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APPARENT ASSOCIATION OF ACTIVITY OF ANTI-INFLAMMATORY DRUGS IN A SULFHYDRYL EXCHANGE REACTION *IN VITRO* AND IN THE GUINEA PIG ERYTHEMA ASSAY

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Abstract—A significant rank correlation was found between the acceleration of a sulfhydryl exchange reaction *in vitro* and protection of guinea pigs from ultraviolet-induced erythema of the skin by anti-inflammatory compounds. Rank correlation was not demonstrated between acceleration of the reaction *in vitro* and ability of anti-inflammatory drugs to reduce carrageenan-induced rat paw edema. The nonsteroidal anti-inflammatory drug-stimulated sulfhydryl exchange reaction *in vitro* was reversed by exposure of the reaction mixture to ultraviolet light. The results seem to indicate an association between the ability of nonsteroidal anti-inflammatory drugs to delay ultraviolet erythema and their ability to accelerate a sulfhydryl exchange reaction *in vitro*.

GERBER *et al.*[1] reported that nonsteroidal anti-inflammatory compounds accelerated a disulfide interchange reaction between protein sulfhydryl groups and 5,5′-dithiobis (2-nitrobenzoic) acid (DTNB) *in vitro*. Inclusion of this assay *in vitro* in a screening program for new nonsteroidal anti-inflammatory drugs has resulted in the finding of an apparent association between the ability of compounds to accelerate this reaction with their ability to delay the erythema occurring in guinea pig skin after ultraviolet irradiation. A correlation of activity of drugs in the exchange reaction *in vitro* with the ability of these same drugs to reduce carrageenan-induced edema of the rat's paw does not appear to be present.

METHODS

In vitro *disulfide interchange reaction.* The assay system *in vitro* was that described by Gerber *et al.*[1] using 2 per cent bovine serum albumin* and final drug concentrations of 2·00, 1·00, and 0·50 mM. Drugs were first dissolved in NaOH and the pH adjusted to 7·2–7·6 before dilution with phosphate buffer (0·1 M, pH 7·4). Appropriate blanks were included for each drug. Absorbance measurements at 412 mμ were made before and 10 min after addition of DTNB. In the experiment in which reaction mixtures were exposed to ultraviolet light, the mixture was contained in 5-ml beakers and the top of the liquid was positioned 3 cm from the ultraviolet light source (Hanovia Lamp Type 7420).

Ultraviolet erythema assay. The method of Winder *et al.*,[2] with minor modifications, was used. Albino guinea pigs (Hartley strain, Simonsen Laboratories, Inc.) of both

* Fraction V, Pentex, Inc., Kankakee, Ill.

sexes weighing 450–650 g were used. All drugs were given by stomach tube 30 min before exposure to ultraviolet light. The three exposed circular areas of skin of each animal were scored separately on a 0 to +3 scale 2 hr after exposure to ultraviolet light and the total score for each animal recorded (maximum score per animal = 9). Animals giving a total score $\leq 5{\cdot}0$ were considered to be protected and the quantal data thus obtained was analyzed by the method of Litchfield and Wilcoxon[3] to obtain estimates of the ED_{50} (dose protecting 50 per cent of the animals from developing erythema).

Carrageenan edema assay. A slightly modified method of Winter *et al.*[4] was used. Female Sprague–Dawley rats weighing 140–160 g were used. All drugs were given orally as 4 per cent acacia suspensions 15 min before injection of 0·1 ml of a 0·5 per cent carrageenan suspension into the plantar surface of the right hind paw. The left hind paw was injected with 0·1 ml saline. The volumes of both hind paws were determined 3 hr after the carrageenan injection with a mercury plethysmometer and the difference between the carrageenan-injected and saline-injected paw for each rat was recorded. Per cent inhibitions of the control volume difference were computed and these inhibitions vs. log dose were subjected to regression analysis. The ED_{50}'s (doses giving 50 per cent inhibition of the control volume difference) were calculated from the regression equations so obtained.

RESULTS

Results obtained in the three assays (sulfhydryl interchange, erythema, carrageenan edema) for the eight anti-inflammatory drugs selected for detailed study are presented in Table 1. Rank correlation analysis of these data gave a correlation coefficient for activities of the compounds in the erythema and sulfhydryl assays of 0·976 ($t = 10{\cdot}977$, $P < 0{\cdot}001$). This rank correlation of activities is shown in Fig. 1. Correlation between activities of the compounds in the carrageenan and sulfhydryl assays yielded $r = 0{\cdot}262$ ($t = 0{\cdot}665$, $P > 0{\cdot}5$). In our screening program we have found agreement between activities in the sulfhydryl and erythema assays for 28 of 37 compounds (75·7 per cent).

TABLE 1. ACTIVITIES OF ANTI-INFLAMMATORY COMPOUNDS IN SULFHYDRYL INTERCHANGE, ULTRAVIOLET ERYTHEMA AND CARRAGEENIN EDEMA ASSAYS

Compound	Acceleration of disulfide interchange reaction (increase in absorbance at 412 mμ)			Anti-erythemic activity	Anti-edemic activity
	(0·5 mM)	(1·0 mM)	(2·0 mM)	(ED_{50}, mg/kg)	(ED_{50}, mg/kg)
Mefenamic acid	0·194	0·697	0·978	65	29
Flufenamic acid	0·154	0·359	0·794	34	58
Diflumidone*	0·094	0·228	0·667	9·4	45
Phenylbutazone	0·183	0·285	0·514	16·2	76
Salicylic acid	0·123	0·162	0·320	103	210
Oxyphenbutazone	0·091	0·102	0·142	>200	64
MBR 4325†	0·103	0·098	0·137	117	9·2
Hydrocortisone acetate	0·088	0·066	0·053	Inactive	7·9

* 3-Benzoyldifluoromethanesulfonanilide, sodium salt.
† 3-Benzoylmonofluoromethanesulfonanilide.

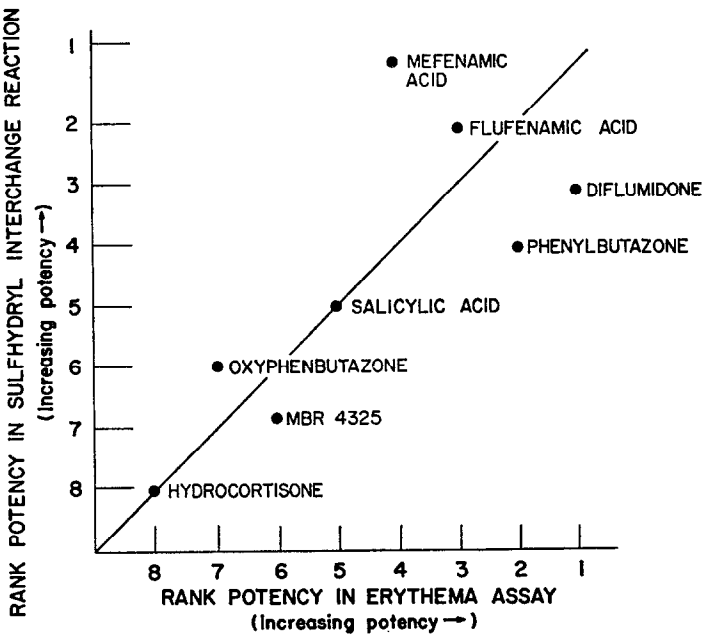

FIG. 1. Rank potencies of compounds listed in Table 1 in the sulfhydryl exchange reaction *in vitro* and in the ultraviolet erythema assay.

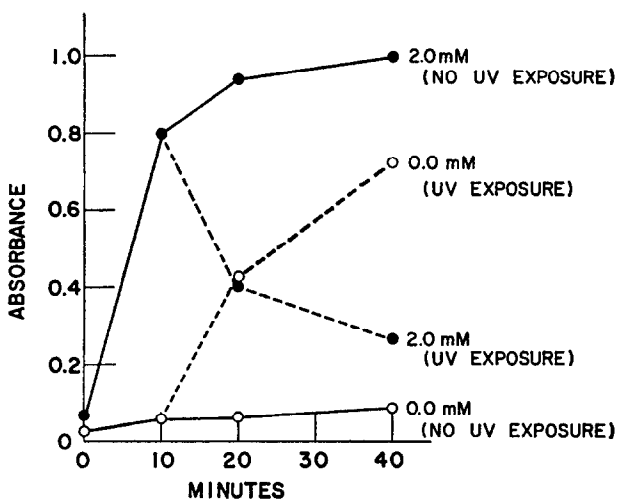

FIG. 2. Effect of ultraviolet irradiation on the sulfhydryl interchange reaction. Bovine serum albumin (2 per cent), dithiobisnitrobenzoic acid (65 μM), and 0 or 2·0 mM diflumidone were allowed to react for 10 min. Half the tubes were than exposed to ultraviolet irradiation for 30 min. Absorbance measurements at 412 mμ were made at 0, 10, 20 and 40 min.

There were eight "false positives" (i.e. sulfhydryl positive and erythema negative) and one "false negative" (sulfhydryl negative and erythema positive). Although in most cases detailed dose–response data were not obtained for these 37 compounds, a relative activity (0 to +3) was assigned to each compound for each assay. Rank correlation analysis of these data gave a correlation coefficient of 0·989 for these compounds in these two assays. The agreement between activities in the sulfhydryl and carrageenan assays has been 150 of 254 compounds (59·1 per cent). There were 76 "false positives" and 28 "false negatives". No steroid structures were included in the screening program.

Because of the apparent correlation between the sulfhydryl interchange reaction and the erythema assay for detection of anti-inflammatory drugs, the effect of ultraviolet light on the drug-stimulated sulfhydryl reaction *in vitro* was examined. Results of one experiment are shown in Fig. 2. A reversal of the reaction *in vitro* was obtained by exposure of the reaction mixture to ultraviolet light. The reversal is even more striking when one considers that the sulfhydryl reagent (DTNB) *per se* is light-sensitive. All appropriate controls were run for the experiment shown (i.e. all possible combinations of components of the mixture with and without ultraviolet exposure) and only DTNB was found to be affected by irradiation. Thus, a solution consisting only of DTNB and buffer, when exposed to ultraviolet light, results in an increase in absorbance at 412 mμ.

DISCUSSION

There is experimental evidence implicating sulfhydryl systems in the reaction of skin to ultraviolet light. Valtonen[5] has summarized the theories relating to ultraviolet-induced erythema and of special interest is that of Wels[6] who stressed the importance of a modification of a disulfide–sulfhydryl redox system which occurs in ultraviolet injury. Flesch[7] reported an immediate decrease in the amount of water-extractable sulfhydryl groups from rabbit skin after exposure to ultraviolet light. Ogura *et al.*[8] postulated a decrease in sulfhydryl groups of the natural inhibitors of tyrosinase, such as glutathione, after ultraviolet irradiation which allowed an increased formation of melanin. An extensive study, particularly as related to the anti-erythemic activity of sulfhydryl positive compounds, is not contemplated and perhaps the selection of compounds for this study was fortuitous. However, these results suggest that activity in the reaction *in vitro* has predictive value for anti-erythemic activity *in vivo*. Just as important is the relatively poor correlation between activity *in vitro* of a compound and its anti-edemic activity *in vivo*.

When considering the vagaries of absorption, distribution, and metabolism of a drug, it is perhaps not so surprising that a number of compounds active in the reaction *in vitro* are found which are not active *in vivo*. These variables, as well as that of solubility of the compound in the system *in vitro*, are important when relative activities in the two assays are compared. A suggestive correlation of relative activities in the two assays was obtained, however.

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