Gene Expression in Rat Skin Induced by Irritating Chemicals

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ABSTRACT: Occupational skin disease is the second most significant cause of occupational disease, after accidents. Irritation from occupational chemicals such as solvents, hydrocarbons, and surfactants are one cause of this disease. Gene expression studies provide useful information about normal processes in the skin and responses of the skin to exogenous chemicals. We exposed rats, cutaneously, to sodium lauryl sulfate (SLS, 1% and 10% aqueous solution), *m*-xylene (pure liquid), and *d*-limonene (pure liquid) for 1 h and measured transcriptional responses at the end of the exposure and 3 h later for comparison with untreated skin samples. Total skin RNA was isolated and analyzed using the Affymetrix RatTox U34 array. Using the Affymetrix software, we found that 234 of approximately 850 genes were detected as present in at least 80% of the normal skin samples. The largest number of these genes was related to metabolism, oxidative/cellular stress, and signal transduction. Limonene caused the largest change in mRNA levels with a total of 34 increased transcripts and 4 decreased transcripts. Xylene treatment resulted in 6 increased transcripts and 14 decreased transcripts, while 10% SLS caused 5 transcripts to increase and 17 to decrease. Only two transcripts were observed to change in skin following a 1% SLS exposure. Sodium lauryl sulfate transcript changes increased with dose and were maximum at 4 h. Limonene transcript changes were more numerous at 1 h than at 4 h. The observed differences may reflect different mechanisms of irritation.
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INTRODUCTION

Irritation of the skin by chemicals and commercial products is a recognized problem that may cause significant work-time loss and an annual cost of $1 billion [1]. Contact dermatitis (irritant and allergic) accounts for over 90% of all occupational skin disease and is the second most significant cause of all occupational disease [1]. Irritant contact dermatitis is a non-immune-related response characterized by direct action of a compound on skin tissues [2]. In contrast, allergic contact dermatitis is a delayed (type IV) hypersensitivity reaction that requires prior sensitization to an allergen [3]. A retrospective study of occupational skin disease over a 10-year period in Singapore showed that irritant dermatitis was more common than allergic dermatitis [4]. Irritant dermatitis can be caused by a wide variety of compounds including solvents, surfactants, hydrocarbons, cutting fluids, and oils [4–7], although the underlying mechanisms resulting in irritation are unknown.

Chemicals and commercial products may cause irritation by interference with structural, molecular, and biochemical events responsible for the normal functions of the skin, including thermoregulation, protection, sensory, and support. Many of the compounds that cause skin irritation are relatively small molecules that might disrupt membranes or interfere with metabolic processes in the viable epidermis or the dermis. Although the most common observed responses in the skin following exposure to irritants are erythema (redness) and edema (swelling), underlying cellular and molecular mechanisms are now appearing to be useful indicators before overt skin irritation is apparent [8,9]. Molecular responses of the skin to irritating chemicals include inflammatory cytokine release [8,10–12], indications of oxidative stress [13–16], effects on prostaglandins [17,18], and activation of transcription factors [19].

Since recent mapping of the human genome, the ability of researchers to understand the regulation of

many molecular processes has increased tremendously for two reasons. First, an understanding of the gene expression in specific tissues gives many insights about the processes and functions in that tissue, because the specific, unique gene expression in that tissue is a result of tissue differentiation. Second, because biological systems are carefully controlled by effectors of the cells, i.e. proteins, perturbations in normal functions would be expected to be reflected in the process of increased or decreased protein synthesis. Gene expression technology has progressed to the stage where thousands of transcripts can be characterized from a single tissue sample using gene array techniques. This technology has not been widely applied to skin yet, but there have been a few studies evaluating thousands of genes. Fletcher and coworkers [20] used a cDNA microarray system to investigate changes in gene expression with sodium lauryl sulfate treatment of a cultured layered skin. Bernard and coworkers [21] compared gene expression profiles using a customized cDNA microarray system in keratinocytes, reconstituted epidermis, and human skin and investigated the effect of retinoid treatments on gene expression. Other studies have looked at smaller numbers of specific gene responses to a variety of treatments in keratinocytes [22–24], cultured skin [20,25], mouse skin [26], and human skin [27].

The purpose of this study was twofold. First, we wanted to characterize basal gene expression patterns in rat skin to help us increase the understanding of normal functioning skin. Second, we wanted to investigate early changes in gene expression following skin exposure to solvents (*m*-xylene and *d*-limonene) and a surfactant (sodium lauryl sulfate). These chemicals are known to cause irritation, and identifying genes that are involved in acute irritation will provide a basis for further investigation into elucidating the mechanism(s) responsible for skin irritation.

MATERIALS AND METHODS

Animals and Exposures

Male Fischer F-344 rats (CDF/CrlBR, Charles River Laboratories, Raleigh, NC; 250–350 g) were housed one per cage and provided food and water ad libitum. The animals were handled in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996, and the Animal Welfare Act of 1966, as amended. On the study day, rats were anesthetized with isoflurane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether) using a vaporizer (Ohio Medical Products, Laurel, MD). The back of the animal was closely clipped of fur, taking care not to damage the skin [28]. A template equivalent in diameter to a Hill Top Chamber® (Hill Top Research, Cincinnati, OH; 2.5 cm in diameter) and permanent marker were used to mark the exposure site on the back of each animal. A Hill Top Chamber® containing either 250 μL of 1% or 10% sodium lauryl sulfate (SLS; 99% purity, Sigma, St. Louis, MO), neat *m*-xylene (99% purity, Eastman Kodak Co., Rochester, NY), or neat *d*-limonene (>97% purity, Sigma) was placed on the exposure site, secured with an elastic adhesive bandage (Elastoplast® Beiersdorf, Inc., Norwalk, CT), and animals were exposed for 1 h. Samples were taken at 1 and 4 h after the beginning of the 1-h exposure. Zero-hour exposures (chemical-loaded chambers placed on the skin and immediately removed) were used as controls. Exposed skin samples were excised to the underlying muscle fascia, and subcutaneous fat was then removed with a sterile scalpel blade. Two to three 8.0-mm biopsy punches were taken from the skin at the exposure site and snap-frozen in liquid nitrogen. For each chemical, two animals per time point were used. Zero-hour skin samples from 10 different animals were used for detecting baseline transcript levels. Skin samples were also processed for routine histological analysis to determine whether chemical-induced pathological changes could be detected in the skin that might correlate with observed transcriptional changes.

Histological Analysis

Skin specimens were fixed overnight in 10% neutral buffered formalin. Following fixation, skin samples were dehydrated, and embedded in paraffin. Seven-micron-thick skin sections were stained with hematoxylin and eosin using routine procedures. Stained slides were assessed for histopathological changes associated with chemical exposure.

Skin Sample Preparation and RNA Isolation

Frozen biopsy punches (~60 mg) were pulverized in a Bessman stainless steel tissue pulverizer (Fisher Scientific, Pittsburgh, PA) prechilled with liquid nitrogen. Each sample was homogenized in TriReagent® (Molecular Research Center, Inc., Cincinnati, OH) using a Tissue Tearor™ electric homogenizer. Total RNA was isolated with TriReagent® according to the manufacturer's protocol, purified using the Qiagen RNeasy mini RNA cleanup protocol, and stored at −80°C in 5 μg aliquots until used for cDNA synthesis.

Transcript Determination

Total RNA (5 μg) was used to synthesize double-stranded cDNA using the Superscript II kit (Life Technologies, Gaithersburg, MD). The cDNA served as a template to synthesize biotin-labeled antisense cRNA using a BioArray T-7 polymerase labeling kit (Enzo Diagnostics, Farmingdale, NY). Labeled cRNA was fragmented and hybridized to the Rat Toxicology U34 array (containing approximately 850 genes) as described in the Affymetrix GeneChip® protocol (Affymetrix, Santa Clara, CA). Each microarray was scanned, visualized, and analyzed for the level of each individual transcript using version 3.3 GeneChip software (Affymetrix).

Data Analysis

For baseline transcript levels in normal skin, only genes that were present or marginally present in at least 80% of 0-h skin samples ($N = 10$ animals) were analyzed. The mean signal intensity ±SEM for each gene was determined. Genes were placed in the following functional categories: cell structure, cytokines/growth factors/receptors, differentiation/cell division, extracellular matrix, metabolism, oxidative/cellular stress, signal transduction, transporters/ligands, miscellaneous, and expressed sequence tags (ESTs).

In skin exposed to either SLS, *m*-xylene, or *d*-limonene, the Data Mining Tool (DMT) version 5.0 software (Affymetrix) was used to determine differences in transcript expression levels between 0-h and 1- or 4-h skin samples. The DMT analysis enabled sorting of transcripts showing a two-fold or greater increase (signal log ratio ≥ 1) or decrease (signal log ratio ≤ -1) in expression for each sample time when compared to the 0-h skin. Selecting the genes changed by treatment was a process of sorting the results using a series of criteria. All pairwise comparisons were made between the treated samples and the sham controls, i.e. there were four pairwise comparisons. We eliminated the genes absent (default DMT values) in both control and treated samples. We selected genes increased or decreased in 75% of the comparisons at each sample time. From these genes we selected ones that had an average (over all comparisons) signal log ratio of greater than 1 or less than -1 (equivalent to twofold changes). From these genes we selected the ones with an average probability of change of <0.05 using the Wilcoxson-signed rank test.

RESULTS

Histological Analysis

The observed histopathological changes in rat skin following exposure to *m*-xylene, *d*-limonene, or 1% or 10% SLS ranged from little to no observable changes to granulocyte infiltration into the skin and epidermal separation from the basement membrane (Figure 1). In

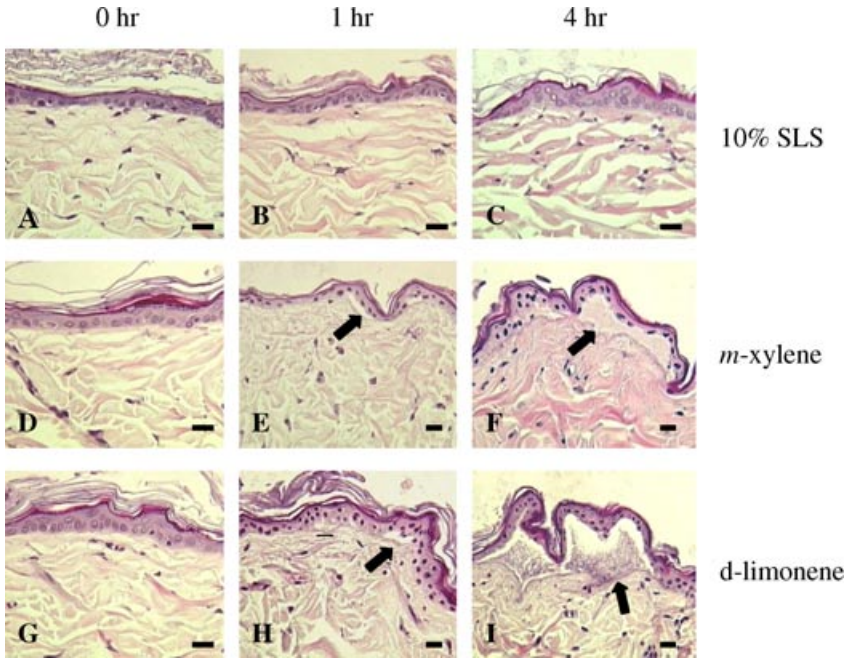

FIGURE 1. Hematoxylin- and eosin-stained sections of rat skin at 0 h (A, D, G), 1 h (B, E, H), and 4 h (C, F, I) following the beginning of a 1-h exposure with 10% SLS (A–C), *m*-xylene (D–F), and *d*-limonene (G–I). Note the presence of epidermal separation from the basement membrane (arrows) in skin exposed to *m*-xylene and *d*-limonene at 1 and 4 h. Bar = 20 μm.

skin exposed to 1% or 10% SLS, we observed no pathological changes when compared to normal rat skin at 4 h. However, histological analysis of skin exposed to *m*-xylene or *d*-limonene revealed pathological changes at 1 and 4 h. At 1 h, skin exposed to *m*-xylene or *d*-limonene showed minor epidermal separation from the basement membrane. By 4 h, epidermal separation was more apparent than at 1 h in skin exposed to *m*-xylene or *d*-limonene. At areas of epidermal/dermal separation, there was accumulation of eosinophilic material between the epidermis and basement membrane.

Transcript Levels in Normal Rat Skin

The analysis of gene expression profiles in 0-h rat skin was conducted to provide information regarding the classification and level of active transcripts in normal skin. Data were placed in generalized functional categories and presented as transcripts present in at least 80% (Table 1) of the normal skin samples. In normal rat skin, there were 234 separate genes present in skin samples from at least 8 out of 10 different animals that were detected using the Rat Toxicology U34 array with a fluorescence signal intensity range of approximately 56–7200 (Figure 2). With respect to the genes that were present in at least 80% of skin samples ($N = 10$) analyzed, 48 separate genes were identified in the metabolism category, with 23, 17, and 13 identified in the oxidative/cellular stress, signal transduction, and miscellaneous categories, respectively. Less than 10 different genes were identified in each of the remaining categories, which included cell structure, cytokines/growth factors/receptors, differentiation/cell division, extracellular matrix, and transporters/ligands. Of the 234 genes, 113 were ESTs, which are short sequences from cDNA clones that are not identified with specific genes.

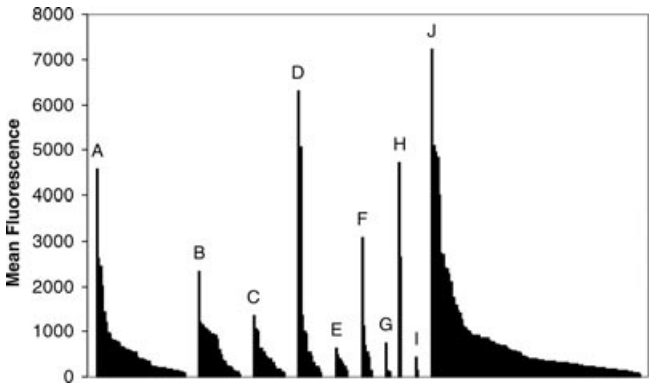

FIGURE 2. Mean fluorescence of genes detected as present or marginally present in 0-h skin samples ($N = 10$). Genes are grouped into metabolism (A), oxidative/cellular stress (B), signal transduction (C), miscellaneous (D), differentiation/cell division (E), cell structure (F), transporter/ligands (G), extracellular matrix (H), cytokines/growth factors/receptors (I), and expressed sequence tag (J) categories.

Transcripts in Skin Exposed to Chemical Irritants

The average signal log ratio and categorization for the transcripts identified in skin exposed to 1% and 10% SLS are listed in Tables 2 and 3, respectively. When compared to the 0-h samples, changes in 2 genes were observed in skin exposed to 1% SLS, while changes in 22 genes were observed in skin treated with 10% SLS. In skin exposed to 1% SLS, no changes in transcript levels were observed at 1 h. However, at 4 h, the minoxidil sulfotransferase gene was upregulated (positive signal log ratio), while the cyclin D2 gene was downregulated (negative signal log ratio). Exposure to 10% SLS led to an upregulation of 1 and 4 genes at 1 and 4 h, respectively (Figure 3). In these samples, a downregulation of 5 and 13 genes was observed at both 1 and 4 h, respectively. For the 10% SLS treatments, the category with

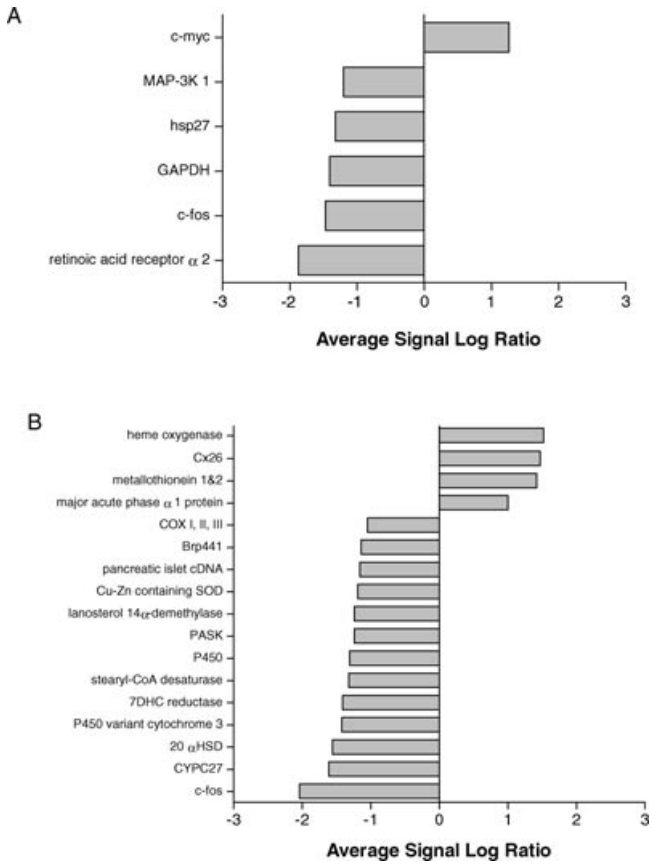

FIGURE 3. Graphical representation of the number and corresponding average signal log ratio of transcripts detected in rat skin exposed to 10% SLS at 1 (A) and 4 (B) h.

TABLE 1. Transcripts Present in at Least 8 of 10 Normal Rat Skin Samples

		Signal Intensity	
Category/Gene	*Affymetrix ID*	*Average*	*Standard Error*
Cell structure			
Alpha tubulin	V01227_s_at	1136	190
Collagen-binding protein (gp46)	M69246_at	428	123
Connexin-31	M59936cds_at	153	39
Connexin protein Cx26	X51615_g_at	556	154
Cytoplasmic beta actin	V01217_at	3092	835
Vimentin	X62952_at	701	156
Cytokines/gowth factors/receptors			
Interleukin-1 receptor type 2	Z22812_at	443	76
Precursor interleukin 18 (IL-18)	AJ222813_s_at	131	17
Differentiation/cell division			
Cyclin D3	D16309_g_at	223	29
Cyclin G	X70871_at	340	55.0
Cyclin-dependent kinase 4	L11007_at	287	49.2
DNA polymerase alpha	M15114_g_at	629	134.1
DNA topoisomerase I	AA848218_at	142	18.4
Nap1	D84346_s_at	390	51.3
Proliferating cell nuclear antigen (PCNA/cyclin)	M24604_at	478	87
Extracellular matrix			
Procollagen, type III, alpha 1	X70369_s_at	4726	1295
Procollagen, type I, alpha 1	Z78279_at	2634	915
Metabolism			
2,4-Dienoyl-CoA reductase	D00569_g_at	140	19
3-Methylcholanthrene-inducible truncated UDP-glucuronosyltransferase	J05132_s_at	603	97
7-Dehydrocholesterol reductase	AB016800_at	244	42
20 Alpha-hydroxysteroid dehydrogenase	L32601_s_at	176	41
Acyl-CoA dehydrogenase long chain	J05029_s_at	179	28
Acyl-CoA dehydrogenase medium chain	J02791_at	544	139
Acyl-CoA oxidase	J02752_at	553	82
Aldehyde reductase	D10854_at	831	113
Aldose reductase	M60322_at	355	33
Bleomycin hydrolase	D87336_g_at	575	67
Carnitine octanoyltransferase	J02844_s_at	367	71
Cox VIa	X72757_g_at	815	184
Cytochrome *c* nuclear-encoded mitochondrial gene	K00750exon#2-3_at	99	18
Cytochrome *c* oxidase subunit IV	J05425cds_s_at	1427	218
Cytochrome *c* oxidase subunit IV	X54081mRNA_i_at	614	136
Cytochrome *c* oxidase subunit Va	X15030_at	975	261
Cytochrome *c* oxidase subunit VIII	L48209_s_at	741	152
Cytochrome *c* oxidase subunit VIII	M28255_s_at	2447	434
Cytochrome oxidase subunits I, II, III	J01435cds#8_s_at	4583	1361
Cytochrome P-450	E00717UTR#1_s_at	119	13
Cytochrome P450IIB12	X63545_at	789	251
Cytochrome P450IIB15	D17349cds_f_at	241	54
CYP45A Rat P-450(1) variant phenobarbital-inducible cytochrome 3	K01721mRNA_s_at	227	51
Cytochrome P-450e	M13234cds_f_at	215	50
Dihydropteridine reductase	J03481mRNA_g_at	206	39
Epoxide hydrolase	M26125_at	132	18
Fatty acid synthase	M76767_s_at	649	176
Glucose-6-phosphate dehydrogenase	X07467_at	642	116
Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH)	M17701_s_at	1200	255
HMG-CoA reductase	X55286_at	144	34
Lanosterol 14-alpha-demethylase	U17697_s_at	539	156
Lanosterol 14-demethylase	AB004096_at	357	71
Lipoprotein lipase	L03294_at	201	25
Minoxidil sulfotransferase	L19998_g_at	216	28
Mitochondrial cytochrome b-245	J01436cds_s_at	2605	695

(Continued)

TABLE 1. Continued

Category/Gene	*Affymetrix ID*	*Signal Intensity*	
		Average	*Standard Error*
Mitochondrial long-chain enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase alpha-subunit of mitochondrial trifunctional protein	D16478_at	798	118
Monoamine oxidase A	D00688_s_at	144	18
NADH dehydrogenase	M22756_at	399	66
NADH-ubiquinone oxidoreductase	D86215_at	263	53
NADH ubiquinone oxidoreductase subunit (IP13)	L38437_at	662	103
NADH-cytochrome b5 reductase	D00636Poly_A_Site#1_s_at	954	182
NADH-cytochrome b-5 reductase	J03867_s_at	405	90
Rieske iron–sulfur protein	M24542cds_at	446	103
Soluble cytochrome b5	AF007107_s_at	204	45
Stearyl-CoA desaturase	J02585_at	2000	601
Steroid 5 alpha-reductase	J05035_g_at	177	29
UDP-glucuronosyltransferase	J02612mRNA_s_at	125	24
UDP glucuronosyltransferase	M74439mRNA_i_at	398	64
Oxidative/cellular stress			
70 kd heat-shock-like protein	M11942_s_at	1149	181
Alpha-crystallin B chain	M55534mRNA_s_at	365	77
Chaperonin 60	U68562mRNA#2_s_at	225	43
Cu–Zn-containing superoxide dismutase	M21060_s_at	597	140
Cu–Zn-containing superoxide dismutase	Y00404_s_at	936	217
DnaJ-like protein	U53922_at	358	48
Glutathione peroxidase	X07365_s_at	1080	175
Glutathione peroxidase I	X12367cds_s_at	825	186
Glutathione *S*-transferase	J03752_at	2329	628
Glutathione *S*-transferase Yb subunit	J03914cds_s_at	208	36
Glutathione *S*-transferase Y-b subunit	J02592_s_at	480	102
Glutathione *S*-transferase Yc subunit	K01932_f_at	121	21
Heat shock protein (Hsp27)	M86389cds_s_at	1057	341
Heat shock protein 60	X54793_at	929	127
Heat shock protein 70 (Hsp70)	L16764_s_at	998	175
Heat shock protein 70 (Hsp70.2)	Z75029_s_at	270	67
Heat shock protein 90	S45392_at	958	240
Heat shock related protein	X82021cds_at	129	19
Mn-containing superoxide dismutase	Y00497_s_at	56	6
Metallothionein-1 and Metallothionein-2	M11794cds#2_f_at	1219	262
Peroxisome proliferator-inducible gene	S83279_g_at	241	43
Stress-inducible chaperone mt-GrpE#1	U62940_at	142	26
Thiol-specific antioxidant	U06099_at	1005	161
Signal transduction			
Calmodulin	X13933_s_at	621	113
Calnexin	L18889_at	164	22
c-*fos*	X06769cds_g_at	424	99
Cyclophilin	M19533mRNA_i_at	1354	316
Cyclophilin B	AF071225_at	184	31
Cyclophilin D	U68544_at	553	136
Extracellular signal-related kinase (ERK3)	M64301_g_at	308	50
Interleukin-1 beta converting enzyme	U14647_at	114	22
MAP kinase kinase	L04485mRNA_s_at	403	53
MAP kinase kinase kinase 1	U48596_g_at	622	182
MAP-kinase phosphatase	AF013144_at	237	33
P38 mitogen activated protein kinase	U73142_g_at	470	68
Phosphatidylinositol 4-kinase	D84667_at	97	11
Phosphoinositide-specific phospholipase C	X12355_s_at	403	91
Protein kinase (MUK)	D49785_at	1021	689
RBL-NDP kinase	M91597_s_at	1077	175
RL/IF-1	X63594cds_at	162	36

(Continued)

TABLE 1. Continued

		Signal Intensity	
Category/Gene	*Affymetrix ID*	*Average*	*Standard Error*
Transporters/ligands			
Mtp1	X57523_g_at	119	19
Na+, K+-ATPase beta-3 subunit	D84450_at	736	166
Transferrin	D38380_g_at	149	15
Miscellaneous			
APEX nuclease	D44495_s_at	235	36
Calcium-binding protein	M86870_at	91	13
Clone par-4 induced by effectors of apoptosis	U05989_at	163	25
DAD-1	Y13336cds_at	553	106
Immunoglobulin heavy chain binding protein (BiP)	M14050_s_at	544	71
Major acute phase alpha-1 protein	K02814_g_at	471	112
PHAS-I	U05014_g_at	315	46
Polyubiquitin	D16554_at	5074	957
Proteasome RN3	L17127_at	1019	210
Proteasome subunit RC7-I	D21799_at	225	37
R2 cerebellum DDRT-T-PCR	U47315_s_at	1342	276
Ribosomal protein S29	X59051cds_s_at	6316	1945
TRPM-2 gene (clusterin)	M64733mRNA_s_at	941	291
EST			
EST105871	AA684963_at	135	31
EST111576	AA686870_i_at	131	34
EST188833	AA799336_at	146	25
EST188971	rc_AA799474_at	457	79
EST188996	rc_AA799499_at	395	61
EST189386	rc_AA799889_at	160	20
EST191305	rc_AA848545_at	159	17
EST194171	rc_AA851403_g_at	713	118
EST195454	rc_AA891651_g_at	629	94
EST195844	rc_AA892041_at	346	51
EST196037	rc_AA892234_at	755	136
EST196051	rc_AA892248_at	873	238
EST196335	rc_AA892532_at	577	91
EST196347	rc_AA892544_s_at	803	98
EST196988	rc_AA893185_at	311	52
EST198184	rc_AA942685_at	113	12
EST199896	rc_AA944397_at	178	26
EST200553	rc_AA945054_s_at	401	108
EST200651	rc_AA945152_s_at	3998	1159
EST201539	rc_AA946040_at	322	66
EST202271	rc_AI007820_s_at	1400	295
EST203583	rc_AI009132_at	1098	201
EST203592	rc_AI009141_at	931	559
EST203598	rc_AI009147_at	293	148
EST203841	rc_AI009390_at	350	54
EST204743	rc_AI010292_s_at	2276	800
EST204822	rc_AI010371_at	102	13
EST205176	rc_AI010725_at	383	61
EST206007	rc_AI011556_s_at	243	51
EST206157	rc_AI011706_at	88	9
EST206502	rc_AI012051_at	241	47
EST207040	rc_AI012589_s_at	857	170
EST207646	rc_AI014091_at	361	112
EST207972	rc_AI013297_at	177	20
EST208509	rc_AI013834_s_at	195	31
EST211794	rc_AI102505_at	1749	345
EST211909	rc_AI102620_at	226	32
EST212157	rc_AI102868_g_at	130	15
EST212685	rc_AI103396_g_at	5105	1371
EST213324	rc_AI104035_s_at	1481	252

(Continued)

TABLE 1. Continued

		Signal Intensity	
Category/Gene	*Affymetrix ID*	*Average*	*Standard Error*
EST213809	rc_AI104520_s_at	1570	240
EST213968	rc_AI104679_s_at	603	81
EST215100	rc_AI169265_at	857	147
EST216621	rc_AI170685_at	314	58
EST217310	rc_AI171355_s_at	2728	750
EST217469	rc_AI171506_at	276	63
EST217507	rc_AI171542_at	917	113
EST217602	rc_AI171630_s_at	236	42
EST218418	rc_AI172411_at	2109	320
EST218462	rc_AI172452_at	329	58
EST219534	rc_AI175959_at	259	51
EST219751	rc_AI176170_at	416	38
EST219890	rc_AI176308_at	648	95
EST220006	rc_AI176422_g_at	181	28
EST220076	rc_AI176491_at	190	29
EST220133	rc_AI176546_at	777	122
EST220250	rc_AI176658_s_at	1271	332
EST220870	rc_AI177256_at	310	38
EST222834	rc_AI179150_s_at	7221	1743
EST223647	rc_AI179916_at	351	65
EST223845	rc_AI180108_at	536	92
EST224582	rc_AI227887_at	100	11
EST225369	rc_AI228674_s_at	4949	1103
EST225433	rc_AI228738_s_at	226	40
EST225986	rc_AI229291_at	346	41
EST226135	rc_AI229440_s_at	941	134
EST226192	rc_AI229497_at	335	44
EST226315	rc_AI229620_s_at	910	165
EST227101	rc_AI230406_at	1029	158
EST227327	rc_AI230632_at	403	31
EST228466	rc_AI231778_at	73	13
EST228995	rc_AI232307_at	714	130
EST229009	rc_AI232321_at	297	58
EST230053	rc_AI233365_at	209	14
EST231166	rc_AI234604_s_at	1768	355
EST231920	rc_AI235358_at	265	43
EST232269	rc_AI235707_g_at	294	65
EST233163	rc_AI236601_at	120	15
EST233357	rc_AI236795_s_at	2699	537
EST233940	rc_AI237378_at	541	98
UI-R-A0-ah-g-06-0-UI.s1	rc_AA818226_s_at	4825	895
UI-R-A0-am-b-09-0-UI.s1	rc_AA818152_f_at	2417	578
UI-R-A0-ap-e-06-0-UI.s1	rc_AA819708_s_at	773	155
UI-R-A0-ar-h-08-0-UI.	rc_AA818858_s_at	2382	523
UI-R-A0-at-d-09-0-UI.	rc_AA818487_s_at	907	144
UI-R-A0-bf-g-10-0-UI.	rc_AA819547_at	459	58
UI-R-A0-bh-h-06-0-UI.s4	rc_AA900199_s_at	212	47
UI-R-A1-ep-a-02-0-UI.s1	rc_AA925473_g_at	1003	218
UI-R-A1-eq-g-04-0-UI.s1 UI-R-A1	rc_AA926137_s_at	565	96
UI-R-A1-eq-h-04-0-UI.s1	rc_AA926149_g_at	181	40
UI-R-C0-gu-e-09-0-UI.s1	rc_AA964320_at	1111	186
UI-R-C0-hy-g-09-0-UI.s1	rc_AA997614_s_at	255	58
UI-R-C0-ig-h-06-0-UI.s1	rc_AA998683_at	519	104
UI-R-C2p-of-f-12-0-UI.s1	rc_AI136891_at	678	115
UI-R-E0-bp-g-09-0-UI.s2	rc_AA933158_at	203	22
UI-R-E0-bq-d-08-0-UI.s1	rc_AA858640_s_at	437	48
UI-R-E0-br-h-03-0-UI.s1	rc_AA866477_at	417	107
UI-R-E0-ca-f-05-0-UI.s1	rc_AA859957_at	853	122
UI-R-E0-ce-b-04-0-UI.s1	rc_AA875268_at	375	70

(Continued)

TABLE 1. Continued

		Signal Intensity	
Category/Gene	*Affymetrix ID*	*Average*	*Standard Error*
UI-R-E0-cf-h-08-0-UI.s1	rc_AA875107_at	280	35
UI-R-E0-ck-g-09-0-UI.s1	rc_AA874919_at	56	6
UI-R-E0-cn-h-05-0-UI.s1	rc_AA875327_g_at	565	65
UI-R-E0-cq-h-04-0-UI.s2	rc_AA899854_at	196	18
UI-R-E0-cv-a-11-0-UI.s1	rc_AA875594_s_at	155	18
UI-R-E0-dl-e-12-0-UI.s1	rc_AA900413_at	155	22
UI-R-A1-ep-a-02-0-UI.s1	rc_AA925473_at	358	69
UI-R-A1-ex-f-01-0-UI.s1	rc_AA955477_g_at	313	48
UI-R-E1-fb-e-12-0-UI.s1	rc_AA955983_at	703	118
UI-R-E1-fi-f-02-0-UI.s1	rc_AA956114_at	232	28
UI-R-E1-gg-h-01-0-UI.s1	rc_AA963674_at	692	155
UI-R-E1-gj-e-08-0-UI.s1	rc_AA963449_s_at	326	80
UI-R-Y0-mg-e-12-0-UI.s1	rc_AI112516_at	384	53
UI-R-Y0-mh-e-09-0-UI.s1	rc_AI112237_at	874	124

Data are expressed as mean ± SEM for each transcript.

the greatest observed change in the number of genes was metabolism, followed by oxidative/cellular stress and signal transduction.

In skin exposed to the volatile organic chemical, *m*-xylene, for 1 h, 14 different transcripts were changed at 1 h and 13 transcripts were changed at 4 h when compared to the 0-h skin samples (Figure 4). The average signal log ratio for each transcript is listed in Table 4 and the data are presented within functional categories previously defined in normal skin. At 1 h, 5 transcripts were observed to be upregulated and the remaining 9 transcripts were downregulated. By 4 h, there were increases in 5 transcripts and a decrease in 8 transcripts. The gene categories of metabolism and cytokines/growth factors/receptors possessed the largest numbers of transcripts changed in skin exposed to *m*-xylene.

In skin exposed to *d*-limonene for 1 h, 33 different transcripts were changed at 1 h and 8 transcripts were changed at 4 h when compared to the 0-h skin samples (Table 5; Figure 5). At 1 h, there was an upregulation of 29 transcripts and a downregulation of 4 transcripts. By 4 h, there was an increase in 8 transcripts and no genes were observed to be downregulated. Skin exposed to *d*-limonene led to the greatest observed changes in the gene categories of metabolism, differentiation/cell division, oxidative/cellular stress, and signal transduction.

TABLE 2. Transcripts Present in Rat Skin at 1 and 4 h Following a 1-h Exposure to 1% SLS

		Average Signal Log Ratio	
Category/Gene	*Affymetrix ID*	*1 h*	*4 h*
Differentiation/cell division			
Cyclin D2	D16308_at		−1.48
Metabolism			
Minoxidil sulfotransferase	L19998_at		1.14

Data are expressed as average signal log ratio compared to 0-h samples.

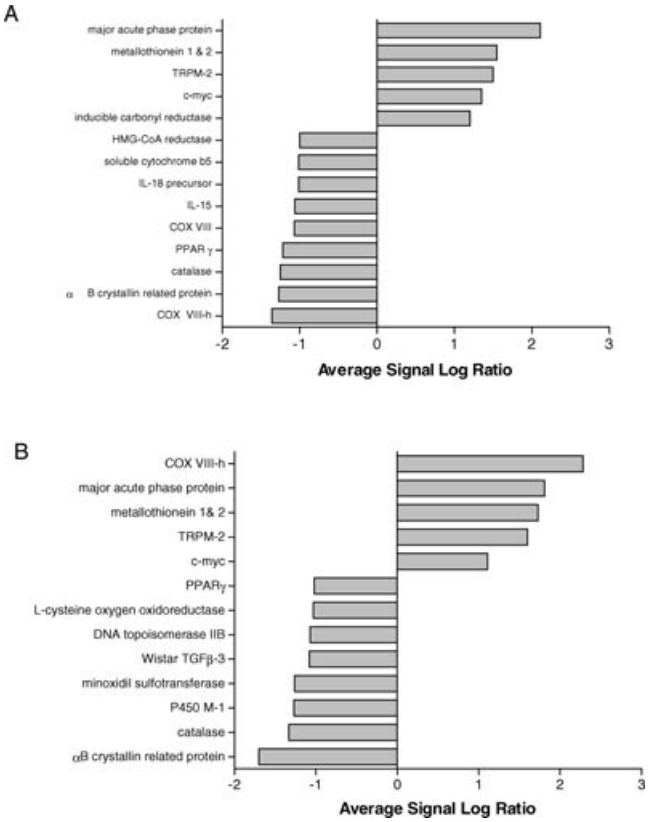

FIGURE 4. Graphical representation of the number and corresponding average signal log ratio of transcripts detected in rat skin exposed to *m*-xylene at 1 (A) and 4 (B) h.

TABLE 3. Transcripts Present in Rat Skin at 1 and 4 h Following a 1-h Exposure to 10% SLS

		Average Signal Log Ratio	
Category/Gene	*Affymetrix ID*	*1 h*	*4 h*
Cell structure			
Connexin protein Cx26	X51615_g_at		1.47
Cytokines/growth factors/receptors			
Retinoic acid receptor alpha 2 isoform	U15211_at	−1.87	
Metabolism			
7-Dehydrocholesterol reductase	AB016800_g_at		−1.41
20-Alpha-hydroxysteroid dehydrogenase	L32601_s_at		−1.56
Cytochrome P-450	E00717UTR#1_s_at		−1.31
Cytochrome P450C27	M38566mRNA_s_at		−1.61
Cytochrome oxidase subunits I, II, III	J01435cds#8_s_at		−1.05
Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH)	M17701_s_at	−1.41	
Heme oxygenase	J02722cds_at		1.52
Liver stearyl-CoA desaturase	J02585_at		−1.32
Lanosterol 14 alpha-demethylase	U17697_s_at		−1.24
P-450 variant cytochrome 3	K01721mRNA_s_at		−1.42
Oxidative/cellular stress			
Cu–Zn-containing superoxide dismutase	M21060_s_at		−1.19
Heat shock protein 27	M86389cds_s_at	−1.32	
Metallothionein-2 & metallothionein-1	M11794cds#2_f_at		1.42
Signal transduction			
c-*fos*	X06769cds_g_at	−1.47	−2.04
c-*myc* oncogene	Y00396mRNA_at	1.26	
MAP kinase kinase kinase 1	U48596_g_at	−1.20	
Protein kinase PASK	D88190_s_at		−1.24
Miscellaneous			
Major acute phase alpha 1 protein	K02814_at		1.00
Pancreatic islet cDNA	C06598_at		−1.16
R2 cerebellum DDRT-T-PCR	U47315_s_at		−1.14

Data are expressed as average signal log ratio compared to 0-h samples.

DISCUSSION

Skin irritation resulting from chemical exposure is a complex process involving gene activation and protein production. The coordinated temporal regulation of mRNA and protein expression resulting in skin irritation is not fully understood. However, there are proteins such as cytokines that have been identified as crucial players in the irritant cascade. These cytokines appear to play an important role in the initiation and amplification of irritation, and the mRNA and protein expression patterns have been monitored in skin in response to irritants [29]. Although activation of these genes and subsequent protein formation in the skin may reflect the onset of skin irritation, other potential genes and gene products may contribute to irritation following exposure to chemicals. A basal level of gene expression exists in the skin to maintain homeostasis and it is important to first identify the basal transcripts that are expressed in normal rat skin to be able to put the changes in perspective.

Skin is a complex and dynamic organ that is involved in temperature regulation (sweating and regulation of blood flow), metabolism (keratin, collagen, melanin, lipid, carbohydrate, xenobiotic, and vitamin D), mechanical support and protection (transport barrier, response to oxidative stress, and immune responses), as well as sensory functions [30]. The level of mRNA transcripts in normal skin may give some indication of the relative proportion and importance of each of these functions. Table 1 and Figure 2 show that transcripts involved in metabolism, oxidative, and cellular stress and signal transduction are the most abundant of the transcripts identified with specific genes. This proportion may reflect the portion of the genome (based on the Rat Toxicology U34 array) involved in each function. The ribosomal protein S29 and polyubiquitin (both in miscellaneous category) were the transcripts with the largest fluorescence values. Fluorescence values may only roughly correlate with the number of molecules of transcript because of differential fluorescence labeling. The breadth and magnitude of the transcripts found in normal skin suggest diverse structure and function.

Gene array technology has been used to monitor expression patterns in whole skin and keratinocyte cultures following ultraviolet light or chemical exposure, or in diseased, injured, or tumor tissue [31–37]. These studies have helped define characteristic

TABLE 4. Transcripts Present in Rat Skin at 1 and 4 h Following a 1-h Exposure to *m*-Xylene

		Average Signal Log Ratio	
Category/Gene	*Affymetrix ID*	*1 h*	*4 h*
Cytokines/growth factors/receptors			
Interleukin 15	U69272_at	−1.06	
Interleukin 18 precursor	AJ222813_s_at	−1.01	
Wistar transforming growth factor beta-3	U03491_g_at		−1.08
Differentiation/cell division			
DNA topoisomerase IIB	D14046_at		−1.07
Metabolism			
Cytochrome oxidase subunit VIII	U40836mRNA_s_at	−1.07	
Cytochrome *c* oxidase subunit VIII-h	X64827cds_s_at	−1.36	2.28
Cytochrome P450 (M-1)	J02657_s_at		−1.27
HMG-CoA reductase	X55286_at	−1.00	
Inducible carbonyl reductase	D89069_f_at	1.20	
L-Cysteine oxygen oxidoreductase	E03229cds_s_at		−1.03
Minoxidil sulfotransferase	L19998_at		−1.26
Soluble cytochrome b5	AF007107_s_at	−1.01	
Oxidative/cellular stress			
Alpha B crystallin related protein	D29960_at	−1.27	−1.70
Catalase	M11670_at	−1.25	−1.33
Metallothionein-1 & metallothionein-2	M11794cds#2_f_at	1.55	1.73
Signal transduction			
c-*myc* oncogene	Y00396mRNA_at	1.35	1.11
PPAR gamma	AB011365_s_at	−1.22	−1.02
Miscellaneous			
Major acute phase alpha 1 protein	K02814_at	2.11	1.81
TRPM-2 (clusterin)	M64733mRNA_s_at	1.50	1.60

Data are expressed as average signal log ratio compared to 0-h samples.

gene expression patterns that reflect skin responses over a broad range of external insults and/or stimuli. The present study demonstrates that the predominant categories of genes that are expressed in normal rat skin include those responsible for metabolism and oxidative/cellular stress, but genes encoding proteins involved in signal transduction and cell division/differentiation, or cell structure are expressed in low numbers. Only one gene, c-*myc*, was upregulated in the skin by all three irritants.

The most widely studied irritant, SLS, induces the expression of many genes and gene products in skin and cultured cells. These transcriptional and protein changes have been associated with skin irritation and inflammation. Exposure of the in vitro model EpiDerm™ to a noncytotoxic dose of 0.1 mg/mL (0.01%) SLS lead to temporal transcriptional changes in gene families such as transporters/receptors, transcription factors, DNA repair and wound healing, tumor suppressors, and metabolism [20]. In the present study, treatment of skin with 1% SLS led to changes in only two genes at 4 h. However, 10% SLS led primarily to the downregulation of transcripts at 4 h, with few observable changes at 1 h. While these changes were mainly associated with metabolic and cellular stress functions, the 10% SLS treatment induced the transcription factor c-*myc*, but c-*fos* was downregulated. The 10% SLS treatment also upregulated some signal transduction genes, such as protein kinases. Since changes in 20 more genes were observed in 10% SLS-treated skin compared to the 1% SLS treatment, these results suggest that there may be a dose-dependent difference with respect to gene activation in the skin and SLS treatment.

When applied to the skin, solvents can induce changes in the skin that include epidermal degeneration, epidermal–dermal separation, leukocyte infiltration, oxidative species formation, and DNA damage [15,38,39]. The molecular responses in this solvent-damaged skin may include mechanisms of oxidative stress and wound healing. In *m*-xylene-exposed skin, we previously reported significant oxidative species formation as early as 2 h following the beginning of a 1-h exposure [15]. In this study, we observed changes in genes associated with oxidative/cellular stress such as the induction of metallothionein-1 and -2 and the downregulation of catalase at both 1 and 4 h. The elevation of metallothionein may also reflect the initiation of a wound healing response, since the induction of metallothionein has been observed in models of wound healing [40]. The downregulation of IL-18 precursor at 1 h may reflect the initiation of an inflammatory response in the skin. Although IL-18 protein is increased significantly during skin inflammation, it has been reported that IL-18 mRNA levels are decreased simultaneously,

TABLE 5. Transcripts Present in Rat Skin at 1 and 4 h Following a 1-h Exposure to *d*-Limonene

		Average Signal Log Ratio	
Category/Gene	*Affymetrix ID*	*1 h*	*4 h*
Cellular structure			
Connexin protein Cx26	X51615_g_at	1.15	
Cytokines/growth factors/receptors			
Retinoic acid receptor alpha 2	U15211_at	−1.43	
Transforming growth factor alpha	M31076_at	1.31	1.10
Differentiation/cell division			
*cdc*2 promoter	X60767mRNA_s_at	1.48	
Cyclin G	X70871_at	1.28	
GADD45	L32591mRNA_at	1.22	
Proliferating cell nuclear antigen	M24604_at	1.21	
Extracellular matrix			
Pro alpha 1 collagen type III	X70369_s_at		1.12
Metabolism			
2,4-Dienoyl-CoA reductase	D00569_g_at	1.37	
Beta-1,2-*N*-acetylglucosaminyltransferase II	U21662_at	1.29	
Cytochrome *c*	K00750exon#2-3_g_at	1.24	
Soluble cytochrome b5	AF007107_s_at	2.32	
Cytochrome *c* oxidase subunit IV	J05425cds_s_at	1.02	
dUTPase	U64030_at	1.17	
Cytochrome *c* oxidase subunit Va	X15030_at	1.11	
Cytochrome oxidase subunit Vic	M27467_at	−1.79	
Cytochrome P450IIB12	X63545_at		1.44
Delta-4-3-ketosteroid 5-beta-reductase	D17309_at	−1.62	
Lanosterol 14-alpha-demethylase	U17697_s_at	1.03	
Stearyl-CoA desaturase	J02585_at	1.32	
Liver arginase	J02720_at	1.72	
Oxidative/cellular stress			
Chaperonin 60	U68562mRNA#2_s_at	1.38	
Cu–Zn-containing superoxide dismutase	Y00404_s_at	−1.19	
DnaJ homolog 2	U95727_at	4.55	
Glutathione *S*-transferase	U86635_g_at	2.42	
Glutathione peroxidase I	X12367cds_s_at	1.94	
Heat shock protein 90	S45392_at	1.91	1.66
Phospholipid hydroperoxide glutathione peroxidase	L24896_s_at	2.11	
Stress-inducible chaperone mt-GrpE#1	U62940_at	1.27	1.11
Signal transduction			
c-*myc* oncogene	Y00396mRNA_g_at	1.43	
Calmodulin (pRCM1)	X13933_s_at	2.00	
Cyclophilin	M19533mRNA_i_at		1.16
Cyclophilin B	AF071225_at		1.46
Extracellular signal-related kinase 3	M64301_at	3.70	
Nuclear factor kappa B	L26267_at	2.89	
p38 mitogen-activated protein kinase	U73142_at	2.80	
Phosphoinositide-specific phospholipase C	X12355_s_at	1.40	
Transporters/ligands			
Na, K-ATPase alpha-1 subunit	M28647_g_at		1.25

Data are expressed as average signal log ratio compared to 0-h samples.

which may serve as a negative feedback mechanism [41]. The transcript for major acute phase alpha 1 protein was upregulated at both 1 and 4 h in skin exposed to *m*-xylene, but only at 4 h in skin exposed to 10% SLS. The major acute phase alpha 1 protein is a member of the kininogen family and plays a role in the inflammatory response. During tissue inflammation, kininogens can induce vasodilatation and increase capillary permeability. The levels of major acute phase alpha 1 protein mRNA and protein change during the acute phase response of inflammation [42]. Therefore, the increases in major acute phase alpha 1 protein that we observed in skin exposed to *m*-xylene and 10% SLS may reflect the early induction of inflammatory mediators in skin in response to irritating chemicals.

In skin exposed to *d*-limonene, the up- or down-regulation of genes occurred primarily within 1 h of exposure. These genes include those involved in metabolism, oxidative/cellular stress, signal transduction, and differentiation/cell division. As observed in

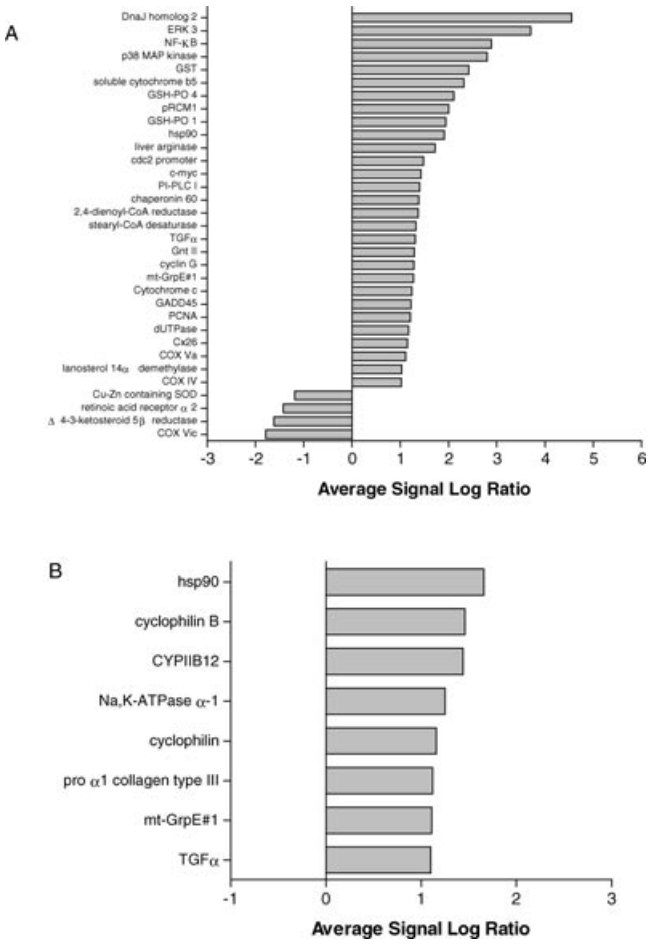

FIGURE 5. Graphical representation of the number and corresponding average signal log ratio of transcripts detected in rat skin exposed to *d*-limonene at 1 (A) and 4 (B) h.

skin exposed to *m*-xylene, a wound healing response may be initiated in skin exposed to *d*-limonene. The induction of genes at 1 h, such as TGF-α and NF-κB, as well as genes associated with cell division and signal transduction are important for the wound healing process. The expression of TGF-α and NF-κB has been implicated as mediators of wound healing in skin [43–47]. The upregulation of the genes proliferating-cell nuclear antigen, cyclin G, and GADD45 suggests that mechanisms controlling cell cycle progression may be activated in response to *d*-limonene-induced skin damage. Moreover, pro alpha 1 collagen type III, a gene involved in extracellular matrix production, was observed to be upregulated by 4 h, and may play a role in structural reorganization of the skin in response to chemical exposure.

Changes in genes associated with metabolism, oxidative/cellular stress, and signal transduction were observed in skin exposed to the irritants SLS, *m*-xylene, and *d*-limonene, with the solvents inducing changes in more genes when compared to either 1% or 10% SLS. For *d*-limonene, these changes were more prominent at 1 h than at 4 h, while more transcriptional changes were observed at 4 h compared to 1 h in skin exposed to SLS. These results suggest that a temporal difference in irritant-induced gene expression may exist following acute chemical exposure, and these changes in gene expression over time may vary between the class of irritant (i.e., solvent or detergent). It is possible that differences in the penetration rates and local chemical concentrations of SLS, *m*-xylene, and *d*-limonene may be important factors in the number and types of genes we observed to be changed following chemical exposure. Solvents can penetrate through the stratum corneum into the viable epidermis within minutes, but it may take much longer for SLS to penetrate the skin. Therefore, the faster-penetrating solvents that were applied as neat solutions may induce more changes in local molecular responses when compared to the slower penetrating, less concentrated (1% and 10%) surfactant. This seems possible since the histological changes we observed in skin exposed to SLS, *m*-xylene, and *d*-limonene suggest that these chemical irritants were eliciting an effect on the skin during our observational time period. Although little to no pathological changes were observed in skin exposed to 10% SLS, we observed changes in 22 genes, while both *m*-xylene and *d*-limonene exposure led to pathological and transcriptional changes in the skin. Therefore, there may be a correlation between observable changes at the histological and transcriptional levels that reflect differences in the irritating effects of SLS compared to the solvents *m*-xylene and *d*-limonene.

In vitro models are currently used for measuring endpoints associated with skin irritation; however, the results typically reflect the responses of a single cell type. These responses include induction and release of proinflammatory cytokines that have been identified as indicators of skin irritation. In the present study, we did not observe many changes in such genes using the Rat Toxicology U34 array that contains ~850 genes. A possible explanation for the results we observed could be that detected genes were derived from transcripts comprising a pool of total RNA from many cell types in whole skin. Therefore, the results we observed in skin exposed to chemical irritants can be assumed to represent an average of transcriptional changes in many cell types of the skin. Additionally, the time points we selected for this study may not encompass a time window in which induction of cytokine and other proinflammatory genes could be detected. In fact, we may be observing changes in molecular events leading to the elicitation of the skin irritation response, as also suggested previously [20].

In summary, gene array studies in normal skin provide a great deal of information about structure

and function of this interesting organ. We found that changes in gene expression with all three skin irritants were rapid, as evidenced by changes in transcript levels after 1 h of exposure. The transcript for the transcription factor, c-*myc*, was increased by all three treatments. Changes in transcripts with SLS at 1% and 10% aqueous solutions suggest a dose-response. Differences in the gene expression profiles with the three chemicals suggest differences in the mechanisms of action. Further studies using quantitative real-time RT-PCR and protein analysis (ELISA) will quantify changes in mRNA and proteins help to elucidate the irritant signaling cascade. It is important to ultimately understand the relationship between the induction of mRNA, the formation of protein products, and the subsequent biological effects that contribute to skin irritation.

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