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ANTI-SCHISTOSOMAL DRUGS: OBSERVATIONS ON THE MECHANISM OF DRUG RESISTANCE TO HYCANTHONE, AND ON THE INVOLVEMENT OF HOST ANTIBODIES IN THE MODE OF ACTION OF PRAZIQUANTEL

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This paper reports recent observations from our laboratory dealing with the anti-schistosome drugs hycanthone (HC) and praziquantel (PZQ). In particular, we discuss a laboratory model of drug resistance to HC in Schistosoma mansoni *and show that drug sensitive and resistant lines of the parasite can be differentiated on the basis of restriction fragment length polymorphisms using homologous ribosomal gene probes. In addition, we summarize data demonstrating that effective chemotherapy of* S. mansoni *infection with PZQ in mice requires the presence of host anti-parasite antibodies. These antibodies bind to PZQ treated worms and may be involved in an antibody-dependent cellular cytotoxicity reactions which result in the clearance of worms from the vasculature.*

Chemotherapy is at present the most effective method of controlling human schistosomiasis (Liese, 1986). A variety of anthelmintics have been used to treat schistosome infections. These originally consisted of toxic antimonial compounds which were later superceded by safer compounds including niridazole, metrifonate, hycanthone, and oxamniquine. However, in many places these agents have now been replaced with praziquantel (PZQ) and this drug is now the treatment of choice for human schistosomiasis (Campbell & Garcia, 1986). In addition, experimental compounds such as amoscanate, oltipraz, and artesunate show promise for future introduction as anti-schistosomal agents (Bennett & Depenbusch, 1984).

Drug resistance in schistosomes

A major risk in the widespread use of anthelmintics is the development of drug resistant parasites. Drug resistance has proved to be a formidable problem in the control of other parasitic diseases by chemotherapy with resistance to chloroquine in malaria being a prominent example (Moore & Lanier, 1961). While no confirmed examples of PZQ resistance in schistosomes have been reported, the compound has been used only on a limited scale to date (Liese, 1986). Moreover, resistance to oxamniquine has been documented in schistosomes in Brazil (Guimaraes et al., 1979) where this drug is still heavily employed. Therefore, it is possible that resistance to PZQ may develop after prolonged application in a manner similar to that which has occurred with oxamniquine. Thus an understanding of the genetic basis of drug resistance would seem to be important in dealing with the problem should it arise through continuing and future campaigns of mass chemotherapy.

Recently we have been studying a laboratory model of drug resistance in which resistance to the thioxanthenone compound hycanthone (HC) is experimentally induced by treatment of immature worms in mice (Rogers & Bueding, 1971). Parasites resistant to HC show cross-resistance to oxamniquine (Jansma et al., 1977). Although chemotherapy with HC itself has been discontinued, because of evidence suggesting that the drug is mutagenic (Hulbert et al., 1974) it is hoped that this research will provide a general strategy for developing probes for detecting resistance to other anti-schistosomal compounds including PZQ should it appear in the field.

A laboratory model of HC-resistance was developed by the late Professor Ernest Bueding at Johns Hopkins University and involves drug treatment of a Puerto Rican strain of *Schistosoma mansoni* in mice at 28 days post infection. Administation of HC to immature worms at 28 days after infection does not result in significant parasite mortality. Nevertheless the eggs produced by these worms give rise in the next generation to parasites which are resistant to HC and the progeny remain so upon passage in the abscence of continuing drug pressure.

Only some strains of *S. mansoni* can be transformed from HC-sensitive to – resistant by this manipulation (Jansma et al., 1977). Worm mating experiments have indicated that the F1 progeny from reciprocal crosses between HC-sensitive and resistant schistosomes are drug sensitive, a finding which indicates that resistance to HC is inherited as an autosomal recessive trait (Cioli & Pica-Mattoccia, 1984). It had been suggested previously that the mutagenic effects of HC in bacteria result from the metabolic conversion of HC *in vivo* to a reactive ester, which upon dissociation, alkylates DNA (Hartman et al., 1971). More recently Cioli et al (1985) presented experimental evidence indicating that this same mode of action may also explain the anti-schistosomal activity of HC. They showed that various HC-resistant forms of schistosomes including immature *S. mansoni,* adult *S. mansoni* from a drug-resistant strain, and *S. japonicum* were killed by treatment with hycanthone N-methylcarbamate, a synthetic ester of HC which acts as an alkylating agent, while as expected, these same forms were not killed by the parent compound (hycanthone methane sulfonate). On the basis of these data they suggested that resistant schistosomes lack the esterifying enzyme responsible for metabolic conversion of HC into the toxic ester. According to their hypothesis, resistance to HC results from drug induced mutation leading to the deletion of functional enzyme activity, a concept which is consistent with the recessive inheritance of the trait (Cioli & Pica-Mattoccia, 1984).

In our research we have been studying of the molecular biology of resistance in Dr Bueding's laboratory model. We obtained the HC-sensitive, Puerto Rican strain of *S. mansoni* from Dr Bueding, as well as a resistant line originally acquired 8 generations after the induction of resistance by drug treatment of 28 day old worms. Using hycanthone methane sulfonate (kindly provided by Dr Sydney Archer, Rensselaer Polytechnic institute) we confirmed the stability of the sensitivity and resistance to the drug of these two lines during the next six generations (Fig.). In addition, here at the NIH we derived another line of resistant worms from Bueding's sensitive strain by treatment of mice with HC at 28 days after infection. The progeny of these drug treated parasites in the next two generations were > 90% resistant to HC in comparison to the parent M strain which remains sensitive to the drug (Fig.). As could be predicted from Bueding's previous results, we have been unable to establish resistance to HC in another strain of *S. mansoni* (NMRI strain) by either of two induction procedures that he described (Fig.).

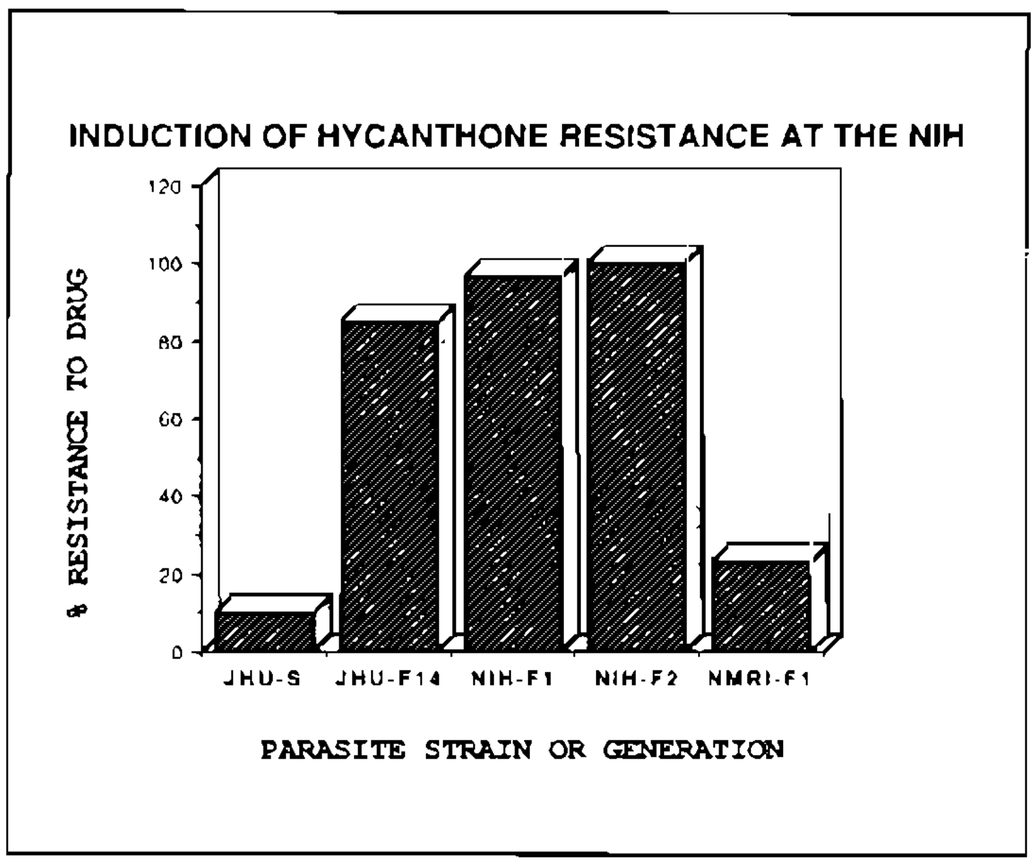

Fig.: Levels of resistance to hycanthone in the drug sensitive JHU-S strain as well as in parasite lines obtained after induction of resistance by treatment of immature JHU-S worms 28 days after infection. JHU-JHU-S, the parental strain; JHU-F14, a resistant line of worms 14 generations after the induction of resistance in Dr Bueding's laboratory: NIH-F1 and NIH-F2, first and second generations of drug resistant worms derived from the JHU-S line at the NIH; NMRI-F1, progeny of drug sensitive NMRI strain worms after treatment with hycanthone according to the resistance induction protocol. As can be seen here, the JHU-S strain is readily and stably transformed from drug sensitive to resistant, whereas the NMRI strain cannot be readily transformed.

At present, we are investigating the genetic basis of resistance to HC by analyzing DNA of individual adult worms, and from populations of HC-sensitive and resistant lines for restriction fragment length polymorphisms (RFLPs), detectable with homologous ribosomal gene probes. One of these probes, plasmid pSM 389 contains a 3.1 kilobase insert comprised of part of the non-transcribed spacer (which separates each of the tandemly repeated ribosomal gene units from each other) as well as a portion of the coding region for the small rRNA gene (Simpson et al., 1984). These probes have been used previously to distinguish by Southern blot analysis, species, strain and gender of schistosomes as well as individuals within strains (McCutchan et al., 1984). Species can be separated on the basis of the electrophoretic mobility of the major band of hybridization which represents the repeating ribosomal gene

itself, whereas minor band differences are used to distinguish strains, individuals or sexes. These minor bands are thought to represent either "orphons", divergent ribosomal sequences residing in regions of the genome distant from the major repeating unit, or may result from hybridization of non-transcibed portions of the ribosomal gene within the probe that have become dispersed throughout the genome.

Using probe pSM 389 we have been able to distinguish HC-resistant and sensitive lines of *S. mansoni* on the basis of RFLPs. Our data suggest that genetic changes occur in Bueding's strain of *S. mansoni* upon induction of HC-resistance and that these changes are stable and heritable. Thus, Hind 111 digested DNAs of each of more than 40 individual adult schistosomes of either sex in the F8 through F14 generations of Bueding's resistant strain contain a 3.8 kb fragment that hybridizes to pSM 389. In contrast, we have never seen this band in Hind 111 digested DNA from a similar number of individual worms from the sensitive strain, from which the HC-resistant line was derived. However, this 3.8 kb band was not observed in a similar analysis of individuals from the resistant line of *S. mansoni* that we derived at the NIH, thereby eliminating it as a reproducible marker of resistance.

More recently we have detected another RFLP which does correlate well with drug resistance. Following restriction of DNA with Bam H1, and hybridization to pSM 389, drug resistant individuals exhibit a 3.5 kb band that is not present in their drug sensitive progenitors. In addition, the drug sensitive worms show a strong band of hybridization at 5.0 kb whereas this band is present only weakly in the Bam H1 digested DNA from drug resistant individuals. Thus the presence of the 3.5 kb band is closely associated with HC-resistance in our laboratory strains of *S. mansoni.*

On a different tack, we are attempting to directly locate sequences in the schistosome genome that encode sensitivity to HC. Thus, in preliminary experiments we have compared the HC-sensitive and –resistant lines for differences in gene products. After cell-free synthesis *in vitro* of poly (A) + mRNA from both sensitive and resistant adult worms in the presence of 35S-methionine, and 2-D electrophoresis of the translation products, differences were evident between the schistosome lines. In particular a 35 kDa, pI 5.5 polypeptide was evident in the translation products from the sensitive line but was not present in those from the resistant line (Brindley & Sher, unpublished). Accordingly, we will now undertake a differential hybridization analysis using mRNA and cDNA from drug sensitive versus drug resistant adult worms in order to isolate nucleic acid sequences correlated with HC-sensitivity. If we are successful with this approach we plan to test these sequences in the form of cloned DNA probes for their utility in detecting resistance to HC and oxamniquine in laboratory strains and field isolates of schistosomes.

Anti-schistosomal antibodies and PZQ

The pyrazino-isoquinoline compound praziquantel (PZQ) is the best anti-schistosome drug in current use. PZQ is active against all species of schistosomes that parasitize people, one oral dose is generally sufficient for effective chemotherapy, and the drug causes only minimal side effects (Bennett & Depenbusch, 1984).

To date, there have not been any carefully documented instances of drug resistance to PZQ in schistosomes, although resistance may appear after prolonged use of this compound in mass chemotherapy campaigns. Some patients are not cured of schistosomiasis by treatment with PZQ. Whereas it seems unlikely at present that this ineffective chemotherapy involving PZQ could be ascribed to infection with PZQ-resistant schistosomes, it may be related to the status of the patient's immunocompetence. We reported recently that the chemotherapeutic effect of PZQ against *S. mansoni* in the mice requires host antibody to be fully effective (Brindley & Sher, 1987). For that study, drug cure with PZQ was examined in u-suppressed versus immunologically intact C3H/HeN mice. These u-suppressed mice are treated with anti-body to u-chain which results in the absence of serum immunoglobulins and, in particular, in the absence of specific anti-schistosome antibodies in infected u-suppressed animals (Lawton et al., 1972; Sher et al., 1982). Accordingly, PZQ therapy may not be (fully) effective in patients who are immunocompromised such that they do not possess suitable anti-parasite antibodies. It appears that in schistosome-infected mice that pre-existing circulating antibodies bind to surface epitopes on the schistosomes following treatment of the mice with PZQ.

Anti-schistosome antibodies do not normally bind to the surface of adult schistosomes *in vivo*, a situation which has classifically been interpreted as due to inhibition or making of parasite antigens by surface adsorbed molecules acquired from the host (Smithers et al., 1965; Sher et al., 1978). However, recent studies, both in this laboratory and elsewhere indicate that the concentrations of host molecules on the surface of *S. mansoni* are not altered substantially during the a 1 hour period following treatment with PZQ (Brindley & Sher, 1987; Harnett & Kusel, 1986).

In these experiments, we have examined adult worms perfused from infected, PZQ-treated nude mice, and have observed that a 200 kDa surface glycoprotein, a carboxypeptidase and an alkaline phosphatase are exposed by the drug treatment (Brindley, Strand & Sher, unpublished; Doenhoff, personal communication). Probably more antigens than these are exposed on dying worms, particularly as the duration following drug treatment increases. Indeed EM studies show that damage to PZQ-treated *S. mansoni* is progressive. We suspect therefore that the antibodies that bind to PZQ-treated worms may not be directly toxic for the drug treated worms. These antibodies, which may be of IgG and/or other isotypes, may be involved in antibody dependent cellular cytotoxicity reactions which eventually kill the parasites (Brindley & Sher, 1987). It has been shown that granulocytes migrate into drug-treated schistosomes in mice within several hours following PZQ administration (Mehlorn et al., 1981). Furthermore, adult *S. mansoni* recover and can repair lesions caused by exposure to sub-lethal concentrations of PZQ *in vivo* (Shaw & Erasmus, 1987).

Thus, at present, it seems reasonable to speculate that worms exposed to PZQ in the absence of anti-schistosome antibodies, or to sub-lethal doses of the drug in immunologically intact animals, can recover from the direct effects of the drug (which include calcium ion flux, tetany, hepatic shift, separation of worm pairs, surface blebbing, vacuolization, exposure of tegumental antigens, and so on [Gonnert & Andrews, 1977; Pax et al., 1978; Xiao et al., 1984; Brindley & Sher, 1987]). In contrast, worms disabled by lethal concentrations of PZQ in immunologically intact mice do not survive because the drug damaged parasites are attacked in the liver by immune responses which kill them and lead to their removal from the vasculature. The dead schistosomes are enclosed by hepatic granulomatous reactions and resorbed (Mehlhorn et al., 1981).

We have shown that the chemotherapeutic effect of PZQ against *S. mansoni* is dependent on host antibody responses in the mouse. If this is true in human schistosomiasis also, knowledge concerning the synergistic role of anti-schistosome antibodies in chemotherapy with PZQ will be important in assessing the failture of drug treatment in certain individuals as well as for the future design of more effective forms of PZQ-based therapy.

REFERENCES

BENNETT, J. L. & DEPENBUSCH, J. W., 1984. The chemotherapy of schistosomiasis, p. 73-131. In J. M. Mansfield, *Parasitic diseases*, vol. 2, *The chemotherapy*. Dekker, New York.

BRINDLEY, P. J. & SHER, A., 1987. The chemotherapeutic effect of praziquantel against *Schistosoma mansoni* is dependent on host antibody response. *J. Immunol., 139:* 215-220.

CAMPBELL, W. C. & GARCIA, E. G., 1986. Trematode infections of man, p. 385-399. In W. C. Cambell & R. S. Rew, *Chemotherapy of parasitic diseases*. Plenum Press, New York.

CIOLI, D. & PICA-MATTOCCIA, L., 1984. Genetic analysis of hycanthone resistance in *Schistosoma mansoni*. *Am. J. Trop. Med. Hyg., 33:* 80-88.

CIOLI, D.; PICA-MATTOCCIA, L.; ROSENBERG, S. & ARCHER, S., 1985. Evidence for the antischistosomal action of hycanthone. *Life Sciences, 37:* 161-167.

GONNERT, R. & ANDREWS, P., 1977. Praziquantel, a new broad spectrum antischistosomal agent. *Z. Parasitenkd., 52:* 129-150.

GUIMARAES, R. X.; TCHAKERIAN, A.; DIAS, L. C. S.; ALMEID, F. M. R. DE; VILELA, M. P.; CABECA, M. & TAKEDA, A. K., 1979. Resistência ao hycanthone e oxamniquine em doentes com esquistossomose forma clínica hepatointestinal. *Revista da Associação Médica brasileira, 25:* 48-50.

HARNETT, W. & KUSEL, J. R., 1986. Increased exposure of parasite antigens at the surface of adult male *Schistosoma mansoni* exposed to praziquantel *in vitro*. *Parasitology, 93:* 401-405.

HARTMAN, P. E.; LEVINE, K.; HARTMAN, Z. & BERGER, H., 1971. Hycanthone: a frameshift mutagen. *Science, 172:* 1058-1060.

HULBERT, P. B.; BUEDING, E. & HARTMAN, P. E., 1974. Hycanthone analogs: dissociation of mutagenic effects from antischistosomal effects. *Science, 186:* 647-648.

JANSMA, W. B.; ROGERS, S. H.; LIU, C. L. & BUEDING, E., 1977. Experimentally produced resistance of *Schistosoma mansoni* to hycanthone. *Am. J. Trop. Med. Hyg., 26:* 926-936.

LAWTON, A. R.; ASOFSKY, R.; HYLTON, M. B. & COOPER, M. A., 1972. Suppression of immunoglobulin class synthesis in mice. 1. Effects of

treatment with antibody to u-chain. *J. Exp. Med., 135:* 277-297.

LIESE, B., 1986. The organization of schistosomiasis control programmes. *Parasitol. Today, 2:* 339-345.

McCUTCHAN, T. F.; SIMPSON, A. J. G.; MULLINS, J. A.; SHER, A.; NASH, T. E.; LEWIS, F. & RICHARDS, C., 1984. Differentiation of schistosomes by species, strain, and sex by using cloned DNA markers. *Proc. Natl. Acad. Sci. USA, 81:* 889-893.

MEHLHORN, H.; BECKER, B.; ANDREWS, P.; THOMAS, H. & FRENKEL, J. K., 1981. *In vivo* and *in vitro* experiments on the effects of praziquantel on *Schistosoma mansoni. Arzneimittelforsch., 31:* 544-554.

MOORE, D. V. & LANIER, J. E., 1961. Observations on two *Plasmodium falciparum* infections with an abnormal response to chloroquine. *Am. J. Trop. Med. Hyg., 10:* 5-9.

ROGERS, S. H. & BUEDING, E., 1971. Hycanthone resistance: development in *Schistosoma mansoni. Science, 172:* 1057-1058.

PAX, R. A.; BENNETT, J. L. & FETTERER, R. H., 1978. A benzodiazepine derivative and praziquantel: effects on musculature of *Schistosoma mansoni* and *Schistosoma japonicum. Naunyn Schmiedbergs Arch. Pharmacol., 304:* 309-315.

SIMPSON, A. J. G.; DAME, J. B.; LEWIS, F. A. & McCUTCHAN, T. F., 1984. The arrangeement of ribosomal RNA genes in *Schistosoma mansoni.* Identification of polymorphic structural variants. *Eur. J. Biochem., 139:* 41-45.

SHAW, M. K. & ERASMUS, D. A., 1987. *Schistosoma mansoni:* structural damage and tegumental repair after *in vivo* treatment with praziquantel. *Parasitology, 94:* 243-254.

SHER, A.; HALL, B. F. & VADAS, M. A., 1978. Aquisition of murine major histocompatibility complex gene products by schistosomula of *Schistosoma mansoni. J. Exp. Med., 148:* 46-67.

SHER, A.; HIENY, S.; JAMES, S. L. & ASOFSKY, R., 1982. Mechanisms of protective immunity against *Schistosoma mansoni* infection in mice vaccinated with irradiated cercariae. 11. Analysis of immunity in hosts deficient in T lymphocytes, B lymphocytes, or complement. *J. Immunol., 128:* 1880-1884.

SMITHERS, S. R.; TERRY, R. J. & HOCKLEY, D. J., 1969. Host antigens in schistosomiasis. *Proc. R. Soc. Lond. (Biol)., 171:* 483-494.

XIAO, S-H.; CATTO, B. A. & WEBSTER, L. T., 1985. Effects of praziquantel on different development stages of *Schistosoma mansoni in vitro* and *vivo. J. Inf. Dis., 151:* 1130-1137.