MORPHOLOGICAL CHANGES AFTER TREATMENT OF BOVINE CYSTICERCOSIS WITH DRONCIT AND OXICHLORON

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Abstract. Droncit and Oxichloron in total doses of 100 (50 mg/kg for two days) and 300 mg/kg (100 mg/kg for three days) of body weight were applied for the treatment of cysticercosis 12 (Droncit) and 6 (Oxichloron) weeks after experimental infection. Four or six weeks after the treatment dead cysticerci or their remnants were found. There occurred a striking vacuolization of the larval tegument, but even six weeks after the treatment, muscles, portions of subtegumental cells and remnants of excretory canaliculer system and microtriches were discernible. The structure of microtriches was still well preserved in some cases. The dominating component of tissue reaction around the dead larvae was the hyperplasia of lymphoid tissue forming sometimes folliculoid structures, and blastic transformation of lymphocytes. Nodules with dead larvae were separated from the surrounding tissue by connective tissue.

According to our experience, the intravital detection of spontaneous bovine cysticercosis by means of serological tests is not quite reliable, though some of the tests can be successfully used for the diagnosis of experimental infection (Bezubik and Machnicka 1970, Walther and Grossklaus 1979, Albert and Hörchner 1978, Hiepe and Buchwalder 1978, Walther and Sanitz 1979, Blažek et al. 1980). The weak infections, however, are most important in the epidemiology of this disease. They can easily escape attention during meat inspection at the abattoir and thus become a source of man infection. The effort to interrupt the infective cycle by cestodocids is therefore justified in some cases. During the last years, Praziquantel (Droncit, Bayer) was found to be highly effective against Taenia saginata larva and in the dose of 100 mg/kg of body weight it produced the death of all cysticerci (Thomas and Gönnert 1977, 1978, Heath and Lawrence 1978, Pawlowski et al. 1978, Walther 1978, Walther and Grossklaus 1979). However, the tissue reaction accompanying the death of the cysticercus has not yet been described in the literature. It has been only stated that the resorption of dead parasites occurs slowly and that 97 days (Hörchner and Albert 1979) or even 20 weeks (Pawlowski et al. 1978) after treatment the muscle tissue contains nodules or detectable residua and the meat of treated animals must be rejected.

The aim of the present work was to detect what is the tissue reaction to dead cysticercus after the treatment with Droncit (= Praziquantel, Bayer) and Oxichloron (developed in the USSR).

MATERIAL AND METHODS

For the experiments with Droncit (Praziquantel, Bayer AG Leverkusen, 2-cyclohexyl-carbonyl-1, 3, 4, 6, 7, 11 b — hexahydro-2H — pyrazine (21-a) isoquinolin — 4) 16 calves at the age of 4—5 months were used. Five of them (4 experimental and 1 control animal) were examined histologically and electron microscopically. The calves were infected perorally with 5,000 eggs of Taenia saginata. Twelve weeks after infection four of them were given Droncit perorally for two days, daily dose being 50 mg/kg of body weight (total dose 100 mg/kg). Four weeks after treatment the calves were killed together with the control animal.
The experiments with Oxichloron (belonging to the group of aromatic phenols, produced by VIGIS, Moscow, 2,2'-diox - 3,3; 5,5'-tetrachlordifenylsulfon) were performed using 20 calves at the age of two months. Four of them (3 experimental and 1 control animal) were examined. The calves were given perorally Oxichloron for three consequent days in the daily dose of 100 mg/kg of body weight (total dose 300 mg/kg). Both experimental and control animals were killed 6 weeks after treatment.

Half of the trunk and inner organs of each experimental animal were cut in 0.5 cm thick sections, the cysticerci were counted and their state and vitality evaluated after immersion into bile. For histological examination, cysts were taken from various muscle groups and from heart, they were fixed for 1 hour in Baker's fixative and in 10 % formaline and then embedded into paraffin using a common technique. The sections were stained by haemalaun-eosin and van Gieson method.

Samples for electron microscopical examination were fixed as mentioned above and additionally fixed in 2 % osmium tetroxide in 0.01 M cacodylate buffer at room temperature and embedded in Vestopal by a common technique. Ultrathin sections contrasted by uranyl acetate and Reynold's solution were examined in Jeol 100 B electron microscope.

RESULTS

Both Droncit and Oxichloron in the used doses caused the death of all cysticerci and the foci with dead cysticerci were almost identical. At the time of histological examination, i.e., four and six weeks after the treatment, the nodular affections contained either dead cysticerci with a rather well discernible and undamaged structure or their single parts. The bladder wall was swollen and oesinophilic (Plate I, Figs. 1, 2). The microtriches were mostly invisible, the subtegumental cells were shadowy and the bladder wall contained deposits of calcium phosphate. Muscle bundles were distinctly visible in the subtegumental region of larva in some cases. However, sometimes the oesinophilic bladder wall was warped and without any structure or only little distinct structureless remnants were found in the nodule. Also scolex exhibited the signs of disintegration. Sometimes there were visible even the suckers (Plate I, Fig. 4), shadowy spiral canal and calcareous bodies, but other times, only the calcareous bodies and fragments of completely calcified tissue were preserved (Plate I, Fig. 3).

In some cases, extensive deposits of calcium salts were encountered in the still well discernible scolex and bladder wall. The deposition of calcium phosphate occurred also in the reduced bladder cavity where eosinophiles accumulated as well. Exceptionally (Droncit 1×) the bladder of cysticercus with microtriches was found in histological sections, whereas in serial sections, necrotic foci were found in the remaining part of bladder and in scolex.

In the electron microscopical picture the bladder tegument was strongly osmiophilic, without microtriches or with their remnants (Plate II, Fig. 1). However, microtriches were found in some transverse and longitudinal sections above the osmiophilic layer, the structure of which could not be discerned with certainty. They occurred single or in groups and their ultrastructure was surprisingly well preserved (Plate II, Fig. 3). The distal cytoplasm appeared like an undulated, relatively high layer of medium electron density. There were numerous vacuoles between it and the osmiophilic layer on the surface. The subtegumental portion contained muscle bundles, sometimes still with a well visible striation (Plate II, Fig. 2), fragments of subtegumental cells, swollen mitochondria arranged in bands and parts of canalicul system and flame cells (Plate II, Fig. 1).

The tissue reaction was characterized by a proliferation of large macrophages and giant multinuclear cells in immediate vicinity of the dead cysticercus, marked activation of lymphoreticular tissue forming nodular formations and conspicuous connective tissue demarcation of the whole nodular affection separating it from the surrounding muscle tissue. This reaction can be described in detail as follows:
The dead cysticercus was sometimes surrounded by a structureless substance with deposits of calcium salts, but more often by spindle-shaped, sometimes vacuolized histiocytes, arranged in a palisade. Giant multinuclear cells of Langhans' type or more often of the type of foreign bodies with phagocyted tissue detritus, calcareous bodies and other parts of larval body were accumulated here (Plate II, Figs. 4, 5, Plate III, Fig. 1). The remnant of bladder cavity contained macrophages and eosinophiles. The whole nodule was usually divided in irregular areas by bands of young connective tissue. The fibrocytes possessed a voluminous, sometimes markedly vacuolized plasma. The nodule consisted mainly of lymphocytes often accumulated in follicular formations, particularly in the peripheral regions (Plate III, Fig. 2). Among them were activated reticular cells with large, bladder-like nucleus or even groups of light large macrophages and multinuclear cells with phagocytized tissue detritus. In this zone were also slit-like blood capillaries, dilated lymphatics filled with lymphocytes and vessels with swollen endothelium and activated cells of media. At the periphery of the wide zone of lymphocytes we found sometimes foci of sparse star-shaped cells resembling "emptied" lymphonodules. The eosinophiles were not numerous here. A larger number of them were accumulated around the cysticercus or, to the contrary, quite at the periphery of the nodule. The plasmacytes were rather numerous. The nodular affection was enveloped from the outside by a wide rim of mature, mostly hyalinized and sometimes strongly calcified connective tissue. In this zone we usually encountered also smaller granulomas with giant cells or only single giant multinuclear cells with phagocytized fragments of calcified substances. The interstitial tissue of the neighbouring muscle was usually only slightly infiltrated with lymphocytes and eosinophiles.

In the electron microscopical picture of the tissue reaction was distinctly visible the activation of lymphocytes and reticular cells, and the lymphocytes often exhibited the signs of blastic transformation (Plate IV, Fig.1). Vacuoles and large lipid drops were found in the plasma of histiocytes (Plate IV, Fig. 2). The granular endoplasmic reticulum of fibroblasts was rich and often formed large cisterns (Plate III, Fig. 4). The plasma of macrophages contained clusters of microtriches enclosed in phagocytic vacuoles (phagosomes) (Plate III, Fig. 4). We encountered numerous plasmacytes and their developmental stages (Plate III, Fig. 3). The cells of the tissue reaction adhered to portions of bladder wall both from inner and outer side. They were often changed by dystrophy.

The control animals contained live cysticerci and the cyst structure corresponded to the stage of morphologically differentiated cysticercus, i.e., without marked cellular reaction. Some of the cysticerci, however, were dead, particularly in the heart. In this case the cyst wall was thickened at the whole periphery, filled with lymphocytes and eosinophiles, and multinuclear cells were accumulated on its inner surface near the everted scolex of cysticercus.

DISCUSSION

Our results show that Drontel given at 50 mg/kg of body weight daily for 2 days and Oxichloron given at 100 mg/kg of body weight daily for 3 days produce lethal effect on larval stages of *T. saginata* at the age of 12 and 6 weeks. Some authors point out that Praziquantel is reliably effective at least 8 weeks after infection (Walter 1978) or state in general that it affects younger larvae more than the old ones (Thomas 1978, Thomas and Gönnert, 1978). Nevertheless, Höchner and Albert (1979) demonstrated that even 28-day-old cysticerci could be killed by Praziquantel. According to our findings the scolex starts to develop at this time.
We have not studied the mechanism of the lethal effect of the drugs, as we
focused our attention more on the process of destruction of dead larvae. The
time which elapsed from the beginning of the therapy to the examination
does not allow to ascertain the character of the early damage. As it is known from
the literature, Praziquantel, like levamisol, thiabendazol and chloroquine,
inhibits the activity of mitochondrial enzymes in the muscles of *Ascaris suum* (Köhler and Bachmann
1978) and first experiments on the mechanism of effect of Praziquantel on
*Hymenolepis diminuta* and *H. nana* showed that it significantly influences the metabolism of carbo-
hydrates in the parasite (Thomas 1977, Thomas and Andrews 1977). Becker
et al. (1980) demonstrated that Praziquantel rapidly affected *Hymenolepis nana*
in vitro. They observed vacuolization and disruption of distal cytoplasm and the
microtriches remained preserved for a long time (like in our experiments). Of interest
was the finding that the changes were most pronounced on the neck, i.e., in
the growth zone of the parasite, whereas mature proglottids were less damaged. We
observed a distinct vacuolization of the tegument even after four and six weeks
and detected microtriches with a well preserved ultrastructure. The microtriches, however,
were disintegrated and some of them were phagocytized by macrophages.
The structure of muscle fibres in the cisticercus remained unchanged for a very long
time. However, the primary damage of larval tissue by the drug was already
obscured by the destructive effect of the intermediate host cells.

The organ reaction to dead parasite after Droncit and Oxichloron treatment
is identical with that occurring after a spontaneous death of the larva, but it differs
from the violent reaction at the early stage of cisticercus development (Blažek et al.,
1981). It is characterized by the presence of a large number of lymphocytes,
often blastically transformed (so-called immunoblasts). It seems to be associated
with the release of somatic antigen after the death of the cisticercus and probably
explains the markedly increased level of humoral antibodies observed one week
(Walther and Grossklaus 1979, Walther and Sanitz 1979) or 2–4 weeks
(Albert and Höchner 1978) after the treatment. Also the presence of plasma
cells and eosinophiles indicates the local immune reaction. Our results show that the
resorption of the dead cisticercus is very slow and that the nodular affections
produced by accumulation of lymphatic tissue and giant multinuclear cells at the
site of dead larva will probably persist in the animal till the end of fattening. This
fact would be important in massive infection (Hörchner and Albert 1979).
Solitary nodular affections, however, are unimportant and though they may be
claimed by the consumer (if they escaped the veterinary inspection), man infection
cannot occur in these cases.

МОРФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ ПОСЛЕ ЛЕЧЕНИЯ
ЦИСТИЦЕРКОЗА КРУПНОГО РОГАТОГО СКОТА ДРОНЦИТОМ
И ОКСИХЛОРОНОМ

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Резюме. Дронцит в дозе 50 мг/кг живого веса, заданный два дня подряд, и оксихлорон
в дозе 100 мг/кг живого веса, заданный три дня подряд, применяли для лечения цисти-
cеркоза телят спустя 12 (дронцит) и 6 (оксихлорон) недель после экспериментального
заражения. Через 4 или 6 недель после лечения обнаружены отмершие цистицерки или их
остатки. Встречалась резкая вакуолизация тегумента личинки, но еще через 6 недель
после лечения можно было различать мышцы, части субтегументальных клеток, остатки
экскреторной каналькулярной системы и остатки микротрихов. Однако в некоторых
случаях структура микротрихов оставалась удивительно не поврежденной. Доминантной
REFERENCES


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Fig. 1. Necrotic bladder of cysticercus (C) surrounded by granulation tissue. HE. Fig. 2. Remnant of bladder wall (C) and detail of cellular reaction in the vicinity. Fig. 3. Calcareous bodies remaining after necrosis of scolex. Fig. 4. Preserved structure of sucker (S). Necrotic scolex, histiocyte proliferation in the vicinity (HI).
Fig. 1. Electronogram of bladder portion of dead cysticercus. A — electron-dense layer of changed microtriches, B — layer of distal cytoplasm, Mi — mitochondria. Fig. 2. Preserved structure of muscle in bladder wall of cysticercus. Fig. 3. Detail of preserved microtriches in transverse section (arrows). Figs. 4. and 5. Tissue reaction around the dead cysticercus. Proliferation of histiocytes and formation of giant multinuclear cells.
Fig. 1. Zone of histiocytes with deposits of calcium and calcareous bodies around the dead cysticercus passing to zone of reticular cells and lymphocytes. Fig. 2. Lymphoreticular proliferation at the periphery of nodule with dead larva. Fig. 3. Detail of plasma cell (PC). Fig. 4. Detail of microrthic group (transverse section) phagocytized in plasma of macrophage (MT). Fig. 5. Detail of extended granular endoplasmic reticulum of a fibroblast.
Fig. 1. Lymphocytes sometimes transformed to blasts. Fig. 2. Lipids in cells of histiocyte type (L).