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Report

The Relationship of pK_a and Acute Skin Irritation in Man

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The relationship between pK_a and skin irritation in man is studied for a homologous series of benzoic acid derivatives, which permeate through human skin at comparable rates (15–88 μg/cm²/hr). Skin irritation and pK_a are correlated for $pK_a \leq 4$. Laser Doppler velocimetric assessment of skin blood flow, color meter readings, erythema, edema, and the primary irritation index are all linearly correlated and related to pK_a; erythema at 24 hr appears to be the most sensitive parameter to variation in pK_a when $pK_a \leq 4$.

KEY WORDS: skin irritation; pK_a; pH, skin permeation; chemical structure/irritation.

INTRODUCTION

Understanding the relationship between chemical structure and skin irritation would aid the selection of drugs and excipients for use in transdermal and topical dosage forms. As a step toward this goal, the effect of penetrant pK_a on skin irritation in man has been studied.

Skin irritation is a complex phenomenon involving interaction among the solution properties of the vehicle, percutaneous transport, the biological response, and the local drug disposition. To elucidate the variables important to the biological response, the mode of delivery, diffusion kinetics, and clearance of the penetrant must be known or carefully controlled. Skin irritation, as determined by erythema and edema (1), seems to have multiple nonspecific and specific mechanisms (2). To isolate the pK_a of a penetrant as a cause of skin irritation is an oversimplification, but it is a useful variable to investigate initially. Previous studies (3–7) have implied that pH does indeed influence skin irritation, and it is the objective of this paper, therefore, to initiate quantitative evaluation of this observation.

MATERIALS AND METHODS

Study Design

To isolate the effect of penetrant pK_a on skin irritation in man, a homologous series of four compounds with a wide range of pK_a values was selected. The four chemicals were salicylic acid, salicylamide, *m*-nitrobenzoic acid, and *m*-anisic (3-methoxybenzoic) acid. The physiochemical properties (melting point, partition coefficient, oil and water solubilities; Table I) of these materials are such that comparable skin permeation rates were expected (8). It was anticipated, therefore, that differences in irritation caused by the compounds would be due to their wide range of pK_a values (~3–8).

Materials

Salicylic acid (Fisher, Springfield, N.J.), salicylamide (Eastman, Rochester, N.Y.), *m*-nitrobenzoic acid (Eastman, Rochester, N.Y.), and *m*-anisic acid (Aldrich, Milwaukee, Wis.) were used as obtained. Hydroxyethyl methacrylate copolymer hydrogels—a stock 65/35 (w/w) mixture, PP batch No.6—were obtained from CIBA-GEIGY. Hydroxyethyl methacrylate (Sipomer, HEMA) and t-butylperoctoate initiator were provided by Dr. K. F. Mueller, CIBA-GEIGY, Ardsley, N.Y.

Methods for Physical Characterization of Test Compounds and Devices

Partitioning and pK_a. The octanol–water partition coefficient, K, and pK_a of each compound were measured using standard procedures (9).

Analytical. Chemicals were assayed by high-performance liquid chromatography (HPLC) (Waters, Milford, Mass.) using a WISP Model 710 with a Model 530 programmable solvent pump and a (Kratos, Paramus, N.J.) Spectroflow Model 783 detector set at 230 nm. The column was a Waters Nova-pak C-18 (3.9 mm × 15 cm × 4 μm) and the mobile phase was 35% methanol, 65% sodium acetate buffer (5 m*M*) adjusted to the appropriate pH with acetic acid (pH 4 for salicylic and *m*-nitrobenzoic acids and pH 5 for the other two). The flow rate was 1 ml/min, the injection volume 20 μl, and the range of detection 1–200 μg/ml. Under these conditions, retention times ranged from 1.9 to 3.6 min, depending on the test compound.

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Table I. Physical Properties of the Test Compounds

	Salicylic acid	Salicylamide	*m*-Nitrobenzoic acid	*m*-Anisic acid
Melting point (°C)	158	140	142	103
MW	138.1	137.1	167.1	152.1
log *K* (octanol–water)				
Experimental	2.22	1.31	1.79	1.90
Literature (Ref. 13)	2.21–2.26	1.25–1.28	1.83	2.02
pK_a				
Experimental	2.95	8.10	3.56	3.99
Literature (Ref. 12)	2.97	8.66 (35°C)	3.45	4.09
Water solubility (mg/ml)[a]	2.5	0.23	3.9	0.18
pH of saturated solution	2.41	5.91	2.57	3.06
Ethanol:water solubility[a] (7:3, v/v) at 38°C (mg/ml)	273	133	555	263
Skin permeation (μg/cm²/hr)	88 ± 10	15 ± 4	60 ± 20	75 ± 2

[a] Capped centrifuge tubes of saturated solutions were rotated for 24 hr before HPLC analysis (in quadruplicate) of the solution phase.

Hydrogel Polymerization. The hydrogels were prepared by dilution of the 65/35 monomer/polymer mixture with HEMA monomer to give an 80:20 mixture. After degassing and addition of 0.20% t-butylperoctoate, films were polymerized by heating at 100°C for 1 hr in polyester-lined glass molds. The films were soaked in water for 24 hr, punched into a 1-cm² disks, and vacuum dried. These disks were Soxhlet extracted in ethanol for 48 hr and again vacuum dried.

Hydrogel Loading with Test Compound. Hydrogel disks were soaked for 24 hr at 38°C in ethanol:water (7:3, v/v) saturated with the test compound. Upon removal from the loading solution, the disk was quickly rinsed (approximately 2 sec) in ethanol:water (7:3, v/v) to remove surface test compound. The disks were blotted and air dried for 24 hr. Immediately prior to use, the loaded disks were soaked for 2 hr in a saturated aqueous solution of the test compound at ambient temperature.

Skin Permeation. Male human skin was obtained frozen from skin banks. After thawing the epidermis was separated from the dermis following exposure of the tissue to water at 60°C for 80 sec (10). The isolated epidermis was rapidly rinsed with hexane to remove surface lipid, then rinsed with water before being mounted between the two halves of a modified static diffusion cell (11). The receptor solution was 0.01% aqueous gentamicin sulfate. The donor phase was either 1 ml of saturated drug solution or a loaded hydrogel disk. The temperature of the epidermis during the experiment was 32°C. Steady-state flux of penetrant was determined by a least-squares fit to the linear portion of a plot of the cumulative amount penetrated versus time.

Methods to Study Human Skin Irritation

Subjects. Sixteen female subjects aged (35 to 45 years) consented to the study. Ten were of Hispanic origin, possessing various degrees of olive-complected skin. Five were Caucasian with type II skin, and one was of Caucasian/Polynesian descent with a slightly olive skin tone. All subjects had reasonably clear backs with no or few comedones, moles, or freckles.

Procedure. Day 1: The subjects acclimated for a half-hour, during which time treatment sites were marked on the back with a felt-tip marker. The scapular regions of the upper back unoccluded by undergarments were tested. A column of five test sites was marked on each side, avoiding the midline. This accomodated two replicates per subject for each of the four compounds and a distilled water control. The position of each treatment within a side of the back was randomized. Baseline scoring and measurements were taken. The relative humidity and temperature were noted. The 12 loaded test disks were applied to the skin and occluded with 3% ethylene vinyl acetate (EVA) membrane, secured with tape (Scanpore Norgeplaster 6, Oslo, Norway).

Day 2: The patches were removed, and the sites again marked. Thirty minutes later, the measurements were repeated. Erythema and edema were scored by standard 0–4 visual scales (1), with an additional rank at 0.5 to signify borderline reaction. Laser Doppler velocimetry (LDV; Med Pacific LD 5000, Seattle, Wash.)-assessed blood flow and color (reflected-light color analyzer Minolta Chroma Meter CR-100) were measured.

Day 3: The procedure of day 2 was repeated. The primary irritation index (PII), defined as the average of the sum of the erythema and edema scores measured on days 2 and 3, was calculated.

RESULTS

Skin Permeation

As expected, the *in vitro* skin permeation rates of the four chemicals from saturated solutions were quite similar, spanning the range 15–88 μg/cm²/hr (Table I). Additionally, *in vitro* skin permeation rates from the loaded hydrogels systems were comparable to those from saturated solutions, indicating that drug release from the hydrogels was rapid compared to percutaneous penetration.

The Skin Irritation Study

There were no statistically significant differences be-

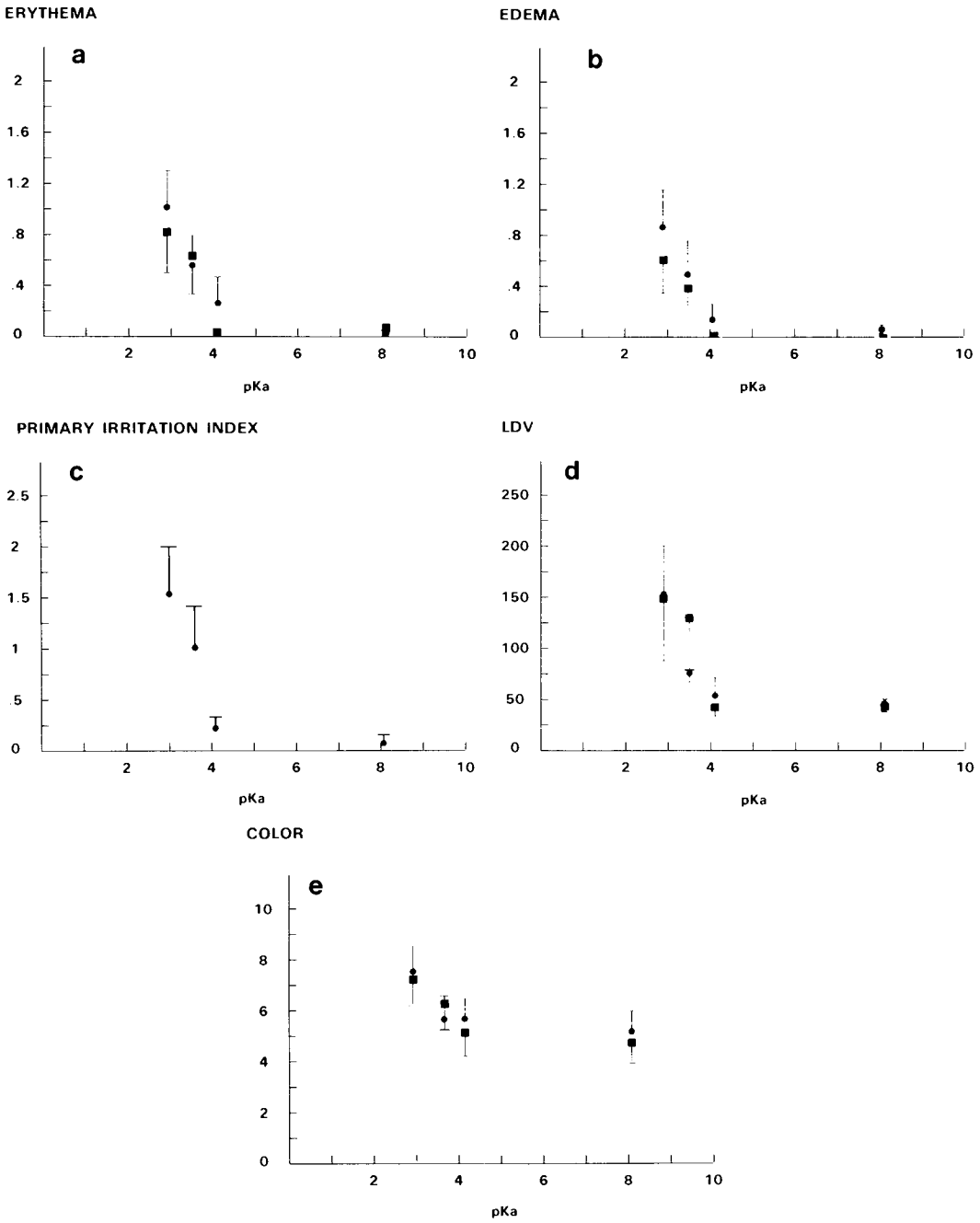

Fig. 1. Measurements of skin irritation versus penetrant pK_a at 24 hr (●) and 48 hr (■). Error bars represent 95% confidence limits. The measurements of skin irritation used are (a) erythema, (b) edema, (c) primary irritation index, (d) LDV, and (e) color meter.

tween the left and the right sides, and consequently, the results were pooled.

In Fig. 1, the average skin irritation measurements for each treatment are plotted against the drug pK_a; a trend of steeply increasing irritation for $pK_a < 4$ is evident. Treatment with salicylamide ($pK_a = 8.1$) was not significantly different from a water control in any of the observables. Erythema at 24 hr appears to be the measurement most sensitive to pK_a ($P < 0.05$ by pairwise t tests); edema at 24 hr is also correlated significantly with penetrant pK_a ($P < 0.05$). The 48-hr measurements were somewhat less sensitive than the 24-hr measurements. None of the instrumental parameters were particularly sensitive to differences in pK_a.

All of the measurements of irritation were strongly correlated with each other ($P < 0.001$ for a linear regression except for 48-hr color with edema and LDV, for which $P < 0.02$).

DISCUSSION

While skin irritation is a complex biological response, the penetrant pK_a for chemicals with $pK_a < 4$ appears pre-

dictive of the degree of local reaction in this one homologous series. For the range of oil and water solubilities and skin permeation rates of this series of compounds, structures with $pK_a < 4.1$ appear to induce skin irritation. The degree of irritation increases as the pK_a becomes smaller. These results agree with subcutaneous injection studies in mice (4–6) and the dependence of irritation on the vehicle pH of hydrocortisone cream (3). On the basis of previous observations (4–6), one might postulate analogous behavior for basic compounds, with irritation becoming significant at $pK_a > 10$ (4–6). To predict skin irritation adequately from chemical structure, percutaneous flux, pK_a, and skin irritation, curves need to be measured for a significant number (e.g., 35–45) of acids and bases. This minimal-variable approach to prediction might also include melting point, molecular weight, and partition coefficient as additional parameters for examination (8).

The effect of pK_a on skin irritation is presumably due to pH effects at the epidermal level and is consistent with a cellular damage mechanism of skin irritation. Such pH-related effects at the epidermal level could result from two potential sources of H^+: (a) different activities of H^+ in the solutions applied to the skin and (b) intact drug that crosses the stratum corneum and then dissociates into ions in the epidermis. In the experiments reported here, the hydrogen ion would have to traverse the stratum corneum with the only available source of counterion, the ionized chemical. The transport of ionized species across the stratum corneum is sufficiently slow (8) that this mechanism is unlikely to apply. Therefore, the local dissociation of the chemical in the epidermis must predominate.

CONCLUSION

The parameters used to assess skin irritation are highly linearly correlated. Erythema at 24 hr appears to be the most sensitive of these parameters to the penetrant pK_a.

In this study, it has been shown that for this homologous series, pK_a is correlated with skin irritation for compounds having a $pK_a \lesssim 4$. Further work to extend this initial base of knowledge to a similar series of bases appears justified.

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REFERENCES

1. J. H. Draize, G. Woodard, and H. O. Calvary. *J. Pharmacol. Exp. Ther.* 82:377–389 (1944).
2. E. Patrick. *Investigations of the Mechanisms of Chemically Induced Skin Irritation,* Ph.D. dissertation 8509113, University Microfilms International, Ann Arbor, Mich., 1984.
3. V. E. Koch, F. Loebich, and P. J. Frosch. *Dermat. Beruf Umwelt* 31:122–126 (1983).
4. K. Bucher, K. E. Bucher, and D. Walz. *Agents Actions* 9:124–132 (1979).
5. K. Bucher, K. E. Bucher, and D. Walz. *Agents Actions* 11:515–519 (1981).
6. D. Walz and K. E. Bucher. *Agents Actions* 12:552–565 (1982).
7. Health Designs, Inc. *Release 1.0,* Rochester, N.Y., 1985.
8. B. Berner and E. R. Cooper. In A. F. Kydonieus and B. Berner (eds.), *The Transdermal Delivery of Drugs,* CRC Press, Boca Raton, Fla., 1987, pp. 41–56.
9. F. H. Clarke. *J. Pharm. Sci.* 73:226–230 (1984).
10. A. M. Kligman and E. Christopher. *Arch Dermatol.* 88:702–705 (1963).
11. E. W. Merrit and E. R. Cooper. *J. Contr. Rel.* 1:161–162 (1984).
12. G. D. Fasman. *Handbook of Biochemistry and Molecular Biology, Vol. I,* 3rd ed., CRC Press, Cleveland, Ohio, 1976, pp. 176–181.
13. C. Hansch. *Substituent Constants for Correlation Analysis in Chemistry and Biology,* John Wiley and Sons, New York, 1979, pp. 212–234.