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CORRELATION BETWEEN *IN VITRO* AND *IN VIVO* MODELS IN ANTI-INFLAMMATORY DRUG STUDIES

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Abstract—Several hundred compounds which had undergone multiple anti-inflammatory testing provided the basis for comparing three *in vitro* and four *in vivo* models. Chi-square analysis for concordance of test results showed highly significant associations among the following: albumin denaturation, sulfhydryl–disulfide interchange, aldehyde binding, broncho-constriction by bradykinin, and acute knee joint synovitis. Results of paw edema or cotton pellet granuloma tests were apparently independent of other test results.

FOR PURPOSES of drug research, an adequate laboratory model of a disease means two things. First, clinically active agents generally perform the action under study, while clinically inactive agents do not. Second, the action itself involves some aspect of the primary disturbance, so that clarification of the way the model works may facilitate the emergence of new drugs.

Although variations in metabolism, species susceptibility, solubility, transport, and excretion place limits on certain drug tests, the major difficulties often lie in the complexity of the disease processes. In the case of inflammation, several models have been devised: paw edema, bronchodilator activity, cotton pellet-induced granuloma, knee joint synovitis, and adjuvant arthritis. Most of the steroidal and nonsteroidal drugs regarded as clinically effective show reproducible activity in all of these systems. Moreover, many of the agents also show activity in isolated systems involving their interaction with macromolecules: oxidative phosphorylation, the uptake of sulfate by cartilage and cornea, the heat denaturation of serum albumin, the binding of aldehydes to albumin amino groups, and the sulfhydryl–disulfide interchange reaction with albumin.

The objective of the present study was to compare the results of various pairs of tests. The findings could take any of three forms: concordance, where there is predominant agreement in the results regardless of whether the drugs are active or inactive; discordance, where the drugs tend to be appreciably more active or inactive in one test than they are in the other; and neither significant concordance nor significant discordance, where the distribution of results is not determined by the particular pairing of tests.

Significant concordance would indicate relatedness between two tests and between the aspects of the inflammation for which they are models. Moreover, it would indicate that one test might predict the results of the other and, if desired, replace it.

Absence of either significant concordance or discordance would denote the two tests as independent. As long as their biological relevance to inflammation remained likely, neither test could replace the other in an adequate characterization of a promising drug.

METHODS

Inhibition of albumin denaturation ("*D*")

The effects of drugs on the heat denaturation of bovine serum albumin (BSA; Fraction V from Pentex, Inc., Kankakee, Ill.) were measured turbidimetrically, as described in an earlier paper.[1]

Acceleration of the sulfhydryl–disulfide interchange reaction ("*S*")

In this reaction a disulfide, R–S–S–R′, reacts with a mercaptan, R″SH, to generate a new mercaptan, R–SH, and a new disulfide, R′–S–S–R″. Our method was derived from that of Gerber, Cohen and Giustra.[2] Serum albumin, containing a single accessible SH group, reacts with 5,5′-dithiobis (2-nitrobenzoic acid) (TNBA) to form a mixed disulfide plus 5-thio-2-nitro-benzoic acid, which is deeply pigmented. The components of the reaction mixture, in their order of addition, were 3 ml of test compound (dissolved in dimethylformamide, DMF, whose final concentration was 2·5%, v/v), 3 ml of 4·1% BSA, and 0·2 ml of 2 mM TNBA, all dissolved in 0·1 M potassium phosphate buffer, pH 7·4. The solutions were incubated at 37°, and absorbance at 412 nm was measured at 0 and 10 min on a B and L Spectronic 20. Drugs were tested initially at 1 mM and, if active, were serially diluted until observed accelerations were less than 20 per cent.

Inhibition of aldehyde binding ("*A*")

The procedure of Skidmore and Whitehouse[3] was used with practically no change. The final reaction mixture consisted of 0·67% BSA, 0·1 mM 2,4,6-trinitrobenzaldehyde (TNBAL), drug at a maximum of 1·0 mM, 2·5% DMF, and 0·1 M sodium phosphate, pH 7·5. After 4 min at room temperature readings were taken at both 425 and 525 nm on a B and L Spectronic 20. Serial dilutions were made until inhibitions fell below 20 per cent.

In vivo *evaluations*

The animal tests were based on the following: granuloma pellet, performed on adrenalectomized rats by the method of DiPasquale and Meli;[4] paw edema, Battle *et al.*,[5] Winter *et al*;[6] bronchodilator, Rosenthale and Dervinis;[7] knee joint synovitis, Faires and McCarty,[8] Rosenthale *et al.*[9]

Selection of the compounds

Three hundred and seventy compounds which had undergone multiple anti-inflammatory testing provided the basis for chi-square analyses of associations between the tests. They were well-characterized and judged to be analytically pure. Most were new compounds, none had been clinically tested, and they were submitted for initial pharmacological testing by the chemists who synthesized them.

Statistical method

A modification of McNemar's test[10] was used to determine the significance of concordance between two procedures. Chi-square was calculated as

$$\chi^2 = (N_c - N_d)^2/(N_c + N_d),$$

where N_c is the total number of test results in concord (+, + and −, −), and N_d is the total number of test results in discord (+, − and −, +). Since no distinction is

made within these two groups, there is only one degree of freedom, and a χ^2 value exceeding 3·84 indicates significance at the 95 per cent confidence level. The hypothesis tested was that the drug assays are independent ($N_c = N_d$, $\chi^2 = 0$). Therefore, χ^2 values differing significantly from zero evince a correlation between the two assay procedures.

RESULTS

Table 1 shows the distribution of positive and negative results among the 21 pairs of tests. The following associations are significant:

Antagonism of bradykinin bronchoconstriction with each of: inhibition of acute knee joint synovitis, inhibition of heat denaturation of albumin, inhibition of aldehyde binding, and acceleration of the SH–SS interchange.

Inhibition of acute knee joint synovitis with each of: inhibition of heat denaturation of albumin, inhibition of aldehyde binding, and acceleration of the SH–SS interchange.

Inhibition of heat denaturation with inhibition of aldehyde binding and acceleration of the SH–SS interchange.

Acceleration of the SH–SS interchange with inhibition of aldehyde binding.

At the same time, the analysis suggests that the following are independent: inhibition of granuloma formation with each of the other six tests; inhibition of paw edema with the granuloma, knee joint, denaturation, aldehyde binding, and SH–SS tests.

TABLE 1. ASSAY RESULTS AND CHI-SQUARE ANALYSES

Models compared*	++	−−	+−	−+	χ^2
G–E	30	39	21	46	0·029
G–K	3	3	10	2	2·000
G–B	3	3	7	1	0·222
G–D	6	4	12	5	1·815
G–S	8	1	5	4	0·000
G–A	6	0	6	5	1·471
E–K	7	7	20	1	1·400
E–B	27	34	91	3	7·026†
E–D	21	19	40	9	0·910
E–S	27	3	15	8	0·925
E–A	19	7	21	7	0·074
K–B	7	18	0	1	22·154†
K–D	8	15	0	3	15·385†
K–S	8	8	0	4	7·200†
K–A	8	8	0	2	10·889†
B–D	15	28	8	5	16·071†
B–S	19	12	3	7	10·756†
B–A	15	17	5	3	14·400†
D–S	22	17	3	11	11·792†
D–A	20	22	5	4	21·353†
S–A	22	11	9	2	11·000†

* G, granuloma pellet test; E, paw edema; K, knee joint synovitis; B, bronchodilator; D, albumin denaturation; S, sulfhydryl–disulfide interchange; A, aldehyde binding.

† Significant at $\alpha = 0·05$.

Only one of the 21 comparisons, that of the paw edema inhibition with the inhibition of bradykinin bronchoconstriction, showed a significant χ^2 value stemming from a predominant discordance.

DISCUSSION

Chi-square analysis shows that the three albumin tests are related to one another and to the bronchoconstriction and synovitis tests. The analysis does not measure the strength of these associations, nor does it explain their meaning. Chi-square discloses strong associations and, given sufficiently large numbers of observations, it can uncover weak ones.

We cannot casually infer from the data in Table 1 that the accelerated sulfhydryl–disulfide interchange is responsible for decreased aldehyde binding to albumin, that bradykinin produces knee joint tenderness as well as bronchial constriction, or that protein denaturation is a causative factor in synovitis. The analysis for concordance of test results offers a basis for forming postulates of this kind, but the supporting evidence and the explanations have to be sought in the chemical pathology of the situation.

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