Comparative studies on the trichomonacidal activity of 5-nitroimidazole-derivatives in mice infected s.c. or intravaginally with T. vaginalis[1]

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Summary. The trichomonacidal efficacy of metronidazole, tinidazole, nimorazole and ornidazole was studied in mice infected s.c., or for comparison purposes intravaginally. In both test systems, the drugs revealed nearly the same order of efficacy, whereby the compounds showed a marked decrease of activity when analyzed in s.c. infected mice.

In a previous paper it was shown that ovariectomized and estrogen- premedicated rats could be infected with Trichomonas vaginalis intravaginally with more reliability when the animals were infected first with Candida albicans topically[2]. This double infection, which, with certain modifications, was found to be applicable to mice also, turned out to be a further useful model for chemotherapeutic tests.

The efficacy of metronidazole, tinidazole, nimorazole and ornidazole was studied in the established model: mouse – T. vaginalis/s.c. infection and for comparison purposes in this new testing technique described in detail subsequently.

Methods. 1. S.c. infection. Female NMRI mice weighing 10–13 g were s.c. infected with 4×10^5 T. vaginalis Δ 2, in each shaved flank. The trichomonads from an over-night culture in CACH-medium[3] were concentrated by centrifugation and resuspended in 0.15–0.2 ml supernatant per inoculum. For each experiment, samples of the axenic stock culture (which was transferred 23 times after isolation before storage in liquid nitrogen) were thawed and passaged 4 times before infection. Systemic treatment was started orally 2 h after the infection and was repeated at 18 and 24 h p.i. The experiments were terminated 6 days after infection. The absence of lesions and microscopically detectable motile trichomonads were used as the criteria by which the activity was measured. The compounds were dissolved in 10% DMSO/0.2% CMC stock solutions followed by a further 2fold dilution with 0.2% CMC solution only. The drugs were given in volumes of 0.1 ml per 10 g b.wt to give concentrations of 50–0.4 mg/kg.

2. Intravaginal infection. NMRI mice 25–30 g in weight were pretreated once with 40 mg/kg estradiolundecylate (Progynon Depot®, Schering AG) in 0.2 ml sesame oil 3 days before infection. The estrogen was administered in two equal doses s.c. and i.p. The inoculum per mouse consisted of 0.05 ml CACH-medium containing approximately 1×10^5 T. vaginalis Δ 2 which was grown as outlined above, and Candida albicans. 1000 IU Na-penicillin G and 1000 µg streptomycin sulfate per ml inoculum were also added. Candida albicans Δ 124 was grown in SAB-broth for 24 h at 30 °C and stored with 5% DMSO in liquid nitrogen[4]. (The concentration of C. albicans in the SAB-culture was about 3.6×10^7/ml.) The thawed yeasts were added to the concentrated flagellates in a ratio of 1:80 v/v immediately before the infection of the animals. The vaginal cavity was stuffed with a plug of sponge (Spongostan®, Ferrosan) to avoid loss of the inoculum. The therapeutic method and the preparation of the compounds were the same as used in s.c. infected mice. The estimation of vaginal infection was based on cultural findings. The vagina of each infected animal was rinsed 4 days p.i. with culture medium which was transferred to tubes containing CACH-medium and a final concentration of 1000 IU Na-penicillin G, 1000 µg/ml streptomycin sulfate and 10 IU nystatin/ml. The seeded tubes were incubated at 37 °C and after 48 h observed for motile trichomonads on an inverted microscope. The efficacy of the compounds was determined by the presence/absence of trichomonads in the tubes.

Results and discussion. The results of the comparative chemotherapeutic studies are summarized in the table. The data presented indicate that all compounds tested are systemically active against T. vaginalis in mice infected intravaginally. By comparison the compounds showed a marked decrease in efficacy when analyzed in s.c. infected mice, although the evaluation was based on native observations only and not on cultural findings which are more reliable. In both test systems, the drugs revealed nearly the same order of efficacy.

The compounds tested are registered drugs for the treatment of trichomoniasis in man. Therefore an activity in mouse models, which are commonly used for screening programmes, was expected. The different degrees of efficacy in both models may be explained by the different locations of the trichomonads in the animal host as well as by the altered physiological conditions of the estrogen treatment. The effect of mixed T. vaginalis/Candida infections on their drug sensitivity requires further studies. However, it has been observed that the degree of efficacy of nimorazole differs far less from that of metronidazole and ornidazole in intravaginally infected mice than in ectopically infected animals; the activity of nimorazole ascertained in ectopically infected animals is only about $^1/_3$ of that of metronidazole, which is in agreement with

Efficacy of several 5-nitroimidazole-derivatives against topical and ectopical infections with T. vaginalis in mice

Substance	Dosage (mg/kg × 3[a])	Model: T. vaginalis inoculation site intravaginally[b]	s.c.[c]
Metronidazole	ED_{50}[d]	3.71 (2.76–4.99)	10.95 (9.10–13.18)
Tinidazole	ED_{50}	1.41 (1.02–1.95)	7.50 (6.19–9.10)
Nimorazole	ED_{50}	5.62 (4.15–7.61)	33.95 (30.62–37.73)
Ornidazole	ED_{50}	4.57 (3.60–5.82	10.59 (9.07–12.37)

[a] 2, 18 and 24 h p.i. orally. [b] 3 × 9 animals at each dosage level, mean infection rate of untreated control groups 94%. [c] 3 × 6 animals at each dosage level, infection rate of untreated control groups 100%. [d] ED_{50} according to Spearman-Kärber: figures in brackets are 95% confidence limits.

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the results of Carneri et al.[5], a fact which was attributed to the pharmacokinetical peculiarities of the drugs in mice and rats. The authors reported that the active fraction of metronidazole in mouse urine was more than twice that of nimorazole, whereas in rats and man the opposite is true. They thus considered that the mouse was not particularly suitable for assessing the therapeutic properties of nimorazole in human infections. This supposition to some extent contradicts the present as well as previous results[6], since the compounds show similar activity to metronidazole in rats as well as in mice, if they are infected intravaginally. The degree of systemic efficacy of nimorazole in the experimental animal does not correlate exactly with the activity in the urine.

Tinidazole was the most effective drug in both models and showed a clear superiority in the new one. These results correlate closely with the dosage of tinidazole used in practice, which are lower than those of the other drugs[7] recommended for a 7-day-course treatment of trichomoniasis. These findings underline the suitability of the intravaginal test systems.

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Effects of 5-bromodeoxyuridine and 2-aminopurine on antheridium differentiation in Anemia phyllitidis L.

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Summary. Both 5-BUdR and 2-Ap cause AG/TC transitions during DNA replication or transcription. However their effects on differentiation of antheridia in the fern Anemia phyllitidis differ totally. Since 5-BdU causes pattern simplification, 2-Ap leads to suppression of correlative cell interactions. This results indicate different targets for this mutagenic compounds in Anemia-DNA.

Gibberellins substitute for the native antheridiogens of the fern Anemia phyllitidis[1,2]. Addition of these phytohormones to culture media causes the premature differentiation of male sexual organs (antheridia)[3]. Although the hormonal induction of cell differentiation is not blocked by inhibitors of protein and nucleic acid synthesis, 5-bromodeoxyuridine (BUdR) has a striking influence on the manifestation of the antheridial pattern[4]. Identical effects are obtained with 5-iododeoxyuridine and 5-bromodeoxycytidine. Addition of these analogues to the culture medium (2×10^{-5} M–5×10^{-4} M) gives rise to the formation of simplified sexual organs. With high inhibitor concentrations, or after long times of application, the antheridia finally resemble vegetative chloronemata (Figure 1 a, b). As in various animal cells[5], the inhibition of cell differentiation precedes the inhibiting effect on rate of cell division.

The presence of BUdR in total DNA of prothallia of Anemia phyllitidis fed with ^{14}C-labelled BUdR was proved by Koop[6]. Since these effects of BUdR on anther-

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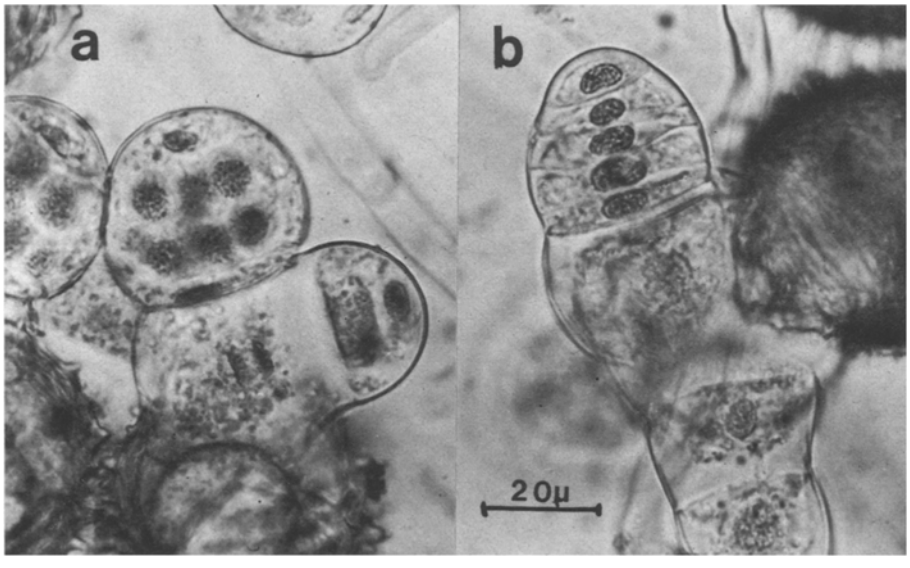

Fig. 1. Effect of BUdR on antheridium differentiation of light grown prothallia of Anemia phyllitidis. a) Control; 10^{-5} g/ml Gibb A_3; b) 2×10^{-5} M BUdR; 10^{-5} g/ml Gibb A_3. 10 d; 20 °C; continuous light ($1{,}2 \times 10^3$ erg cm^{-2} sec^{-1}).