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Multicentre trials of praziquantel in human schistosomiasis: design and techniques

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This paper outlines the experimental design and techniques used in the initial multicentre clinical experiences with praziquantel in the treatment of human infections due to Schistosoma haematobium, S. mansoni, *and* S. japonicum. *Trials were conducted in Brazil, Japan, the Philippines, and Zambia.*

Close professional cooperation between informed representatives of the manufacturers of the drug and WHO led to the use of a standard clinical trial design and agreed technical protocols, although parasitological methods of therapeutic assessment varied with the species of infecting parasite. Double-blind studies of tolerance were conducted at three different dose levels and subsequently, in Brazil and Zambia, single-blind trials of parasiticidal efficacy were carried out. The results of the various trials are reported separately.

This type of close professional cooperation is a useful model for initial clinicopharmacological studies of parasiticidal drugs—an area beset with difficulties for both industry and international agencies.

The accumulated experiences of the many national or international programmes of research into optimum methods of schistosomiasis control conducted during the past 30 years have indicated that, in many and perhaps the majority of endemic areas, population-based chemotherapy will become increasingly important in both transmission and disease control.

The desirable criteria for schistosomicidal drugs that might be used in large-scale chemotherapeutic programmes are well known and can be summarized as:

(*a*) *Experimental.* Evidence of schistosomicidal activity in animal models equivalent or superior to that exhibited by known drugs in current use; absence of adverse toxicological or pharmacodynamic effects in the customary preclinical screening procedures; absence of adverse general biological effects of either short-term (e.g., teratogenicity) or long-term nature (mutagenicity, carcinogenicity).

(*b*) *Clinical.* High curative efficacy against the different stages of the three common schistosomes infecting man; favourable pharmacokinetic characteristics in man particularly in relation to rapid excretion and non-cumulation of drug and/or metabolites; high patient-acceptance rate (which infers an oral dosage form); few major side effects; minimal treatment time—preferably one dose, in any case not more than a one-day treatment.

(*c*) *Pharmaceutical.* Minimum interactions with other drugs, dietary constituents, alcohol, tobacco; acceptable production costs, favourable selling prices, and a long shelf-life in hot and humid climates.

Despite the major advances in the drug treatment of schistosomiasis made during the last 15 years, none of the current schistosomicides meet all the criteria desirable for chemotherapeutic use in large-scale control campaigns. Undoubtedly, effective and well-tolerated drugs are available but, regrettably, each has deficiencies that have stimulated the continuing search for new and improved chemotherapeutic compounds.

For the purposes of schistosomiasis control when large-scale or population-based chemotherapy is to be used as a major tactical tool, the antimonials and

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lucanthone hydrochloride are of historical interest only, although widespread use of antimonials persists in Egypt. Adverse toxicological findings, particularly those demonstrating mutagenicity in various experimental test systems, have tended to limit more widespread use of niridazole and hycanthone. Niridazole was carcinogenic after oral administration to Swiss mice and Syrian golden hamsters, whether or not previously infected with *Schistosoma mansoni* (*1, 2*). Hycanthone was reported to produce a significant increase in hepatic hyperplasia and induction of hepatocellular carcinomas in mice previously infected with *S. mansoni* (*3, 4*), findings at variance with the results of another study in which mice were infected with *S. mansoni* by a different route and given different doses of the drug, but in which there was a low survival rate (*5*).

Furapromidium, widely used in China (*6*), was mutagenic in bacterial, fungal, and mammalian cell systems (*7*). The chemotherapy of *S. japonicum*, whether for individual or large-scale treatment remains unsatisfactory (*8*).

Metrifonate is currently in widespread use in large-scale chemotherapy campaigns against *S. haematobium*, and oxamniquine is employed similarly against *S. mansoni*. Both suffer from the defect that they are effective only against one species of parasite. While each drug is extremely well tolerated in field use, neither is totally free from the suspicion of mutagenicity, since both have shown mutagenic activity in tests with *Salmonella typhimurium* strains TA98 and TA100 under *in vitro* and *in vivo* (host-mediated) conditions (*9*). There is however, evidence that the antischistosomal and mutagenic properties of a drug may not necessarily be associated (*10, 11, 12*).

Praziquantel is a newly developed compound that is not structurally related to previously used schistosomicides (Fig. 1).

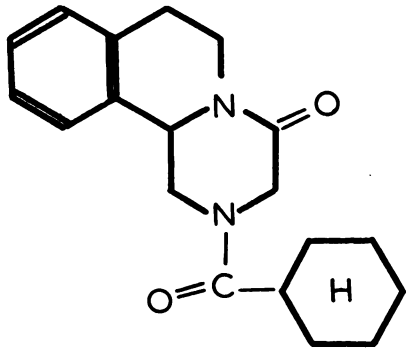

Fig. 1. Structural formula of praziquantel ($C_{19}H_{24}N_2O_2$).

In various animals, tolerance to praziquantel was good in preclinical toxicity studies (*13, 14*), and it is highly active experimentally and clinically against a wide range of cestodes and against all species of schistosome pathogenic to man (*15–21*). A wide range of studies has not revealed any mutagenic activity of praziquantel in tissue-, host- and urine-mediated assays with *Salmonella typhimurium* strains (*22*), in dominant lethal and micronucleus tests in mice and in spermatogonial tests in Chinese hamsters (*23*), and in a battery of investigations with *S. typhimurium, Schizosaccharomyces pombe, Saccharomyces cerevisiae, Drosophila melanogaster,* and cultured V79 Chinese hamster cells, in the presence or absence of a metabolic activation system where appropriate (*12*).

In man, praziquantel is rapidly absorbed after oral dosage and, after a pronounced first-pass biotransformation process, metabolites are excreted mainly in the urine. In healthy volunteers, maximum serum concentrations are reached in 1–2 hours. By means of an isotope-measuring technique using ^{14}C-labelled praziquantel and a specific gas-chromatographic assay, it was shown that the elimination half-life from the serum was 1–1½ hours and that for praziquantel plus metabolites was 4–5 hours. The renal elimination half-life for praziquantel plus metabolites was 4–6 hours and the cumulative renal excretion of praziquantel within 4 days was in excess of 80% of the dose, 90% of which was eliminated on the first day (*24, 25*).

Cooperative multicentre clinical trials of tolerance to praziquantel and of its therapeutic effect against the three common species of schistosome infecting man were conducted jointly by WHO and Bayer AG in Africa, Japan, the Philippines, and South America. These studies were carried out on *Schistosoma haematobium* infections in Zambia, at the clinical pharmacology unit of the WHO Tropical Diseases Research Centre, Ndola Central Hospital, Ndola; on *S. mansoni* infections at the Centro de Pesquisas "René Rachou", Belo Horizonte, Brazil; and in the case of *S. japonicum* infections, double-blind studies of tolerance were conducted in the Koma-Kyoritsu Hospital, Yamanashi-Ken, with the advice of the National Institute of Health, Tokyo, Japan, complemented by clinical trials of tolerance and efficacy performed by the Schistosomiasis Control and Research Service team at Palo, Leyte, Philippines. The results of these trials are reported separately (*26-29*).

MATERIALS AND METHODS

A standard trial design and a common protocol were used, although techniques of therapeutic assessment naturally varied with the species of infecting parasite.

The design was kept as simple as possible in view of the known practical difficulties of multicentre trials. Initially, groups of potential participants were screened parasitologically and entrants to the trial, selected on the basis of having at least a specified mean urinary or faecal schistosome egg output, calculated from multiple excretal examinations, were randomly allocated, from a table of random numbers prepared in the Federal Republic of Germany before the start of the studies, to one of two treatment groups, either active drug or placebo. For inclusion in the trial, participants were also required not to have any serious acute coexistent diseases or complications, not to have had any other treatment within the previous 6 months, to be over 6 years of age, and, in the case of females, not to be either pregnant or lactating.[a]

Double-blind studies of tolerance were conducted at three different dose levels: a single oral dose of praziquantel of 20 mg/kg body weight, two oral doses each of 20 mg/kg, and three oral doses each of 20 mg/kg (total dose 60mg/kg) compared with the same dosage frequency of placebo. Doses were given at intervals of 4 hours.

Tablets were formulated as white, odourless preparations of 400 mg which could easily be quartered, thus enabling the dose to be rounded to the nearest 100 mg. Clinical investigators were unaware of the identity of the tablets, which were simply coded as "A" and "B". Tablets were given personally by participating physicians, washed down with water, and mouths were inspected to ensure that the dose had been swallowed.

In Zambia and Brazil, after the completion of the double-blind studies in *S. haematobium* and *S. mansoni* infections respectively, the code was broken and two further single-blind trials were conducted on the efficacy of two regimes of 3 × 20 mg/kg and 50 mg/kg given as a single dose. In *S. japonicum* infections, only double-blind studies of tolerance were performed in Japan. In the Philippines, a trial of both tolerance and efficacy of a dose of 3 × 20 mg/kg was conducted by a double-blind technique against placebo and the results were contrasted with those resulting from a single oral dose of 50 mg/kg. Patients initially given placebo were followed up parasitologically and then subsequently treated with praziquantel. They thus served as their own "within-patient" parasitological control and confirmed that there was no spontaneous disappearance of eggs from the excreta during the period of initial assessment of efficacy of the drug.

[a] Full protocols are available from Dr D. H. G. Wegner.

TECHNIQUES

Only the techniques common to all trial centres are described here. Entrants to the trials were admitted to hospital in order that intensive clinical supervision could be guaranteed. On admission, a full history was taken, body weight and height were recorded, each patient was subjected to a detailed physical examination by the participating physician, and a questionnaire on symptoms was completed. A pretreatment electrocardiogram, complete blood count, clinical chemical profile, routine urinalysis, and appropriate quantitative and qualitative parasitological examinations were performed before treatment. All female patients over 12 years were screened with a commercially available urinary pregnancy test. None were positive.

Examinations additional to these minimal requirements were necessary in different trial groups, e.g., screening for haemoglobin abnormalities, chest X-ray, electroencephalography, and these are described in separate papers (*26-29*) where details of specific techniques are also given.

After treatment with either drug or placebo in the double-blind studies, and after drug treatment in the efficacy trials, a questionnaire on post-treatment symptoms was completed, regular physical examinations were performed, and serial clinicopathological measurements were made at 24 or 48 hours to monitor haematological, biochemical, and electrocardiographic functions.

In addition, venous blood samples were withdrawn at various intervals after dosage, and the serum was separated, kept at −20°C, and flown to the Federal Republic of Germany in vacuum flasks for gas-chromatographic studies of blood levels of praziquantel and metabolites. These pharmacokinetic studies were performed blind and the investigator did not receive details of whether a sample was from a drug- or placebo-treated patient in the trials of tolerance until the end of the series of estimations.

Parasitological techniques and criteria

General criteria for entry to the trial were, in *S. haematobium* infections, a geometric mean egg excretion rate of 50 per 10 ml of urine, calculated from a minimum of two urine examinations performed immediately before treatment; in *S. mansoni* and *S. japonicum* infections, a geometric mean excretion rate of 100 eggs per gram of stool, also calculated from two immediate pretreatment stool samples. In each of the three infections, it was necessary also to demonstrate at least one positive pretreatment hatching test to confirm egg viability. Details of the techniques are reported separately for each trial (*26-29*).

Assessment of therapeutic efficacy was made by parasitological examinations for 3 consecutive days at 1, 2, 3, 4–6, and 12 months after treatment. Since all techniques used were quantitative, calculations of residual egg excretions could be made in the usual fashion.

CONCLUSION

Close professional cooperation between informed representatives of major pharmaceutical companies and technical staff of the World Health Organization led to agreed protocols for clinical trials of a new schistosomicide, a more rapid completion of initial clinicopharmacological studies than would have been the case if either partner had attempted their conduct individually, and fewer problems in communications than might have been anticipated. Such cooperation, which depends largely on mutual interest and trust, is recommended as one, but not the sole, useful mechanism in an area beset with difficulties for both industry and international agencies.

RÉSUMÉ

ESSAIS MULTICENTRES DU PRAZIQUANTEL POUR LE TRAITEMENT DE LA SCHISTOSOMIASE HUMAINE: CONCEPTION ET TECHNIQUES

L'expérience des campagnes de lutte contre la schistosomiase a montré que la chimiothérapie de masse est appelée à prendre une importance croissante du double point de vue de la lutte contre la transmission et de la lutte contre la maladie. L'article expose brièvement les faiblesses des composés actuellement disponibles. Des résultats toxicologiques défavorables, notamment ceux qui démontrent la mutagénicité de ces composés dans divers systèmes d'épreuve, ont limité l'emploi du niridazole et de l'hycanthone. Le furapromidium, largement utilisé en Chine, est également mutagène dans de nombreux systèmes d'épreuve. Le métrifonate et l'oxamniquine, actuellement utilisés dans des campagnes sur le terrain contre *Schistosoma haematobium* et *S. mansoni* respectivement, ont le défaut de n'être efficaces que contre une seule espèce de parasite.

Le praziquantel est un composé de mise au point récente dont la tolérance s'est révélée bonne au cours d'études préliminaires de toxicité à court et à long terme chez l'animal. Il s'est également révélé hautement efficace lors d'études expérimentales et d'études cliniques contre une gamme étendue de cestodes et contre toutes les espèces de schistosomes pathogènes pour l'homme. Au cours de nombreuses études réalisées dans une grande variété de systèmes d'épreuve, aucune activité mutagène n'a pu être démontrée. Chez l'homme, l'absorption rapide après administration orale est suivie par une biotransformation avec montée brusque du taux sérique («first-pass effect») et l'élimination rapide du composé et de ses métabolites, principalement dans l'urine.

Une coopération étroite entre les représentants des fabricants du composé et l'OMS a conduit à mettre au point un protocole standard d'essai clinique et des modes opératoires approuvés, bien que les méthodes parasitologiques d'évaluation thérapeutique varient selon l'espèce en cause.

Les participants aux essais, choisis parmi des sujets ayant un taux donné d'excrétion urinaire ou fécale d'œufs de schistosomes, calculé d'après les résultats de nombreux examens préalables d'excreta, ont été répartis au hasard dans l'un ou l'autre des groupes de traitement. Des études de tolérance en double insu ont alors été réalisées avec trois posologies: une dose orale unique de praziquantel de 20 mg/kg de poids corporel, deux doses orales de 20 mg/kg et trois doses orales de 20 mg/kg (dose totale 60 mg/kg), les doses multiples administrées à intervalle de quatre heures, avec, dans chaque cas, un groupe témoin recevant un placebo selon la même posologie.

Des mesures biochimiques, hématologiques, électrocardiographiques et, dans certains cas, électroencéphalographiques, ont été faites avant et après le traitement, et des questionnaires ont été utilisés pour évaluer d'éventuels symptômes liés à l'administration du médicament.

En Zambie et au Brésil, après réalisation d'études en double insu dans des infections à *S. haematobium* pour la Zambie et à *S. mansoni* pour le Brésil, les résultats ont été décodés et deux essais en simple insu ont été réalisés pour tester l'efficacité de deux posologies, l'une à trois fois 20 mg/kg et l'autre avec une dose unique de 50 mg/kg. Pour les infections à *S. japonicum,* seules des études de tolérance en double insu ont été faites au Japon. Aux

Philippines, un essai de tolérance et d'efficacité en double insu a été réalisé avec une dose de 3×20 mg/kg et un placebo, et les résultats ont été comparés à ceux obtenus après administration orale d'une dose unique de 50 mg/kg. Le plus grand nombre possible de malades ont subi des examens cliniques et parasitologiques réguliers pendant au moins un an après le traitement. On a réalisé un suivi parasitologique des malades ayant d'abord reçu le placebo afin de vérifier qu'aucun arrêt spontané de l'excrétion d'œufs ne s'était produit; ces malades ont ensuite été traités par le praziquantel.

Ce type de coopération professionnelle étroite constitue un modèle utile pour les études clinico-pharmacologiques initiales des médicaments parasiticides, dont la réalisation est difficile à la fois pour l'industrie et pour les organismes internationaux.

REFERENCES

1. URMAN, K. H. ET AL. *Cancer letters,* **1**: 69-74 (1975).
2. BULAY, O. ET AL. *Journal of the National Cancer Institute,* **59**: 1625-1629 (1977).
3. HAESE, W. H. ET AL. *Journal of pharmacology and experimental therapeutics,* **186**: 430-440 (1973).
4. HAESE, W. H. & BUEDING, E. *Journal of pharmacology and experimental therapeutics,* **197**: 703–713 (1976).
5. YARINSKY, A. ET AL. *Toxicology and applied pharmacology,* **27**: 169-182 (1974).
6. Report of the American Schistosomiasis Delegation to the People's Republic of China. *American journal of tropical medicine and hygiene,* **26**: 427-457 (1977).
7. TONG-MAN ONG, ET AL. *Mutation research,* **48**: 37-42 (1977).
8. WHO Technical Report Series, No. 515, 1973, p. 20.
9. BATZINGER, R. P. & BUEDING, E. *Journal of pharmacology and experimental therapeutics,* **200**: 1-9 (1977).
10. HARTMANN, P. E. ET AL. *Science,* **172**: 1058-1060 (1971).
11. HULBERT, P. B. ET AL. *Science,* **186**: 647-648 (1974).
12. BARTSCH, H. ET AL. *Mutation research,* **58**: 133-142 (1978).
13. MURMANN, P. ET AL. *Veterinär-medizinische Nachrichten,* **21**: 142-153 (1976).
14. FROHBERG, H. In: *XIV Congresso da Sociedade Brasiliera de Medicina Tropical. João Pessoa, 19-23 February 1978* (In press, 1979).
15. THOMAS, H. & GONNERT, R. *Zeitschrift für Parasitenkunde,* **52**: 117-127 (1977).
16. GONNERT, R. & ANDREWS, P. *Zeitschrift für Parasitenkunde,* **52**: 129-150 (1977).
17. WEBBE, G. & JAMES, C. *Zeitschrift für Parasitenkunde,* **52**: 169-177 (1977).
18. DAVIS, A. In: *Kurzfassungen der Tagung deutschsprachiger tropenmedizin Gesellschaften, Lindau, 24-26 March 1977,* 1977, p. 45.
19. GROLL, E. *Boletín chileno de parasitología,* **32**: 27-31 (1977).
20. DAVIS, A. In: *XIV Congresso da Sociedade Brasiliera de Medicina Tropical. João Pessoa, 19-23 February 1978* (In press, 1979).
21. KATZ, N. In: *XIV Congresso da Sociedade Brasiliera de Medicina Tropical. João Pessoa, 19-23 February 1978.* (In press, 1979).
22. OBERMEIER, J. & FROHBERG, H. *Archives of toxicology,* **38**: 149-161 (1977).
23. MACHEMER, L. & LORKE, D. *Archives of toxicology,* **39**: 187-197 (1978).
24. LEOPOLD, G. ET AL. *European journal of clinical pharmacology,* **14**: 281-291 (1978).
25. BUHRING, K. U. ET AL. *European journal of drug metabolism and pharmacokinetics,* **3**: 179-190 (1978).
26. DAVIS, A. ET AL. *Bulletin of the World Health Organization,* **57**: 773-779 (1979).
27. KATZ, N. ET AL. *Bulletin of the World Health Organization,* **57**: 781-785 (1979).
28. ISHIZAKI, T. ET AL. *Bulletin of the World Health Organization,* **57**: 787-791 (1979).
29. SANTOS, A. T. ET AL. *Bulletin of the World Health Organization,* **57**: 793-800 (1979).