The third evolution of ionic liquids: active pharmaceutical ingredients†‡

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A modular, ionic liquid (IL)-based strategy allows compartmentalized molecular level design of a wide range of new materials with tunable biological, as well as the well known physical and chemical, properties of ILs, which thus deserve consideration as 'tunable' active pharmaceutical ingredients (APIs) with novel performance enhancement and delivery options. IL strategies can take advantage of the dual nature (discrete ions) of ILs to realize enhancements which may include controlled solubility (*e.g.*, both hydrophilic and hydrophobic ILs are possible), bioavailability or bioactivity, stability, elimination of polymorphism, new delivery options (*e.g.*, slow release or the IL-API as 'solvent'), or even customized pharmaceutical cocktails. Here we exemplify this approach with, among others, lidocaine docusate (LD), a hydrophobic room temperature IL which, when compared to lidocaine hydrochloride, exhibits modified solubility, increased thermal stability, and a significant enhancement in the efficacy of topical analgesia in two different models of mouse antinociception. Studies of the suppression of nerve growth factor mediated neuronal differentiation in rat pheochromocytoma (PC12) cells suggests potential differences between LD and lidocaine hydrochloride at the cellular level indicating an entirely different mechanism of action. Taken together these results suggest that the unique physiochemical properties of ILs in general, may confer a novel effect for the bioactivity of an API due to (at least) slow-release properties in addition to novel delivery mechanisms.

Introduction

Ionic liquids (ILs) are defined generally as salts with melting points below 100 °C (many liquid at ambient temperature), and whose melts are composed of discrete cations and anions.[1] ILs *per se* have been known for over a century,[2] but they have come under intense worldwide scrutiny only relatively recently due to implications for the use of these compounds as solvents,[1,3] where the accessible physical property set (*e.g.*, non- or low volatility, thermal stability, or large liquid ranges)[4] achievable with many ILs (Fig. 1: [Generation 1]) are often unique. There is now, however, growing interest in the materials applications of ILs which utilize novel tunable physical and chemical property sets (Fig. 1: [Generation 2]) for such applications as energetic materials, lubricants, metal ion complexation, *etc.*[5] ILs make a unique architectural platform on which, at least potentially, the properties of both cation and anion can be independently modified, enabling tunability in the design of new functional materials, while retaining the core desired features of an IL.

While a tremendous amount of recent research has focused on the physical properties of ILs, and more recently the chemical properties, the toxicity, a biological property, has been one of the most highly debated topics in this field.[6] Indeed, toxicity is also a tunable property of ILs, and given the similarities between many common IL building blocks and active pharmaceutical ingredients (APIs) or API precursors, one wonders why the potential to utilize the biological properties of ILs has received passing interest at best. Biologically active ions have been used to make new ILs; however, the primary driver for these materials has been the use of ions of known low toxicity to obtain the IL physical property set.[7] ILs of antimicrobial quaternary ammonium cations have been known for quite some time and recently, these have been shown to retain their biological activity.[8]

Results and discussion

Let us then consider here, biological activity, as the primary IL property and look at ILs as APIs (Fig. 1: [Generation 3]). Currently, the pharmaceutical industry and government regulatory agencies rely on crystalline APIs, which may be

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† The HTML version of this article has been enhanced with colour images.

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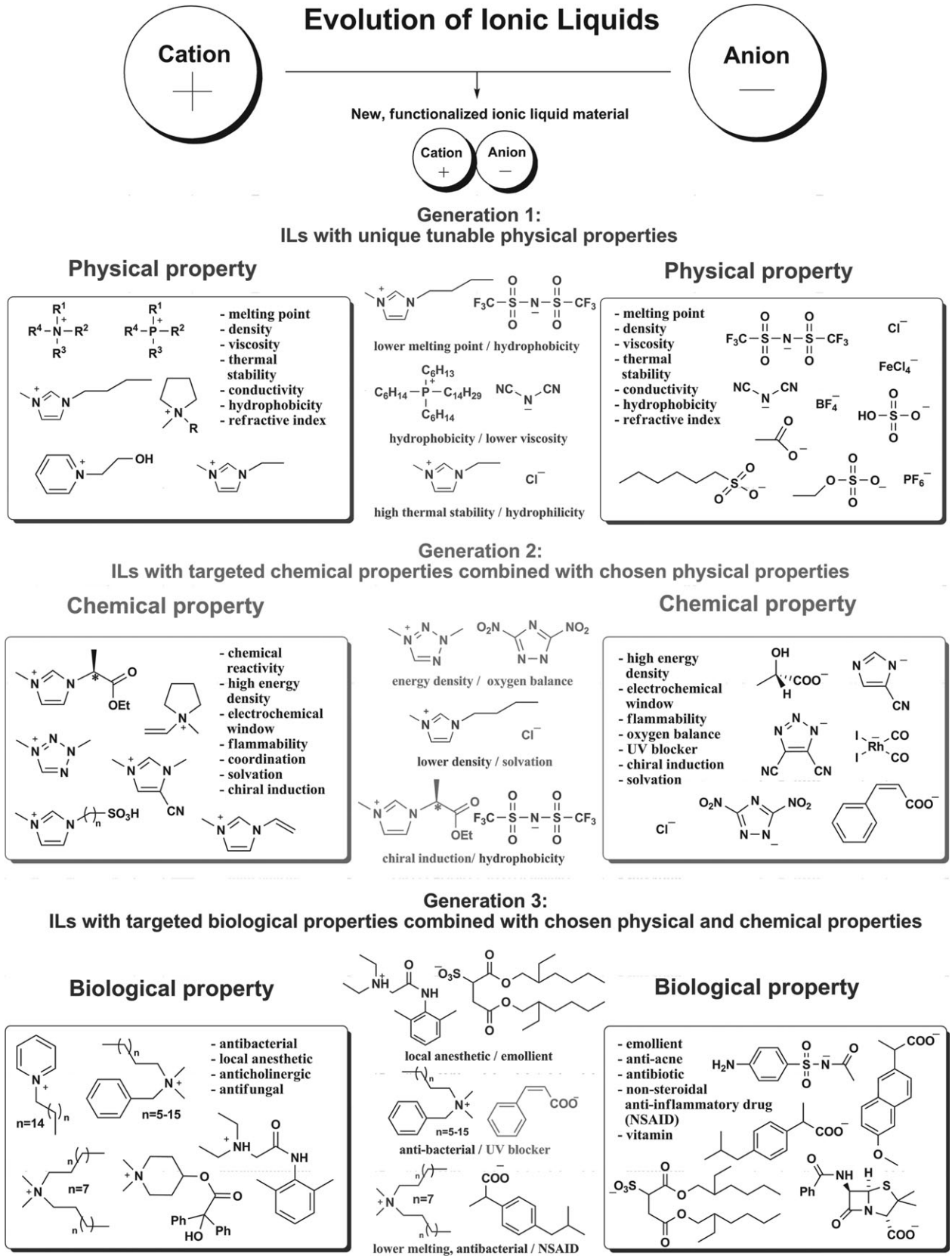


Fig. 1 The evolution of the scientific focus on ILs from unique physical through unique chemical and now biological property sets.

approved as neutral compounds or salts and solvates of neutral compounds or salts. Typically, the solid form of a pure pharmaceutical salt is selected by screening salts[9] of pharmaceutically accepted counter ions, to ultimately yield APIs with ease of manufacturing, coupled with controlled crystal size, and product solubility. However, solid forms of APIs often suffer from polymorphic conversion, low solubility, and a variety of factors which affect bioavailability associated with the final solid form.[10] Many Phase II trials of new APIs end in failure due to their efficacy, often related to bioavailability and thus, solubility.[11] Given the tremendous increase in knowledge directed toward understanding 'salts' generated by the IL community, targeted alterations of a final drug form based on the various property sets obtainable through an IL approach may help to enhance efficacy, while retaining the biological activity, or even introduce a second biological activity.

There is a growing body of knowledge addressing the structural and physical characteristics likely to lead to IL formation.[12] Selection of pairs of ions likely to form ILs is commonly carried out with candidate ions that are low symmetry, charge diffuse ions; traits also characteristic of many typical APIs. Even the nitrogen-containing heterocycles, so commonly used in ILs today, are frequently found in APIs or API precursors.[13] Still, care must be taken when choosing appropriate IL-forming ion pairs. Many of the important APIs are not permanent ions, but rather are protonated or deprotonated to form the commonly used salts; thus suitable pK_a differences need to be considered.[14] Although MacFarlane and Seddon[15] have recently proposed that protic ILs only be considered 'ILs' if the pK_a difference is such that more than 99% of the salt exists in ionized form, for an API, such a distinction may not be needed since there may be advantages in having the ability to tune the exact amount of API present in ionized and neutral form. Additionally, IL-APIs might require a more stringent liquid range than normal for 'ionic liquids', such as salts that are liquid at room temperature or at or below body temperature.

We have tested modifications of API physical properties and biological efficacy with ranitidine docusate (RD) and lidocaine docusate (LD). Ranitidine hydrochloride (RHCl), an anti-ulcer drug by GlaxoSmithKline and commonly known as Zantac™, has been the subject of extensive litigation over polymorphic forms and purity.[16] Lidocaine hydrochloride (LHCl) is in broad use as a topical, regional anesthetic for the treatment of post-surgical and neuropathic pain. Sodium docusate (sodium dioctylsulfosuccinate) has been previously shown to produce ILs,[17] but is also a dispersing agent shown to be absorbed in epithelial intestinal cells.[18]

Syntheses‡

The syntheses discussed here consist of metathesis reactions, although more elegant routes to pure IL-APIs can be envisioned.[19] The cation and anion in their available salt forms were separately dissolved in a solvent (*e.g.*, water or methanol) allowed to stir with heating (to *ca.* 90 °C if necessary) or at room temperature. The products (Table 1) were extracted typically with chloroform. Following that step, the chloroform phase was then washed with water to remove any inorganic salt (*e.g.*, NaCl, which was monitored by a silver nitrate test), and solvent was removed with a rotary evaporator. The resulting product was placed on a high vacuum line to remove any residual solvent. The structure and purity of each IL was confirmed by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and silver nitrate test. Additionally, the compounds were characterized with Karl-Fischer analysis, thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC).

Polymorphism

All three room temperature IL-APIs in Table 1 demonstrate that an API can be quite easily converted into a room temperature IL, which by its very nature as a pure liquid will not exhibit polymorphism (of the type which was highlighted in the Zantac™ litigation), or crystallization at all for that matter. This is in common with all room temperature IL-APIs, however, a major challenge in adopting the IL-API approach lies in determining enhanced, reduced, synergistic, or even equivalent performance as an API, and for that we need to examine other tunable 'IL' attributes as they relate to APIs.

Solubility and thermal stability‡

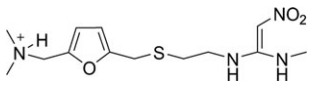
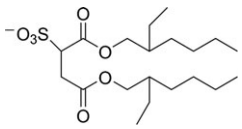
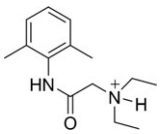
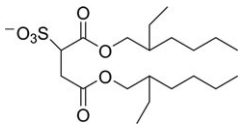
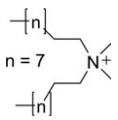
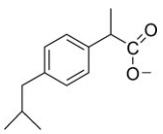
Lidocaine hydrochloride (LHCl) is a white crystalline solid that has been used as a local surface anesthetic in dentistry, and more recently for the topical treatment of *Herpes zoster* neuralgia[20] and postherpetic neuralgia.[21] Transdermal preparations of local anesthetics or analgesics depend on drug-retaining layers that typically contain a water-soluble gel base that can release an active substance.[22] The IL-API strategy brings together the relatively hydrophobic lidocaine cation with a hydrophobic anion, docusate (an emollient) to produce a hydrophobic IL salt, which exhibits reduced or controlled water solubility and thus should exhibit extended residence time on the skin.

DSC analysis of LD (Table 1) indicates no melting point, but a glass transition (T_g) at −29 °C and a liquid–liquid transition at 78 °C. TGA data suggest a one-step decomposition temperature ($T_{5\%onset}$) of 222 °C, which is significantly higher than that of LHCl ($T_{5\%onset}$ = 155 °C). The decomposition temperature for LD is similar to that observed for sodium docusate ($T_{5\%onset}$ = 219 °C).

LD is both hygroscopic (in common with many ILs)[23] and hydrophobic (that is exhibiting limited water solubility). The maximum water solubility of LD (neutral free-drift pH) is 1.24 mM (0.813 g L^{-1}), which is lower than either starting material, LHCl (673.7 g L^{-1} or 2.488 M)[24] or sodium docusate (15 g L^{-1} or 33.7 mM).[25] This behavior is common with many hydrophobic ILs, where the water solubility is a tunable property.[26]

Even though LD is hydrophobic, it is still hygroscopic and thus will absorb moisture from the atmosphere. When saturated, the water content of LD is 9.6 wt%. The water content can be reduced to 3 wt% after drying under high vacuum at 80 °C for 100 h, with the expected increase in viscosity (suggesting future drug delivery options). All of the observed behavior for LD is consistent with the behavior of hydrophobic ILs reported in the literature,[26] where a growing body of

Table 1 Examples of the IL-API approach[a]

Biological properties	Cation source	Anion source	IL
Histamine H_2-receptor antagonist, emollient	Ranitidine hydrochloride Function: histamine H_2-receptor antagonist 	Sodium docusate Function: emollient 	Ranitidine docusate Form: dark red gel Melting point: liquid at RT T (glass transition): −12 °C T (liquid–liquid transition): 29 °C
Pain reliever, emollient	Lidocaine hydrochloride Function: pain reliever 	Sodium docusate Function: emollient 	Lidocaine docusate Form: colorless gel Melting point: liquid at RT T (glass transition): −29 °C T (liquid–liquid transition): 78 °C
Antibacterial, anti-inflammatory	Didecyldimethylammonium bromide Function: antibacterial 	Sodium ibuprofen Function: anti-inflammatory 	Didecyldimethylammonium ibuprofen Form: yellow liquid Melting point: liquid at RT T (glass transition): −73 °C T (liquid–liquid transition): 69 °C

[a] Ranitidine hydrochloride was investigated to show that a well known polymorphic API could be reformulated as a room temperature IL. Didecyldimethylammonium ibuprofen was prepared to provide support for the approach that the primary biological activity could be chosen as the anion rather than the cation.

knowledge allows one to choose appropriate techniques for complete drying and control of water content.[27]

Antinociception assays‡

LD produced a longer duration of antinociceptive effect than LHCl as indicated by an overall analysis of the efficacy of the drugs administered at different concentrations in 90% DMSO–10% H_2O (Fig. 2). For the intact mouse model, statistically-significant group differences in the antinociceptive area under the analgesic curve (AUC) were found: $F_{(5,66)} = 3.177$, $p < 0.05$ (analysis not shown). The effect of the 100 mM dose reflects greater antinociception in mice exposed to LD as indicated by Fischer's LSD *post hoc* analysis (Fig. 2a). Repeated measure ANOVA (analysis of variance) demonstrated group differences for the 100 mM dose: $F_{(5,66)} = 3.0967$, $p < 0.05$, as well as a significant effect of time ($F_{(4,264)} = 20.486$, $p < 0.05$; Fig. 2b). Notably there was also a significant group by time interaction ($F_{(20,264)} = 2.226$, $p < 0.05$) indicative of prolonged antinociception.

Enhanced antinociception produced by 100 mM LD over 100 mM LHCl was also found for the thermal injury, but over a longer time interval (Fig. 3). Significant group differences in AUC reflect enhanced efficacy and duration of the LD formulation: $F_{(1,47)} = 8.991$, $p < 0.01$. An ANOVA repeated measure analysis which captured the tail-withdrawal responses

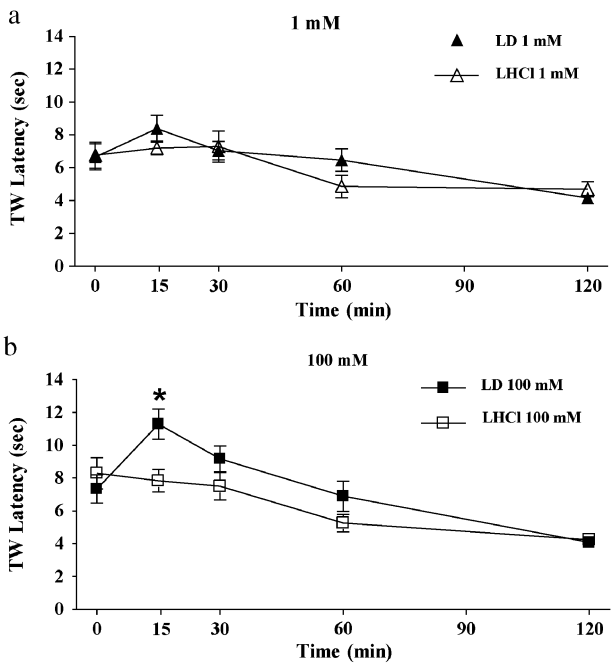


Fig. 2 Effects of topical LD and LHCl on the warm-water mouse tail-withdrawal (TW) latency response (*$p < 0.05$, Fisher's LSD Test).

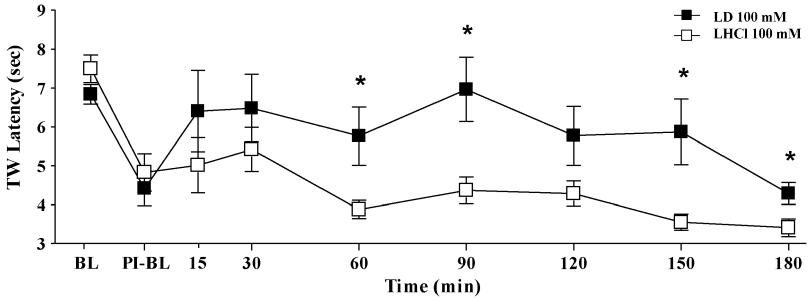


Fig. 3 Effects of topical LD and LHCl on the warm-water tail-withdrawal latency response of mice bearing a tail wound about 1 cm^2 which was placed in a 47 °C water bath (BL = baseline, PI-BL = post injury baseline). The drugs were administered 20 min after PI-BL (*$p < 0.05$, Fisher's LSD Test).

20 min after injury, and for 180 min following lidocaine exposure, demonstrates that LD produces greater antinociception. This was supported by the repeated measures analysis, with a main effect of group $F_{(1,47)} = 6.515$, $p < 0.05$, of time ($F_{(8,376)} = 7.377$, $p < 0.01$), and a significant interaction ($F_{(8,376)} = 2.317$, $p < 0.05$). These results indicate that LD, in comparison with LHCl, was more effectively absorbed through the skin of the mouse tail to reach afferent free-nerve endings for the production of antinociception.

Suppression of PC12 neuritic outgrowth by LD and LHCl‡

Local anesthetics suppress nerve growth factor (NGF) mediated neuronal differentiation in rat pheochromocytoma (PC12) cells. This was used as a bioassay for detecting potential differences between PC12 cells treated with LD or LHCl. About 600 μM lidocaine is required to block afferent nociceptive fibers that include Aδ and C fibers in the rat sciatic nerve.[28] Therefore 4000 or 400 μM corresponds to the concentrations necessary for regional anesthesia.

Fig. 4 shows the effects of LHCl and LD on neuritic outgrowth in PC12 cells exposed to NGF. No neurites were found without NGF added to the media, while in the presence of NGF alone, about 30 neurites were counted. Most cells were killed at 4000 μM LHCl and all cells were killed at the same concentration with LD. At 400 μM LHCl an average of about 10 neurites were found, where with 400 μM LD, about twice as many neurites were found. There was a statistically-significant difference between the number of neurites produced with 50 mg mL^{-1} NGF alone and 50 mg mL^{-1} NGF in the presence of either LHCl or LD ($p < 0.05$). There was also a significant difference between the number of neurites found with 400 μM LD and LHCl ($p < 0.05$). Overall, even though cell death was complete at 4000 μM LD, at the lower 400 μM LD, cells appeared healthy and well organized.

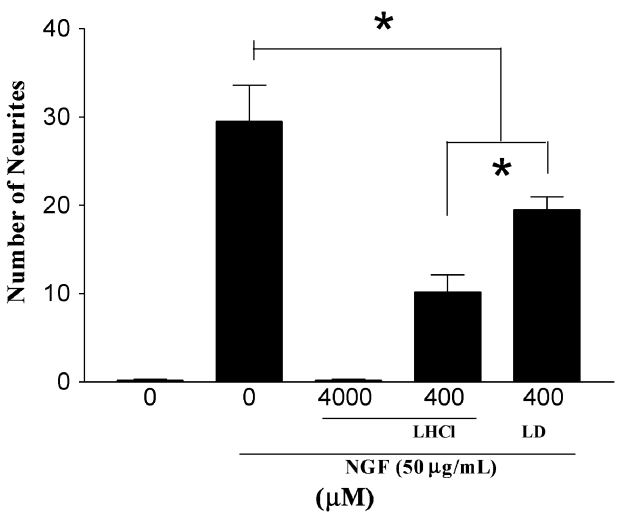


Fig. 4 LHCl or LD suppression of neuritic outgrowth in PC12 cells treated with 50 ng mL^{-1} NGF (*$p < 0.05$, Bonferroni's Multiple Comparison Test).

The PC12-NGF data suggest potential differences between LD and LHCl at the cellular level and indicate a mechanism of action entirely different for LD than that for LHCl. Docusate may enhance membrane permeability as has been shown in bacteria,[29] which may suggest at least one mechanism associated with the apparent increase in LD efficacy *in vivo*. However, while an increase in permeability may enhance transdermal transport and account for the longer duration and greater efficacy of LD *in vivo*, the longer duration of LD on the mouse tail-withdrawal indicates an alternative mechanism.

The PC12 data also suggest that the kinetics for LD are unique. One possible scenario is for lidocaine to be released from a hydrophobic-induced pairing of the lidocaine and docusate ions, for example a 'leaching' of the component ions into solution as observed for many hydrophobic ILs.[26] A slow release of lidocaine from the hydrophobic docusate interaction would account for the PC12 data if relatively small, and thus ineffective concentrations of lidocaine were available to the cell surface over time. This would allow for the formation of neuritic processes even in the presence of lidocaine in the media.

Slow release

Bramer *et al.*[30] have shown that mixtures of lidocaine hydrochloride and sodium docusate in physiological saline form micelles and/or vesicles which exhibited slow-release kinetics in gels. The hydrophobicity we observe for LD would also account for the increased duration of LD over LHCl as observed in our *in vivo* models and this may constitute a slow-release mechanism unique to any hydrophobic IL. Taken together with the solvent properties of hydrophobic ILs in general, where, for example, additional API solutes could be

dissolved in the IL-API, one can envision a general platform for the slow release delivery of a variety of APIs.

Conclusions

Even though biological testing was conducted here only *via* topical application, taken as a whole, the results above suggest that the unique physicochemical properties of ILs in general, may confer a novel effect for the bioactivity of an API due to (at least) slow-release properties in addition to novel delivery mechanisms. In the case of LD, such enhancement of API bioactivity from topical application appears to occur in addition to, or apart from, the possible enhancing effects of the surfactant on membrane permeability. These observations provide a foundation for the formulation of controlled and novel API bioactive characteristics using IL technology.

Although dual functioning salts (including blending of salts), and salts of APIs that fit the general current definition of ILs are known, the knowledge base being generated within the field of ILs has not been applied to these systems, or perhaps even recognized. We thus propose that, in addition to the traditional crystalline salt screening and selection that most APIs undergo, pure liquid salt forms of APIs should be considered as a design strategy to overcome potential problems such as polymorphism, solubility, and bioavailability. We are convinced that the tunability inherent to the field of ILs is entirely appropriate and applicable to the field of pharmaceuticals. ILs can not only provide the solutions to the problems often faced by the solid drug, but can also introduce new treatment or delivery options which are not available through use of solid APIs or traditional approaches. Using such a modular design, it should be possible to prepare a plethora of IL-APIs which can be customized to fit individual needs, while tuning the physical and chemical properties of these ILs. Importantly, by pairing a failed API with the appropriate cation or anion, one may be able to overcome the specific problem that led to this compound's failure (*e.g.*, poor solubility).

Interestingly, synergistic effects as a result of pairing two biologically active ions may be both a blessing and a curse. While IL-APIs can indeed be prepared quite easily through proper ion selection, it is not clear how readily such formulations could be adopted for use. Synergistic biological effects (as observed for LD here) may not be predictable and regulatory agencies may require significant testing on such salt forms. By the same token, such synergistic or additive effects may lead to new treatment options or enhanced drug efficacy or delivery, not possible from crystalline APIs. Regardless of one's point of view, we feel that an IL modular design strategy should at least be added to the considerable portfolio of process options currently employed by the pharmaceutical industry.

Methods

Lidocaine docusate

In a 100 mL round-bottomed flask charged with a magnetic stirbar, 1.552 g (3.49 mmol) of sodium dioctylsulfosuccinate (sodium docusate; Aldrich, St. Louis, MO) was dissolved/suspended in 25 mL of reagent grade acetonitrile. To the stirred mixture was added, in one portion, 0.945 g (3.49 mmol) lidocaine hydrochloride (Sigma-Aldrich, St. Louis, MO). The suspension was stirred overnight, after which time the solvent was removed *in vacuo*. The white residue was extracted with 2×25 mL portions of dichloromethane, which were combined and flash filtered through small plugs of Celite and silica. The colorless solution was evaporated to leave 1.88 g (2.86 mmol, 82%) of the desired lidocaine docusate salt as a clear, colorless syrup. The product tested negative for the presence of chloride anions with silver nitrate.

Ranitidine docusate

In a 50 mL round-bottomed flask charged with a magnetic stirbar, 1.917 g (4.31 mmol) of sodium dioctylsulfosuccinate (sodium docusate; Aldrich, St. Louis, MO) was dissolved/suspended in 20 mL of reagent grade acetonitrile. To the stirred mixture was added, in one portion, 1.513 g (4.31 mmol) ranitidine hydrochloride (Sigma-Aldrich, St. Louis, MO). The deep yellow-brown suspension was stirred overnight, after which time it was filtered to remove precipitated NaCl. The solvent was removed under vacuum and the deep red-brown residue extracted with dichloromethane, the resulting solution then being flash chromatographed on silica, eluting with dichloromethane. Removal of the dichloromethane yielded the product (1.621 g, 3.83 mmol, 89%) as a deep, red-brown syrup.

Didecyldimethylammonium ibuprofen

Didecyldimethylammonium bromide (0.001 mol) (Sigma-Aldrich, St. Louis, MO) was dissolved in 60 mL of distilled water by gentle heating and stirring. Sodium ibuprofen (0.001 mol) (Sigma-Aldrich, St. Louis, MO) was dissolved in 60 mL of distilled water by gentle heating and stirring. The two solutions were combined and the reaction mixture was heated and stirred for 30 min. Afterwards, the reaction mixture was cooled to room temperature, 60 mL of chloroform was added, and the mixture was stirred for an additional 30 min. The two phases were separated and the chloroform phase was washed several times with cool distilled water to remove any inorganic salt. The presence of chloride anions was monitored by silver nitrate test. The solvent was removed on a rotary evaporator and the product, didecyldimethylammonium ibuprofen, was obtained with 91% yield.

Mouse antinociception tests

The warm water tail-withdrawal assays were conducted with mice at Furman University in accordance with guidelines from the Institutional Animal Care and Use Committee. Relative differences in the potency of LHCl and LD were assessed in adult naïve Swiss Webster mice of both sexes obtained from Taconic Laboratories (Germantown, NY). The mice were housed 4–5 per Plexiglas cage and maintained on a reverse 12 : 12 light–dark cycle (lights off 0700) at an ambient temperature of 22 ± 2 °C. Water and food (Harlan Mouse Chow) were provided *ad libitum*.

Antinociception was assessed using a modification of the tail-withdrawal procedure established by D'Amour and Smith.[31] Two antinociceptive models were used: warm water tail-withdrawal from 49 °C water in intact mice, and warm water tail-withdrawal from a 47 °C bath, following tail injury. A 12 s cut-off latency was used for intact mice to prevent tissue damage, and due to the reduced intensity of the heat stimulus used for mice with tail injury, cutoff time was set at 20 s. The hyperalgesia produced by the tail injury approximates the physical conditions under which lidocaine has been widely used for regional analgesic therapy. For the tail injury, a hyperalgesic state was induced by lightly taping the distal 3 cm of the tail to a 52 °C hotplate for 60 s. In both cases, pre-drug baselines and all subsequent nociceptive measures were determined by averaging two tail-withdrawal latencies (separated by *ca.* 60 s) taken while the mouse was lightly restrained in a cloth pouch with the distal third of its tail protruding into the water bath.

LHCl or LD were dissolved in 90% DMSO : 10% H_2O for transdermal administration according to the method of Kolesnikov *et al.*[32] The animals were restrained in these same cloth pouches by submerging the distal half of the tail for 60 s in the drug solution. In order to minimize possible stress resulting from this restraint (unlike the tail-withdrawal testing, which takes only a few seconds and the mice are free to squirm, tails need to be more or less stationary for 60 s) mice were habituated to this procedure on three separate occasions during the week before testing.

In the thermal injury test, mice were assessed for baseline sensitivity and then immediately exposed to the heat stimulus. Twenty minutes after injury, a second (post-injury) nociceptive baseline was determined, immediately followed by transdermal drug administration. Tail-withdrawal latency was subsequently measured at 15, 30, 60, 90, 120, 150, and 180 min. Between procedures mice were returned to their home cages. Three concentrations of each drug (1.0, 10.0, or 100.0 mM) were used to generate dose–response curves in the initial characterization and the higher dose (100 mM) was evaluated following injury.

An ANOVA was used to assess group differences in tail-withdrawal latencies at each drug concentration (by repeated measures analysis across time; *post hoc* analysis was determined by Fischer's LSD method). In all cases, the criterion for significance was set at $p \leq 0.05$.

Suppression of PC12 neuritic outgrowth

Data collection was done using methods consistent with Takatori *et al.*,[33] who report ~5% neurite-bearing cells at day 3 and ~20% at day 4 using 100 ng mL^{-1} NGF and counting process $\geq 1\times$ cell body diameter. PC12 cells were purchased from American Type Culture Collection (Manassas, VA). Cells were grown in RPMI medium supplemented with 15% normal horse serum and 5% fetal bovine serum and 25 U of penicillin and streptomycin in 1 cm diameter wells for 3 days at 37 °C under 5% CO_2 and 95% air. Cells were exposed to either no treatment (0), NGF (50 μg mL^{-1}) alone, 400 and 4000 μM LHCl, or 400 and 4000 μM LD in the presence of NGF. Most cells were killed at 4000 μM LHCl and all cells were killed at 4000 μM LD. There were a total of 6 counts per bar/well. Cells were stained with nuclear red and cell processes were counted using phase-contrast microscopy. A minimum cluster range of 80–100 cells were selected mid-center of the well-floor that were examined per data point. Only processes with lengths equivalent to 3–4× diameter of cell body were counted.

Acknowledgements

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