# Altered Response of Strain of *Schistosoma mansoni* to Oxamniquine and Praziquantel

**Patrícia Ivana Pires Bonesso–Sabadini, Luiz Cândido de Souza Dias+**

Departamento de Patologia Clínica, Faculdade de Ciências Médica, Universidade Estadual de Campinas, Caixa Postal 6111, 13083-970 Campinas, SP, Brasil

*The susceptibility of a fourth generation Ouh strain (Paranapanema Valley, São Paulo, Brazil) of* Schistosoma mansoni *to oxamniquine (OXA) and praziquantel (PZQ) was studied. Ten groups of 13 female albino mice each were infected with 70 cercariae per animal. These mice were medicated orally on the 50th day after infection. Five groups were given OXA doses of 0, 100, 200, 300 and 400 mg/kg (single doses) and the rest were treated with PZQ doses of 0, 100, 200, and 250 mg/kg/5 days. Each group was sub-divided: 8 animals underwent perfusion after 15 days treatment, 5 mice followed up for oviposition and their feces were tested every 15 days for miracidia hatching. The efficacy of the OXA doses of 100 and 200 mg/kg was 66% and 91.4%, respectively and for the 100 mg/kg PZQ dose it was 90.1%. The follow-up groups with 100 and 200 mg/kg of OXA and PZQ, 100 and 150 mg/kg, showed that they re-established the oviposition after a period of 60 to 75 days of treatment. The* $ED_{50}$ *was 69.6mg/kg OXA and 39.4 mg/kg PZQ. The results show the tolerance of the Ouh strain to a dose of 100 mg with both drugs and they appoint the need for a dose review during the follow up of the oviposition and in monitoring phenomena in the field.*

Key words: *Schistosoma mansoni* - susceptibility - praziquantel - oxamniquine - experimental chemotherapy

In Brazil, schistosomiasis mansoni is an important endemic disease that affects 11% of the country's territory (Silveira 1989). There are about 6.3 million Brazilians suffering from *Schistosoma mansoni* (Katz & Peixoto 2000). The studied strain was isolated in the State of São Paulo where there was a low endemic parasitic focus (Dias et al. 1994).

The chemotherapy used to treat intestinal schistosomiasis, today, is based on two drugs, oxamniquine (OXA) and praziquantel (PZQ). In control programs the parasitological cure with these drugs is generally satisfactory. Since 1976, OXA has been used on a large scale in control programs (Silveira 1989). About 10 million Brazilian *S. mansoni* carriers have been treated with this drug (Almeida Machado 1982). It has been reported human strains of *S. mansoni* that have altered their susceptibility (resistance and/or tolerance) to OXA (Katz et al. 1973, Dias et al. 1978). The tolerance to PZQ has been reported in Senegal (Stelma et al. 1995) and in Egypt (Ismail et al. 1996), and even in Brazil, where it is rarely used (Gomes et al. 1993, Araújo et al. 1996).

These findings support Kinoti's (1987) supposition that *S. mansoni* has a great capacity to develop resistance to therapeutic doses of a determined drug, especially when the parasitic population is under continuous pressure from schistosomicides (Coles & Bruce 1990).

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+Corresponding author. Fax: +55-19-3788.9434. E-mail: luizcan@fcm.unicamp.br

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It was not possible yet to identify genetic resistance markers using molecular techniques (Dias Neto et al. 1993, Araújo et al. 1996). Therefore experimental in vivo chemotherapeutic research is indispensable in evaluating the susceptibility of *S. mansoni* to schistomocides.

This study deals with in vivo chemotherapy of the human strain (Ouh) of *S. mansoni*. Gomes et al. (1993) showed that the first generation of this strain in mice is tolerant to OXA and PZQ and with 69.7% and 87.5% of efficacy respectively.

## MATERIALS AND METHODS

*S. mansoni strain* - The 4th generation Ouh human strain that was isolated in 1992 from 3 *S. mansoni* carriers was used (Gomes et al. 1993). These patients were from the Ourinhos county (Paranapenema Valley, State of São Paulo, Brazil) and had never been treated with schistosomicides. These patients were treated with PZQ in the Health Center.

*Definitive host* - Four week old female mice were used. They were individually infected, through the tail, with 70 cercariae of the 4th generation of the Ouh strain (Olivier & Stirewalt 1952).

*Intermediate host* - Three hundred specimens of *Biomphalaria glabrata*, melanic and sympatric to the strain were used. They were individually exposed to 5 miracidia (Standen 1952).

*Experimental groups* - Ten groups of 13 mice each were used in this study. Five groups were treated with OXA and the rest with PZQ. Each group had two subgroups – one with 8 mice that underwent perfusion after 15 days and the other of 5 mice that were maintained in order to follow up the oviposition of the trematode (follow-up group).

*Treatment* - The treatments started 50 days after infection using single doses of 100, 200, 300 and 400 mg/kg of OXA and 100, 150, 200 and 250 mg/kg/5 days of PZQ.

Both the drugs were administered in a 1% Cremophor EL aqueous suspension by oral gavage (Coles et al. 1986). Pure salt was used in both drugs. Two groups were kept as untreated control. Therefore, of the 10 groups, 4 groups were treated with different doses of OXA and 4 groups with different doses of PZQ.

*Follow-up groups* - An individual follow up was carried out for a year on 5 animals from each group. This was done by examining the feces every 15 days for the hatching of the miracidia (Standen 1952).

*Perfusion* - Fifteen days after treatment, the animals were sacrificed and portally perfused (Yolles et al.1947). After perfusion of the liver and mesentery, the worms were collected and placed in Petri dishes in a 0.85% saline solution. They were counted and classified according to their location, sex and vitality. After perfusion, the liver was crushed between glass plates to check for worms that may have been left behind during perfusion (Hill 1956).

*Evaluation of susceptibility* - The following parameters were used for evaluation: (a) the distribution of worms – the percentage of worms found in the intrahepatic veins was calculated in relation to the total number of worms collected. The worms found in the portal vein were considered as pertaining to the mesentery; (b) percentage efficacy (Kemp et al. 1956) – was determined by use of the following equation: (a - b)/a x 100, where a = average number of live worms recovered from untreated animals and b = number of live worms from treated animals; (c) percentage oogram change (Pellegrino & Katz 1968) – was calculated based on the number of mice with altered oogram. The oogram was considered altered when one of the five maturity degrees, based on the embryo size and morphology, was absent; (d) effective doses (ED) – dose response curves were generated where response was plotted as % efficacy on the y-axis against dose (total mg/kg) on the x-axis. The values of ED were obtained by observing the dose with intersected the curve dose (Drescher et al. 1993).

## RESULTS

A high rate of live worms (males, females, couples) (54.9%) were found when a dosage of 100 mg/kg OXA was used (Table I); of the total number of worms, 6.9% remained in the mesenteric veins after perfusion (Table II) and in the case of one animal, the oogram was found to be normal, with eggs in different stages.

When the dose of OXA was 200 mg the percentage of

TABLE I

Total number of worms recovered 15 days after treatment, in the oxamniquine (OXA) and praziquantel (PQZ) groups of mice infected with 70 cercariae each of the fourth generation Ouh strain of *Schistosoma mansoni*

| | | Number of | Worms collected | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Dose | mice | Live | | Total (live and dead) | | Mean number of live worms | | | |
| Drug | mg/kg | examined | N | % | N | Mean | Male | Female | Couples | Total |
| OXA | 0 | 7 | 245 | 98.4 | 249 | 35.6 | 7.4±5.2 | 5.3±3.5 | 11.1±6.4 | 34.9 |
| OXA | 100 | 8 | 95 | 54.9 | 173 | 21.6 | 2.0±2.9 | 9.4±5.4 | 0.3±0.5 | 12 |
| OXA | 200 | 7 | 21 | 23.3 | 90 | 12.8 | 0.0±0.0 | 3.0±3.8 | 0.0±0.0 | 3 |
| OXA | 300 | 8 | 0 | 0.0 | 146 | 18.2 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0 |
| OXA | 400 | 8 | 1 | 0.7 | 149 | 18.6 | 0.0±0.0 | 0.1±0.4 | 0.0±0.0 | 0 |
| PZQ | 0 X 5 | 4 | 134 | 100.0 | 134 | 33.5 | 5.8±4.6 | 3.3±3.6 | 12.3±8.4 | 33.7 |
| PZQ | 100 X 5 | 7 | 23 | 13.3 | 176 | 25.1 | 0.6±1.5 | 1.9±2.1 | 0.4±0.8 | 3.3 |
| PZQ | 150 X 5 | 5 | 4 | 2.4 | 169 | 33.8 | 0.2±0.4 | 0.2±0.4 | 0.2±0.4 | 0.8 |
| PZQ | 200 X 5 | 7 | 4 | 2.5 | 158 | 22.6 | 0.0±0.0 | 0.6±1.5 | 0.0±0.0 | 0.6 |
| PZQ | 250 X 5 | 4 | 5 | 5.3 | 94 | 23.5 | 0.0±0.0 | 1.3±1.5 | 0.0±0.0 | 1.3 |

OXA: single dose, orally; PZQ: 5 consecutive days of treatment, orally

TABLE II

Schistosomocidal activity of oxamniquine (OXA) and praziquantel (PZQ) in mice infected individually with 70 cercariae of the fourth generation Ouh strain of *Schistosoma mansoni*

| Drug | Dose | % of worms recovered | | % of dead | % of the alteration | Efficacy |
|---|---|---|---|---|---|---|
| | (mg/kg) | Liver | Mesentery | worms in the liver | of the oograms | (%) |
| OXA | 100 | 93.1 | 6.9 | 77.0 | 87.5 | 66.0 |
| OXA | 200 | 98.9 | 1.1 | 75.6 | 100 | 91.4 |
| OXA | 300 | 100 | 0 | 100 | 100 | 100 |
| OXA | 400 | 100 | 0 | 99.3 | 100 | 99.7 |
| PZQ | 100 | 96.6 | 3.4 | 86.9 | 83.3 | 90.1 |
| PZQ | 150 | 100 | 0 | 97.6 | 100 | 97.6 |
| PZQ | 200 | 100 | 0 | 97.5 | 100 | 98.2 |
| PZQ | 250 | 98.9 | 1.1 | 94.7 | 100 | 96.1 |

OXA: single dose, orally; PZQ: 5 consecutive days of treatment, orally

female worms was relatively high (23.3%) (Table I) and according to the oogram there was an interruption of the ovoposition in all the mice (Table II). The percentage of efficacy in the case of 100 mg/kg and 200 mg/kg doses was 66% and 91.4% respectively (Table II). Those groups of animals that were treated with 300 mg/kg and 400 mg/kg doses reported practically a total absence of live worms, which resulted in a 100% efficacy (Table II). The percentages of worms recovered were calculated according to live and dead worms combined. The number of dead worms in the liver was recovered by perfusion and crushing.

In the case of PZQ, when the dosage was 100 mg/kg/5days, the number of live worms (females, males and couples) was relatively high (13.3%) (Table I). When the dosage was 150 mg, 2.4% of live worms of all kinds were found but when the dosage was higher, it is important to note that only live female worms were found (Table I). Mice treated with 100 mg/kg showed the least efficacy (90.1%) and for the rest of the groups the efficacy was higher than 96.1% (Table II).

The follow up in the case of the 2 control groups, OXA (5 mice) and PZQ (5 mice), was possible for just 105 days as most of them died between the 90th and 105th day. The test showed the presence of fully developed trematode eggs.

Two groups of mice medicated with single doses of 100 mg/kg and 200 mg/kg of OXA regained oviposition. This took place 75 days after being medicated with 100 mg/kg OXA in one animal and the condition was maintained until the experiment terminated (360 days, follow-up). With a dosage of 200 mg/kg, the oviposition was recovery in two mice after 60 days and continued till the end. In the case of the remaining doses – 300 mg/kg and 400 mg/kg, the oviposition was not re-established.

In those groups treated with PZQ, the process of egg elimination through the feces was re-established in 2 mice, 60 days after receiving 100 mg/kg/5days and in 1 animal that was medicated with 150 mg/kg/5days and this condition was maintained for 360 days. The elimination of eggs through the feces did not take place in the animals medicated with 200 mg/kg/5days and 250 mg/kg/5days.

The effective doses ($ED_{50}$) were: a single dose of 69.6 mg/kg OXA and 39.4 mg/kg of PZQ for 5 consecutive days.

## DISCUSSION

Our results show that the susceptibility of the Ouh strain to OXA and PZQ has changed when compared to results obtained for Brazilian strains by other Brazilian authors (Katz et al. 1973, Dias et al. 1978, Araújo et al. 1996). Gomes et al. (1993) showed that efficacy of OXA and PZQ on the $F_1$ generation of the parasite was 69.7% and 87.5%, respectively. The doses used on mice were only 100 mg/kg for both drugs.

We studied the 4th generation of this same strain and the OXA and PZQ efficacy obtained was 66% and 90.1%, respectively (Table II). In the case of the Brazilian strains the efficacy is generally more than 95% and other parameters show a high susceptibility to these schistosomicides (Dias et al. 1982, 1988, Drescher et al. 1993). Recently, Araújo et al. (1996) studied ten isolates of *S. mansoni* derived from patients in an endemic area in Bahia, Brazil. They were treated with OXA and later with PZQ and were not parasitologically cured. The in vivo therapeutic response of these isolated types was significantly different among them, but not enough to be able to characterize any of them as being resistant. The authors reported that a single dose of 100 mg/kg OXA showed an efficacy between 48% to 92.4% and a single dose of 400 mg/kg PZQ an efficacy between 30.4% to 90.2%.

It is important to stress the fact that we used a therapeutic scheme that consisted of curative doses administered when the worms were at the adult stage (50 days old). This condition is generally found in regions where the endemicity of schistosomiasis mansoni is low. Situations in which mice with younger worms (35 to 37 days after infection) were treated with sub-curative doses of schistosomicides (Fallon et al. 1997) did not show to represent the reality observed in the case of human in the field.

It was observed that perfused animals had adult live worms (males, females and couples) (Table I) during therapeutic treatment with OXA and PZQ, especially when the dosage was 100 mg/kg/single dose and 100 mg/kg/5 days.The male is more susceptible to the 2 drugs than the female, which explains the survival of a larger number of females (Goldberg et al. 1980, Popiel & Erasmus 1982).

Recovery of the oviposition of the worms that survived after treatment (60 to 75 days) was faster than that shown by Rogers and Bueding (1971), which was a period of 6 to 12 months in the case of the *S. mansoni* strain in which resistance to hycanthone was induced. The period was more than 100 days for adult trematode worms in mice treated with sub-curative doses of PZQ (Shaw & Erasmus 1987). This distinct behavior of our strain could be related to the care taken in using a recently isolated field helminth, thus maintaining the original characteristics that are usually lost when strains are kept in the laboratory for a long time.

The ED of OXA that killed 50% of the worms was a single dose of 69.6 mg/kg given orally. This value was 34 mg/kg greater than that found by Drescher et al. (1993) for the BH strain (Belo Horizonte, Brazil) but was closer to the value of 62 mg/kg obtained for the K strain (Kenya). Both strains are considered sensitive to OXA. In relation to the resistant strains to this drug, the values obtained by the above authors were 320 mg/kg for MAP (Belo Horizonte, Brazil) and 512 mg/kg for MPR-1 (Porto Rico). Araújo et al. (1996) worked on 10 isolates obtained from patients who were treated and not cured by OXA and PZQ, found that in mice the $ED_{50}$ varied between 30 mg/kg to 100 mg/kg with OXA administered in single dose.

In the case of PZQ, using 100 mg/kg/5 days we obtained an $ED_{50}$ of 39.4 mg/kg. Drescher et al. (1993) worked with the strains BH, K, MAP and MPR-1, previously mentioned and obtained an $ED_{50}$ of 42, 36, 13 e 46 mg/kg/5 days, respectively, by oral route. Araújo et al. (1996) worked with single doses of PZQ obtained in $ED_{50}$ that varied between 92 mg/kg and 640 mg/kg. Bennett et al. (1997) worked with isolates from infected Egyptian villagers, that in the field tolerate high doses of PZQ and could not be cured after 3 doses, obtained $ED_{50}$ values ranged between 123 to 680 mg/kg in a single dose.

When studying the susceptibility of *S. mansoni*, the following factors have to be observed: identifying the drug pressure, using curative dosage when the worms are adults, follow up of the oviposition, changes in the oogram and parameters like percentage of efficacy and effective doses. Taking into consideration the above points, the Ouh strain showed a change in susceptibility to OXA and PZQ. This degree of susceptibility shown by the mice to the two drugs and the recovery of eggs strengthens the preoccupation about an increase of insensitivity in the field (Cioli 2000).

Faced with these problems, the doses for both drugs have to be reviewed, the Ouh sub-strains have to be isolated using drug pressure and new schistomocides have to be tested (Penido et al. 1994). It is important to study the possible alterations at a genomic level of this strain to PZQ in comparison to another that is sensitive or resistant to OXA/hycanthone (Brindley et al. 1991, Pica-Mattoccia et al. 1993, Cioli 2000).

As a result of our findings and of those that showed that the strains of *S. mansoni* were tolerant and/or resistant to PZQ (Gomes et al. 1993, Stelma et al. 1995, Ismail et al. 1996, Fallon et al. 1997), there should be rigorous monitoring of these phenomenona (Cioli 2000). It is clear that only two drugs, OXA and PZQ are available for use in mansonic schistosomiasis control programs. We should remember that chemotherapy is just one of the means used in reducing infection and morbidity. Long term measures like education, sanitation, permanent primary health care and safe water supply should also be provided.

## REFERENCES


Almeida Machado P 1982. The Brazilian program for schistosomiasis control. *Am J Trop Med Hyg 31*: 76-86.

Araújo N, Souza SP, Passos LKJ, Simpson AJG, Dias Neto E, Pereira TR, Cerutti Jr C, Alencar FEC, Dietze R, Katz N 1996. Suscetibilidade aos agentes quimioterápicos de isolados de *Schistosoma mansoni* oriundos de pacientes tratados com OXA e PZQ e não curados. *Rev Soc Bras Med Trop 29*: 467-476.

Brindley PJ, Heath S, Waters AP, McCutchan TF, Sher A 1991. Characterization if a programmed alteration in a 18S ribosomal gene that accompanies the experimental induction of drug resistance in *Schistosoma mansoni*. *Proc Natl Acad Sci USA 88*: 7754-7758.

Bennett JL, Day T, Feng-Tao L, Ismail M, Farghaly A 1997. The development of resistance to anthelmintics: a perspective with an emphasis on the antischistosomal drug PZQ. *Exp Parasitol 87*: 260-267.

Cioli D 2000. PZQ: is there real resistance and are there alternatives? *Curr Opin Infect Dis 13*: 659-663.

Coles GC, Bruce JI 1990. Resistance in Schistosoma. Round Table Conference. VII International Congress of Parasitology, Paris, August, p.1-12.

Coles GC, Bruce JI, Kinoti GK, Mutahi WT, Dias EP, Katz N 1986. Drug resistance in schistosomiasis. *Trans R Soc Trop Med Hyg 80*: 347.

Dias LCS, Glasser CM, Marçal Jr O, Bonesso PIP 1994. Epidemiologia da esquistossomose mansônica em área de baixa endemicidade. *Cad Saúde Públ 10* (Suppl. 2): 254-260.

Dias LCS, Pedro RJ, Deberaldini ER 1982. Use of PZQ in patients with schistosomiasis mansoni previously treated with OXA and/or hycanthone: resistance of *Schistosoma mansoni* to schistosomicidal agents. *Trans R Soc Trop Med Hyg 76*: 652-659.

Dias LCS, Pedro RJ, Rigo E, Goto MM, Mafra GL 1978. Linhagem humana de *Schistosoma mansoni* resistente a esquistossomicidas. *Rev Saúde Pública 12*: 110.

Dias LCS, Bruce JI, Coles GC 1988. Strain variation in the infectivity of *Schistosoma mansoni* for *Biomphalaria glabrata*. *Rev Inst Med Trop São Paulo 30*: 86-90.

Dias Neto E, Souza CP, Rollinson D, Katz N, Pena SDJ, Simpson AJG 1993. The random amplifiation of polymorphic DNA allows the identification of strains and species of schistosome. *Mol Biochem Parasitol 57*: 83-88.

Drescher KM, Rogers EJ, Bruce JI, Katz N, Dias LCS, Coles GC 1993. Response of drug resistant isolates of *Schistosoma mansoni* to antischistosomal agents. *Mem Inst Oswaldo Cruz 88*: 89-95.

Fallon PG, Mubarak JS, Fookes RE, Niang M, Butterworth AE, Sturrock RF, Doenhoff MJ 1997. *Schistosoma mansoni*: maturation rate and drug susceptibility of different geographic isolates. *Exp Parasitol 86*: 29-36.

Goldberg M, Gold D, Flescher E, Lengy J 1980. Effect of OXA on *Schistosoma mansoni*: some biological and biochemical observations. *Biochem Pharmacol 29*: 838-840.

Gomes E, Dias LCS, Takaku L, Stangenhaus G 1993. Biological and morphological characteristics of *Schistosoma mansoni* from the Paranapanema Valley (Ourinhos), São Paulo, Brasil. IV International Symposium on Schistosomiasis, p. 51.

Hill J 1956. Chemotherapeutic studies with laboratory infections of *Schistosoma mansoni*. *Ann Trop Med Parasitol 56*: 39-48.

Ismail M, Metwally A, Farghaly A, Bruce J, Tao L-F, Bennett JL 1996. Characterization of isolates of *Schistosoma mansoni* from egyptian villagers that tolerate high doses of PZQ. *Am J Trop Med Hyg 55*: 214-218.

Katz N, Peixoto SV 2000. Análise crítica da estimativa do número de portadores de esquistossomose mansoni no Brasil. *Rev Soc Bras Med Trop 33*: 303-308.

Katz N, Dias EP, Araújo N, Souza CP 1973. Estudo de uma cepa humana de *Schistosoma mansoni* resistente a agentes esquistossomicidas. *Rev Soc Bras Med Trop 7*: 381-387.

Kemp HA, Hunter GW, Wilkins OP, Smalley H, Dashiel MA 1956. Some ointments examnined for protection against *Schistosoma mansoni* cercarie in preliminary tests. *Milit Med 119*: 1-10.

Kinoti GK 1987. The significance of variation in the susceptibility of *Schistosoma mansoni* to the antischistosomal drug OXA. *Mem Inst Oswaldo Cruz 82* (Suppl. IV):151-156.

Olivier L, Stirewalt MA 1952. An efficient method for exposure of mice to cercariae of *Schistosoma mansoni*. *J Parasitol 38*: 19-23.

Pellegrino J, Katz N 1968. Experimental chemotherapy of schistosomiasis mansoni. *Adv Parasitol 6*: 233-290.

Penido MLO, Nelson DL, Vieira LQ, Coelho PMZ 1994. Schistosomicidal activity of alkylaminooctanethiosulfuric acid. *Mem Inst Oswaldo Cruz 89*: 595-602.

Pica-Mattoccia L, Dias LCS, Moroni R, Cioli D 1993. *Schistosoma mansoni*: genetic complementation analysis shows that two independent hycanthone/OXA-resistant strains are mutated in the same gene. *Exp Parasitol 77*: 445-449.

Popiel I, Erasmus DA 1982. *Schistosoma mansoni*: the survival and reproductive status of mature infections in mice treated with OXA. *J Helminthol 56*: 257-261.

Rogers SH, Bueding E 1971. Hycanthone resistance: development in *Schistosoma mansoni*. *Science 172*: 1057-1058.

Shaw MK, Erasmus DA 1987. *Schistosoma mansoni*: structural damage and tegumental repair after *in vivo* treatment with PZQ. *Parasitology 94*: 243-254.

Silveira AC 1989. Controle da esquistossomose no Brasil. *Mem Inst Oswaldo Cruz 84* (Suppl. I): 91-104.

Standen OD 1952. Experimental infection of *Australorbis glabratus* with *Schistosoma mansoni*. I. Individual and mass infection of snails, and the relationship of infection to temperature and season. *Ann Trop Med Parasitol 46*: 48-53.

Stelma FF, Talla I, Sow S, Kongs A, Niang M, Polman K, Deelder AM, Gryseels B 1995. Efficacy and side effects of PZQ in an epidemic focus of *Schistosoma mansoni*. *Am J Trop Med Hyg 53*: 167-170.

Yolles TK, Moore PV, Deginsti DL, Ripson CA, Meleney HE 1947. A technique for the perfusion of laboratory animals for the recovery of schistosomes. *J Parasitol 33*: 419-426.