed on top of the skin as the donor phase. Samples of 0.5 ml of receptor phase were withdrawn at appropriate time intervals and the receptor phase was replenished with 0.5 ml of buffer. The samples were diluted with 0.5 ml of acetonitrile, centrifuged and analyzed by HPLC for the drug and metabolites. A control experiment was performed in which the donor phase contained 1 ml of buffer without any drug. The permeability coefficients (K_p) were calculated according to the established methods based on Fick' laws of diffusion.[15]

Results and Discussion

The *in vitro* anticholinergic activity of **1** and the compounds are summarized in Table 1. The most potent compound of the series, **3a**, is about one log unit less potent than **1**. The anticholinergic activity is found to decrease with the increasing side chain length. This could be due to increased steric bulk preventing proper interaction with the receptor. The slopes of the plot, -log (antagonist concentration) vs log (dose ratio - 1), are around unity, showing the pure antagonistic nature of the soft drugs.

Table 1. Anticholinergic activity determined by guinea pig ileum assay. The regression coefficients and slopes are from Schild plot [-log (antagonist) vs log (dose ratio -1)]. pA_2 value is the *x*-axis intercept

Compound	pA_2	r^2	Slope
1	8.95	0.991	-0.878
3a	7.85*	---	---
3b	7.53	0.970	-0.899
3c	7.15	0.967	-0.946
3d	6.75	0.932	-0.995
3e	6.40	0.984	-0.873
3f	7.35*	---	---
3g	6.17	0.967	-1.217
3h	5.75	0.980	-0.968
3i	7.61*	---	---
2	6.07*	---	---

*Readings from Ref. 7.

The *n*-octanol/water partition coefficients are listed in Table 2. The partition coefficients are found to increase with the increasing side chain length for normal alcohols. The increase in partition coefficients for branched chain compounds is not consistent with the increase in the number of carbons in the alcohol structural moiety. Thus, the *n*-hexyl compound (**3e**) is 2.2 times more lipophilic than *c*-hexyl compound (**3f**). This could be due to the masking of quaternary head by the wrapped over hydrophobic side chain, making the molecule more lipophilic. This masking is not feasible with a branched side chain.

The *in vitro* penetration of **1** and the soft drugs (**3a–g**) across hairless mice skin was studied. The time vs cumulative amount penetrated curves show a linear profile after an initial lag period. The representative time course of penetration curves are depicted in Figure 1. The permeability coefficients (K_p) are listed in Table 2. The lag periods ranged from 3 to 8 h without significant differences between the compounds and **1**.

The soft drugs were found to metabolize to a great extent during their penetration across the skin. In an *in vitro* study carried out with transdermal penetration of steroid esters across fuzzy rat skin,[16] considerable retention of enzymatic activity has been shown to exist even after prolonged periods of study. No noticeable metabolism was seen with **1**. The K_p of **1** determined in this study (4.1×10^{-5} cm/h) is about five times higher than the value reported for human skin permeability (8.6×10^{-6} cm/h).[17] The permeability coefficients ranged from 1×10^{-5} cm/h to 120×10^{-5} cm/h, with the most lipophilic compound, **3e**, penetrating to the highest extent. The amount that accumulated in the receptor chamber in all the studies accounted for less than ten percent of the applied drug. Thus essentially sink conditions were maintained throughout the period of study. The maintenance of sink conditions in the receptor fluid are essential in order to maintain zero-order flux conditions. A linear correlation was obtained ($r^2 = 0.83$) between log partition coefficients (log K_p) and log permeability coefficients (log P) for all the compounds tested (Figure 2). A linear correlation was obtained ($r^2 = 0.995$) between log P and log K_p for the compounds with normal side chain (**3a–e**).

The general principle behind the design of compounds for dermal or transdermal delivery has been to increase the lipophilicity of the compounds so that they can traverse the hydrophobic stratum corneum to reach the inner layers of the skin or the systemic circulation, respectively. In general a parabolic relationship has been shown to exist between log P and log K_p with the maximum permeability attained typically at a log P value in the range 2.0–2.5. Thus an essentially linear relationship[18] is shown to exist at lower P values with log K_p increasing with log P. In the present study, the P values of the compounds ranged from 1.10×10^{-2} to 28.5×10^{-2}, which are well below the optimal P values reported for maximal permeability and are in the ascending arm of the parabola. Hence, as expected a linear correlation exists between log P and log K_p. The permeabilities of soft drugs which are very hydrophilic are comparable to the permeabilities reported for other polar compounds.[17] It has been postulated that their phospholipid bilayer, which constitutes the stratum corneum, comprised largely of fatty acid zwitterions, may solubilize the organic electrolytes, thus facilitating their absorption through the skin.[17]

Table 2. Partition coefficients and permeability coefficients of methatropine and soft drugs. Each value of permeability coefficient is the mean of four readings ± S.E.M.

Compound	Partition coefficient ($X 10^{-2}$)	Permeability coefficient ($X 10^{-5}$ cm/hr)	Lag period hrs
1	1.30	4.10 ± 0.35	4.0
3a	1.10	1.02 ± 0.15	6.0
3b	2.12	4.12 ± 0.40	5.5
3c	4.35	9.60 ± 0.55	7.5
3d	12.20	32.50 ± 1.25	4.5
3e	28.50	119.00 ± 5.50	3.5
3f	12.90	8.50 ± 0.65	3.0
3g	15.10	31.60 ± 1.55	7.5
3h	6.20	2.70 ± 0.30	5.0

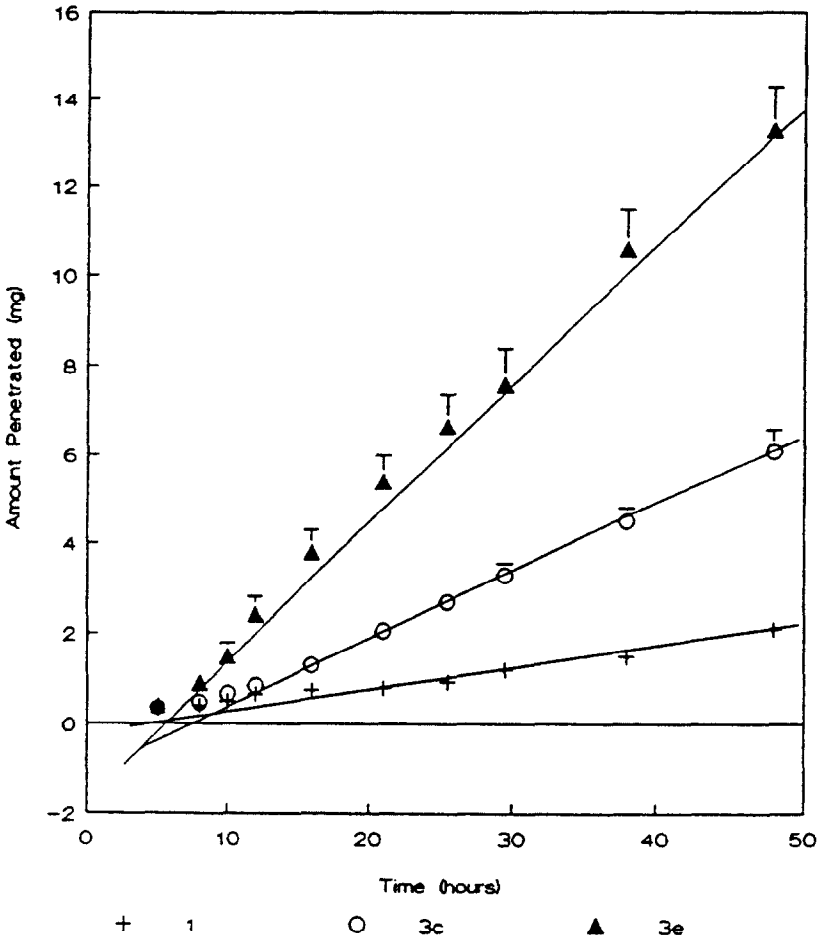


Figure 1. A representative diagram of the time course of penetration of methatropine and soft drugs **3c** and **3e** across the hairless mice skin. Each value is the mean of four determinations. Error bars indicate the S.E.M.

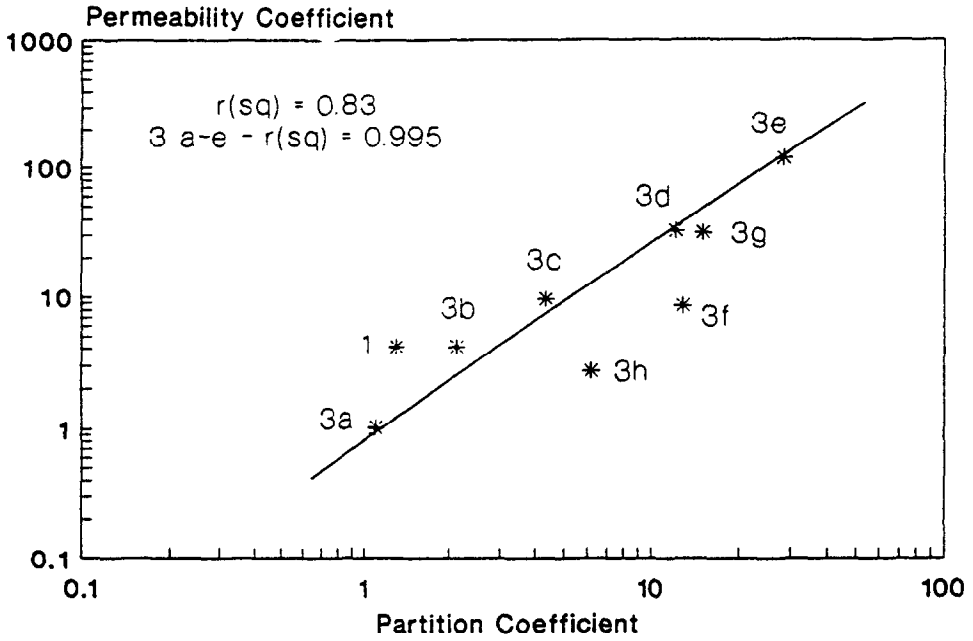


Figure 2. Relationship between log [*n*-octanol/water partition coefficient] and *in vitro* determined permeability coefficient across the hairless mice skin

Conclusion

The soft drugs synthesized are potent anticholinergics and are metabolized to an inactive polar metabolite (**2**) during the transdermal penetration process in contrast to the non-metabolizable nature of **1**. When these compounds are applied topically, for e.g. skin, they will act locally (on eccrine sweat glands) and will be metabolized to the inactive metabolite in the systemic circulation, thus possibly minimizing any systemic side effects. Experiments are underway to study the antisecretory activities of these compounds.

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