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Letter to the Editor

Statistical assessment of the synergistic effect between a vaccine and a drug

ELIAS ZINTZARAS, *Laboratory of Mathematical Biology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK*

The determination of the interaction between drugs or other treatments is widespread and important in biomedicine. There is disagreement over concepts and approaches for assessing such interactions (Berenbaum 1989). This report illustrates the biological concept of the interaction between a vaccine and a drug, and describes approaches for its statistical assessment. Schistosomiasis is a disease caused by the parasitic worm *Schistosoma mansoni*. The disease is common in many parts of the tropics. Praziquantel is a drug for controlling schistosomiasis in man (Flisser et al 1989). Vaccination of animals with irradiated cercariae (immature forms of the parasite) provides partial protection against infection. This paper examines a study of the possible synergistic effects of vaccination and drug treatment.

Materials and methods

Experimental data. The data comprised measurements, on each of 83 mice, of the numbers of cercariae recovered from mice after being subjected to challenge by live cercariae. The mice were naive (i.e. unvaccinated) or vaccinated previously with irradiated cercariae of *S. mansoni*. Praziquantel was administered to groups of naive and vaccinated mice.

The same dose of praziquantel was administered intradermally on day 1 post-challenge (regimen A) or intramuscularly on day 6 post-challenge (regimen B). For each regimen two experiments, with different numbers of challenges, were performed. Each experiment consisted of four groups of mice: naive; vaccinated only; drug-treated only; vaccinated and drug-treated. The parasites harvested from each mouse were counted (see Table 1).

Synergism. Two types of interaction between drugs or treatments are commonly investigated: synergy, where the drugs or treatments are working together; and antagonism, where they work against each other.

Synergy is variously defined to exist in the following instances: (a) when the combined effect of the drug and the vaccine is greater than the additive sum of the drug and the vaccine effects; (b) when the combined effect is greater than the multiplicative product of the individual effects; (c) when the combined effect of the drug and the vaccine is greater than both the drug effect and the vaccine effect (Berenbaum 1989). Smith et al (1987) argued, however, that the last instance might not be appropriate for defining synergism and it will not be considered here.

The problem that the experimenter is often faced with is to decide which of the above definitions applies to the data set. The definition must be specified before drawing any conclusions as to the supposed nature of the interaction, preferably when the study is designed.

Two criteria can help in deciding the definition of synergy: if the experimenter is interested in the alteration in pharmaceutical effect (e.g. reduction in number of worms recovered) by a fixed amount, when the experimental units (e.g. mice) are treated with the vaccine or the drug, then synergy is defined as in instance (a). Then the drug and vaccine effects represent actual amounts (e.g. differences in numbers of worms recovered). However, if the experimenter is interested in the alteration in pharmaceutical effect by a fixed factor then synergy is defined as in instance (b).

The significance of the interaction can be tested using analysis of variance. When synergy is defined in terms of the multiplicative effect, then the data set must be transformed into logarithms so that the multiplicative effects are converted into additive ones. If the synergy is defined in terms of the additive effect, a log transformation must not be considered even if other statistical considerations suggest such transformation is desirable. Rothman (1974) claims that arbitrary transformation of the scale of observation may falsely suggest or mask the presence of interaction.

Results

The experimenter was interested in the proportional reduction of the number of worms when the mice are treated with the vaccine and drug (K. P. Piper, personal communication). Thus in the absence of interaction, the combined effect of the vaccine and drug will equal the multiplicative product of the individual effects.

The data from the two drug treatment regimens (A: day 1 post-challenge, intradermally; B: day 6 post-challenge, intramuscularly) were analysed separately. Each analysis was done as a three-factor analysis of variance, the factors being: (i) experiments, with two levels (each experiment also involved a different challenge number); (ii) vaccine, with two levels (i.e. presence/absence); (iii) drug, with two levels (i.e. presence/absence).

The data were logarithmically transformed before the analysis of variance. A small proportion of zeros was observed in the row data, and this problem was overcome by adding one to each worm count.

For regimen A, the combined effect of the drug and vaccine was 1·60 ln units, i.e. a reduction in worm count by 80%, as compared with 1·05 ln units (65% reduction) for the sum of the drug and vaccine effects. The interaction of drug and vaccine was non-significant ($P > 0.05$) indicating no synergism.

Table 1. Table of means (m) and (s.e.) of the number of recovery worms for each group of mice in the absence or presence of vaccine and drug. The number of mice (n) in each group is also shown.

	Vaccine	
	Absence	Presence
Regimen A		
absence	m = 52·8 (13·9)	m = 27·22 (2·98)
drug	n = 8	n = 9
presence	m = 26·75 (2·63)	m = 10·45 (1·73)
	n = 8	n = 11
Regimen B		
absence	m = 41·33 (13·9)	m = 24·31 (3·55)
drug	n = 12	n = 13
presence	m = 17·82 (2·33)	m = 3·45 (1·1)
	n = 11	n = 11

For regimen B the combined effect was 2·77 ln units (95% reduction) as compared with 1·58 ln units (79% reduction) for the sum of the individual effects. The interaction was significant ($P < 0.05$), suggesting synergism.

Discussion

The appropriate definition of synergism depends on the nature of the data, and this choice must be made before statistical analysis. In the present case study, the primary interest was in the proportional reduction in worm recovery, leading naturally to a definition of synergism on a multiplicative basis. The analysis was performed on the log transformed data.

If synergism had been incorrectly defined in terms of the additive effect then, for regimen B, the combined effect would be 37·88 units, as compared with 40·53 units for the sum of the individual effects, suggesting antagonism instead of synergism.

This example illustrates how the different definitions may give different results for the same data set.

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References

Berenbaum, M. C. (1989) What is Synergy? Pharmacol. Rev. 41: 93–141

Flisser, A., Delgado, V. S., McLaren, D. J. (1989) *Schistosoma mansoni*: enhanced efficacy of praziquantel in immune mice. Parasite Immunol. 11: 319–328

Rothman, K. J. (1974) Synergy and antagonism in cause-effect relationships. Am. J. Epidemiol. 99: 385–388

Smith, G. H., Williams, F. L. R., Lloyd, O. L. (1987) Respiratory cancer and air pollution from iron foundries in a Scottish town: an epidemiological and environmental study. Br. J. Indust. Med. 44: 795–802

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Book Review

Pharmacokinetic Modelling Using STELLA on the Apple Macintosh
By Clive Washington, Neena Washington and Clive G. Wilson
Published 1990 Ellis Horwood, London
118 pages
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The title of this book, as well as being rather cumbersome, would seem to suggest it would be of limited appeal to the casual browser. It must first of all attract those interested in pharmacokinetic modelling who have access to an Apple Macintosh and who also happen to have a copy of the program Structured Thinking Experimental Learning Laboratory with Animation (STELLA) installed. The most likely users of this book, then, would be students in an academic department where the staff are enthusiastic about STELLA, and where it would be advantageous for students (and staff) to have their own personal copies. Indeed, the book itself has grown out of manuals developed by the staff at Nottingham University.

The authors have the laudable aim of teaching the concepts of pharmacokinetics without introducing complex mathematical equations. This is done by imagining the disposition of a drug in the body as a series of boxes containing the drug, with simple rules determining how the drug is transferred from one box to another. For example, a drug could be imagined to be in the gastrointestinal tract, systemic blood, body tissue and urine as the four boxes with the rules governing transfer including irreversible transfer from gastrointestinal tract to blood, reversible transfer from blood to tissue, and irreversible transfer from blood to urine. This simple classical pharmacokinetic model has a surprisingly complex mathematical solution; simulation programs would be expected to deal with the complex mathematics once the investigator has defined the concepts.

Interestingly, STELLA itself does not use the pure mathematical approach for the simulation. An initial state is imagined; in the example given above, this would imagine all the drug in the gastrointestinal tract with none in the blood, tissues or urine. STELLA applies the rules to establish where the drug will be next, i.e. a proportion of the drug in the gastrointestinal tract would be transferred to the blood, but as there is no drug in the blood or tissue in the initial state, then none is transferred to tissue or urine. STELLA then recalculates for the next step, again transferring a proportion from the gastrointestinal tract to blood, and proportions from blood to tissue and urine. The third step calculates the same transfers plus one involving transfer from tissue back to blood. Computers can happily perform these calculations thousands of times in a few seconds and if the time scale of each step is chosen to be small enough, then the resulting plot of the results is indistinguishable from the same plot using the pure mathematical approach.

The STELLA method is referred to by the authors as numerical analysis or number crunching. In other disciplines it may be termed force field analysis, where all the forces acting on a system are considered determining any change in the system over a short time period. In this particular context, we might have a Catch-22 situation; if the computer does all the calculations, then why not let it use the pure mathematical approach? If the numerical approach is used then the researcher has to know how it works to avoid such howlers as transferring drug from boxes which are already empty.

However, once the user has mastered the elements of STELLA, and has played with a few simple what-if scenarios, the possibilities appear limitless. The great strength of the method is that quite complex models can be created. The authors rightly warn against drawing conclusions from a complex model (such as rate constants) when the precision of available data does not justify such conclusions. Nevertheless, in this what-if world, such exercises are justifiable in testing the plausibility of postulates.

Nearly half the book is used to present the basics of STELLA and of pharmacokinetics, as is to be expected for a manual. The second half presents detailed examples of more complex situations, including the correlation of pharmacokinetics and the behaviour of sustained-release preparations in the gastrointestinal tract. Individuals who take up this method enthusiastically will surely find most satisfaction in applying it to their own particular field of interest; if STELLA gives the unexpected prediction, then they will have learned something new, or may be forced to reconsider the original concepts. Either way, their knowledge and understanding will have been increased.

JOSEPH CHAMBERLAIN