Cross-Resistance of Trichomonads to 5-Nitroimidazole-Derivatives

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Summary. The strain *T. foetus* KV 1/M 100, a sub-strain of *T. foetus* KV 1, was made highly resitant to metronidazole by several passages in mice under increasing drug pressure. This drug resistance was accompanied by a remarkable cross-resistance to all 5-nitroimidazole-derivatives tested in vitro and in vivo.

Experiments have shown that trichomonads can develop, under certain circumstances, drug resistance to metronidazole, a 5-nitroimidazole-derivative[1–6]. As the chemotherapy of trichomonads depends at present almost exclusively on the use of 5-nitroimidazole-derivatives, we were interested to establish to what extent cross-resistance to these systemically effective chemotherapeutics can be expected if resistance to metronidazole develops. In order to clear up this question, we carried out chemotherapeutical experiments in vivo and in vitro with a strain of *Tritrichomonas foetus* resistant to metronidazole, with special reference to the following 5-nitroimidazole-derivatives[7]:

1. 1-(2-Hydroxyethyl)-2-methyl-5-nitroimidazole (Metronidazole).
2. 1-(2-Hydroxy-3-chloropropyl)-2-methyl-5-nitroimidazole (Ornidazole).
3. 1-(2-Ethylsulfonyl)ethyl-2-methyl-5-nitroimidazole (Tinidazole).
4. 1-(N-2-morpholino-ethyl)-5-nitroimidazole (Nitrimidazine).
5. 1-(2-(γ-Pyridyl)ethyl)-2-methyl-5-nitroimidazole (Panidazole).
6. 1-(2-Hydroxyethyl)-2-(*p*-fluorophenyl)-5-nitroimidazole (Flunidazole).
7. 1-Methyl-2-(*p*-fluorophenyl-5-nitroimidazole (MK-910).
8. 1-Methyl-2-carbamoyloxymethyl-5-nitroimidazole (Ronidazole).
9. 1,2-Dimethyl-5-nitroimidazole (Dimetridazole).

Table I. Comparison of the efficacy of several 5-nitroimidazole-derivatives against a metronidazole-sensitive (KV 1) and a metronidazole-resistant (KV 1/M 100) strain of *T. foetus* in vivo (mouse)

Substance	DC_{50}[a] (mg/kg)[b]		Rf[c]
	KV 1	KV 1/M 100	
Metronidazole	11.7	>800	>68
Ornidazole	6.5	180	28
Tinidazole	3.5	140	40
Nitrimidazine	24.3	>800	>33
Panidazole	7.9	137.5	17
Flunidazole	12.1	302	25
MK 910	2.5	80	32
Ronidazole	1.7	42	25
Dimetridazole	7.5	200	27

[a] DC_{50} derived from 2 experiments with 6 animals at each dosage. [b] 2, 18, 24 h p. i. [c] Resistance factor (DC_{50} – KV 1/M 100: DC_{50} – KV 1).

Table II. Comparison of the efficacy of several 5-nitroimidazole-derivatives against a metronidazole-sensitive (KV 1) and a metronidazole-resistant (KV 1/M 100) strain of *T. foetus* in vitro

Substance	MLC (µg/ml)		Rf[a]
	KV 1	KV 1/M 100	
Metronidazole	1.6	100	63
Ornidazole	3.1	50	16
Tinidazole	0.8	12.5	16
Nitrimidazine	1.6	50	31
Panidazole	0.4	12.5	31
Flunidazole	0.4	12.5	31
MK 910	0.1	3.1	31
Ronidazole	0.4	6.3	16
Dimetridazole	0.8	25	31

[a] Resistance factor (MLC – KV 1/M 100: MLC – KV 1).

Methods. 1. Induction of metronidazole-resistance in *T. foetus* in vivo. In order to develop a resistant strain of *Tritrichomonas foetus* KV 1[8], *T. foetus* was passaged through mice under increasing drug pressure. For this purpose, female NMRI-mice weighing 12–15 g were infected intraperitoneally with 8×10^5 trichomonads and treated orally with metronidazole 2, 18 and 24 h after infection. As soon as the animals showed the characteristics of the disease, the experiment was interrupted to prepare the inoculum from the abdominal exudate containing the trichomonads. Starting with 2.5 mg/kg body weight, the dosage was increased by 2.5 mg/kg body weight at each passage. After the 40th passage, in which the trichomonads tolerated 3×100 mg/kg body weight of metronidazole, the strain, now designated KV 1/M 100 was cultivated continuously without drug pressure in vitro in fluid thioglycollate medium (FTG/BBL). Over a period of 60 weeks, during which more than 170 drug-free passages in vitro were made, we checked the degree of resistance to metronidazole several times in vitro and in vivo. Up to now, the strain KV 1/M 100 has been completely stable in its resistance to metronidazole in vitro as well as in vivo.

2. In-vitro test. The determination of the trichomonacidal effect of the compounds in vitro was based on the determination of the minimal lethal concentration (MLC) in a serial dilution test. The lowest concentration in which no living, i.e. motile, trichomonads in a 48-hour-

[1] R. Samuels, J. Protozool. *8*, 5 (1961).
[2] B. M. Honigberg and M. C. Livingston, in *Proc. First Int. Congress of Parasitology* (Ed. A. Corradetti; Pergamon Press, Oxford 1966), vol. 1, p. 365.
[3] I. de Carneri, in *Proc. First International Congress of Parasitology* (Ed. A. Corradetti; Pergamon Press, Oxford 1966), vol. 1, p. 366.
[4] P. Actor, D. S. Ziv, J. F. Pagano, Science *164*, 439 (1969).
[5] F. Benazet and L. Guillaume, Lancet *2*, 982 (1971).
[6] I. de Carneri and F. Trane, Arzneimittel Forsch. (Drug Res.) *21*, 377 (1971).
[7] Compounds not available on the market were kindly supplied by the corresponding pharmaceutical firms.
[8] The strain KV 1 was kindly provided by J. Kulda, Prague, 1970.

FTG-culture containing an inoculum of 6×10^5 microorganisms/ml could be detected, was regarded as the MLC.

3. In-vivo test. Female NMRI-mice weighing 10–15 g were treated orally 2, 18 and 24 h after intraperitoneal infection with 8×10^5 trichomonads of a 24 hour culture. The results were evaluated 5 days after the last treatment. The efficacy of the compounds was determined by the presence or absence of trichomonads in the abdominal cavity, as tested by re-isolation.

Results. The comparative analyses of the efficacy of different 5-nitro-imidazole-derivatives against the *T. foetus*-strains KV 1 and KV 1/M 100 in vivo and in vitro are summarized in Tables I and II. It can be seen (Table I) that the strain KV 1/M 100 is both resistant to metronidazole and also shows different decrease of sensitivity to all 5-nitroimidazole-derivatives. Differences are revealed by comparison of their resistance factors. As far as metronidazole and nitrimidazine are concerned, these factors represent, however, only a minimal value, as we have not determined the DC_{50} owing of the high dosages necessary.

Similar results were obtained from the experiments carried out in vitro (Table II). Here also the strain KV 1/M 100, proved less sensitive to metronidazole, showed to be less sensitive to all 5-nitroimidazole-derivatives in comparison with the strain KV 1.

Discussion. A comparison of the efficacy of several 5-nitroimidazole-derivatives against the metronidazole resistant strain *T. foetus* KV 1/M 100 shows that an extremely high metronidazole resistance is accompanied by a clearly marked cross-resistance to all 5-nitroimidazole-derivatives studied in the experiments. This corresponds to the findings of McLOUGHLIN[9], who not only observed a cross-resistance of a dimetronidazole resistant *T. foetus* strain to metronidazole, but also to aminitrazole, a nitrothiazole derivative. It confirms also the findings of BENAZET and GUILLAUME[5], who, by using both a metronidazole-resistant and a nitrimidazine-resistant strain of *T. vaginalis*, described a complete cross-resistance between these two 5-nitroimidazole-derivatives. On the other hand, CARNERI[10] found only a slight cross-resistance between these two drugs on testing nitrimidazine, using different metronidazole-resistant strains of *T. vaginalis*. This phenomenon might be related to the degree of metronidazole resistance of the strains used. Both our experiments and those of BENAZET and GUILLAUME[5] have been carried out with highly metronidazole-resistant strains with factors of > 68 and > 48 respectively, while the resistance factors of the strains used by CARNERI[10] were in the range of 1.5 to 12.1.

Under in-vitro conditions, the strain KV 1/M 100 was also found to be cross resistant to different degrees to all 5 nitroimidazole-derivatives tested. Here, as under in-vivo conditions, the strain KV 1/M 100 demonstrated the highest resistance factor to metronidazole. However, we did not find an exact correlation between the factors of resistance observed in vitro and those found in vivo. Such a correlation could hardly be expected, as the results of the experiments in vivo are influenced qualitatively and quantitatively by the specific pharmacokinetics and biotransformation of the drugs in animals.

[9] D. K. McLOUGHLIN, J. Parasit. *53*, 646 (1967).
[10] I. DE CARNERI, Trans. R. Soc. trop. Med. Hyg. *65*, 268 (1971).

The Problem of Detecting a Free Glutamate Decrease in the Dorsal Sensory Neuron Following Dorsal Root Crush[1]

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Summary. Dorsal root crush results in a highly significant decrease in the free glutamate/total free amino acid concentration ratio over an extended time period. Perhaps this is a good measurement to use in glutamate crush studies.

Since glutamate is a potent spinal cord excitant[2], and free glutamate levels are higher in the dorsal root than in the ventral root[3–5] or distal sensory root[4,5], this amino acid is presently a transmitter candidate at the dorsal root terminals[3,6]. In analogy with the cholinergic transmitter system where nerve section or crush has led to definite changes in the regulatory enzymes[7,8] or in the acetylcholine level[9], it has been tested whether this would be true for the glutamate content of the dorsal root after such injury[10–12]. Although there is a decrease in free glutamate per g tissue in the root following injury, neither proximally[12] nor distally[10,11] is this decrease significant. This can be interpreted to support any one of three alternative possibilities: 1. glutamate is not a transmitter candidate here, 2. the glutamate transmitter system is different from the cholinergic system in this respect, or 3. the usual means of expressing such amino acid data has not been able to uncover any significant free glutamate change. The purpose of this study is to analyze free glutamate changes and total free amino acid changes proximal and distal to a dorsal root crush in order to discern if there is any way to detect a significant free glutamate change as a result of the crush. Such a study has not been carried out using several time periods over the time when maximal crush effects should be seen[8].

Adult cats (3.5–4.2 kg) were anesthetized with sodium pentobarbital (Nembutal, 35 mg/kg) and the lumbosacral spinal cord and roots were exposed. The dura was carefully cut rostrocaudally in order to expose the dorsal roots of L7 and S1. The rootlet bundles of L7 and S1 on one side were crushed with a fine forceps 13 mm from the cord. The dura was then stitched and the skin was closed with wound clips after stitching the underlying fascia. Skin-Hesive liquid skin cement (United Surgical) was applied to the wound area to facilitate complete closure. 300,000 units of procaine Penicillin-G were given i.m. to each animal. After times ranging from 2 to 25 days after crushing, the cat was sacrificed and the root and ganglionic tissue of L7 and S1 were removed rapidly and frozen in isopentane in dry ice. The 13 mm segment of