

Efficacy of closantel for treatment of naturally-acquired and experimentally-induced *Fasciola hepatica* infections in cattle

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소에 자연 또는 인공감염된 간질(*Fasciola hepatica*)에 미치는 closantel의 구충 효과

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초록 : Closantel이 간질(*Fasciola hepatica*)에 감염된 소에 미치는 치료 효과를 알아보기 위하여 간질 및 쌍구흡충에 자연감염된 41두의 한우 암컷에 체중 kg당 closantel 5mg을 1회 경구투여하였다. 치료 후 2주째부터 일주일 간격으로 3회 실시한 분변검사서서 closantel로 치료한 군은 치료 후 2주째까지는 음성이었으나 치료 후 3주 및 4주째에는 3마리의 소의 분변에서 간질충란이 검출되어 97.7%의 음전율을 나타냈다. 치료군 41두 중 30두는 임신중이었으나 임신우로부터 또는 이들로 부터 태어난 송아지에서 아무런 부작용이나 이상증세가 관찰되지 않았다. 검사한 모든 소에서 쌍구흡충란이 검출되었으나 closantel 치료에 의하여 제거되지 않았다. 두번째 실험에서는 16두의 Holstein종 송아지에 실험적으로 300개의 간질 피낭유충을 인공감염시켰으며, 감염후 14주째에 이르러 모든 개체의 분변에서 간질 충란이 검출되었다. 이들에게 감염 후 18주에 체중 kg당 5mg의 closantel을 경구투여하였으며, 치료 후 2주째부터 1주일 간격으로 3회 실시된 총란검사서서 치료군의 모든 송아지가 100% 음전율을 나타냈다. 본 실험의 결과, 간질에 자연 또는 인공감염된 소에 체중 kg당 5mg의 closantel을 경구투여했을 경우, 97% 이상의 치료효과가 있음이 관찰되었다.

Key words : *Fasciola hepatica*, *Paramphistomum*, closantel, chemotherapy, cattle, Korea

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Introduction

Closantel(N-5-chloro-4-[(4-chlorophenyl) cyanomethyl]-2-methylphenyl-2 hydroxy-3, 5-diiodobenzamide) is a salicylanilide antiparasitic compound that has been efficacious against several species of nematodes and trematodes which include *Fasciola hepatica*, *F. gigantica* and *Haemonchus spp* of ruminants¹. In Korea, *F. hepatica* infection in cattle has been reported since 1915. Prevalence ranges from 20 to 50% among Korean cattle and up to 56% among imported Holstein cattle². The economic loss due to the condemnation of the livers of infected cattle was estimated as over 87 million dollars during 17 months of 1982 and 1983. Chemotherapy has been a major method of control and several flukicidal compounds have been tested and commercially available².

The purpose of this study was to evaluate if closantel(Flukiver™, Janssen Pharmaceutica, Beerse, Belgium) could successfully remove *F. hepatica* infection in cattle with a single treatment. The present study is divided into two parts; closantel treatment of Korean cattle naturally infected with *F. hepatica*, and of Holstein calves experimentally infected with *F. hepatica* metacercariae.

Materials and Methods

Animals : For the study of naturally-acquired *F. hepatica* infection, 195 Korean cattle, from 40 small-size farms(up to 36 head per farm) out of three villages where *F. hepatica* infection in cows had been previously reported, were screened for the presence of *F. hepatica* eggs in their feces. These villages have been designated as breeding units of Korean cattle by the government and only Korean cattle have been allowed to be raised and reproduced within these villages. Forty-six cows were found to be infected with *F. hepatica*, as determined by fecal examination. Average size of these eggs were $154.3 \pm 9.5 \mu\text{m}$ (mean \pm SD) in length and $78.4 \pm 7.3 \mu\text{m}$ in width. The average age of the treated group was 4.7 ± 2.3 years and 354.0 ± 71.0 kg body weight. Thirty of the 41 treated cattle(73%)

were at various stages of gestation. The mean gestation period of the 30 pregnant cows was 4.2 ± 2.5 months(range: 1.5-8 months). Fecal examination also revealed that all 195 cattle including the 46 *Fasciola*-positive cows, were heavily infected with paramphistomes with a mean number of eggs per gram of feces(EPG) of approximately 260.

Sixteen healthy Holstein calves, five male and 11 female at 7.2 ± 1.5 months of age and 173.1 ± 33.7 kg body weight, were used for experimental infection. They were born and raised at a university-affiliated farm in Kangwon Province of Korea that is located in a *Fasciola*-free area. These animals were free of *Fasciola spp* or paramphistomes, as determined by fecal examination and were dewormed prior to experiment to eliminate any nematode infection. They were housed in a barn and were fed a balanced ration of hay and concentrate; salt and water were available *ad libitum*.

***Lymnaea viridis* culture and metacercarial production** : Culture of *Lymnaea viridis*, the snail host for *F. hepatica* in Korea, has been standardized in this laboratory and are described in detail in previous reports³.

Snail infection with *Fasciola hepatica* miracidia : *F. hepatica* eggs were obtained from the feces of an experimentally-infected goat with *F. hepatica* metacercaria. Eggs were collected using Flukefinder™(Visual Difference, Moscow, ID, USA) and were then washed off into a beaker with tap water. The beaker containing the eggs was maintained at 24°C. Mass hatching was induced 15 days later by exposing the eggs to light. The tenth generation of laboratory-cultured young *L. viridis* snails was selected from the culture vessels and were exposed the eggs to one to five miracidia in 3ml of water in a well of a 24-well plate(Nunc, Denmark). Penetration of miracidia into the snails was observed both directly and by washing each snail and confirming that no miracidia remained in the washings. All the infected snails were returned to the culture vessels and maintained at room temperature.

Cercarial shedding : After infection two snails were kept in one culture vessel. They were moved at frequent intervals to fresh culture vessels, which con-

tained vigorous growth of blue-green algae. Each snail from day 25 PI on was placed separately in a dish 11cm in diameter and cercarial shedding was induced by adding fresh distilled water daily. The snails which shed cercariae were maintained in individual dishes, fed with algae taken from culture vessels and received fresh distilled water daily until they ceased cercarial shedding and died.

Infection of calves with metacercariae : The 16 Holstein calves were inoculated with 300 metacercariae each. Filter papers containing known numbers of metacercariae were placed in gelatin capsules and administered *per os* by use of a balling gun. Patency was monitored weekly by fecal examination after seven weeks post-infection(PI).

Chemotherapy : Of the 46 naturally-infected cows, 41 were treated by administering once orally with 5mg/kg body weight(BW) closantel(Flukiver™, Janssen Pharmaceutica, Beerse, Belgium), and five left as untreated controls. Fourteen experimentally-infected calves were treated at 18 weeks PI once orally with 5mg/kg BW closantel and two left as untreated controls.

Efficacy determination : Efficacy of closantel was monitored weekly by fecal examination of all infected animals after the second week post-treatment(PT) for three weeks. All fecal samples were collected from the rectum of each animal to prevent contamination with feces of adjacent animals. A quantitative fecal ex-

amination was carried out in the laboratory using Flukefinder™ and the EPG was calculated as described in the instruction for the kit. An EPG value represents the mean of two examinations of each sample.

Results

Natural infection : As shown in Table 1, cattle treated with closantel did not contain *F hepatica* eggs in the feces at second week PT. On the third and fourth weeks PT, however, 3 of the 41 treated cattle contained *Fasciola* eggs in their feces with a mean EPG of 0.1 and 0.2, respectively(2.0 and 2.6 for 3 cattle excreting eggs). All animals in the untreated control group produced *F hepatica* eggs at 2nd, 3rd and 4th week after treatment as well as prior to treatment. The efficacy of closantel administered orally at a level of 5mg/kg BW against *F hepatica* was 97.7%, as calculated by the percentage of cows with no *F hepatica* eggs in the feces after closantel treatment.

Side effects such as abortion or other adverse reactions were not observed from any of the treated cows with closantel and no congenital defect of calves born from the treated cows was reported. Closantel was not effective against paramphistomumes, because it did not affect on the egg production of paramphistomumes in the treated cows(data not shown).

Table 1. Efficacy of closantel treatment against naturally-acquired *Fasciola hepatica* infection in Korean cattle

Fecal exam	Treated group*		Untreated control	
	No of positive animals (% efficacy)	EPG** (mean±SD)	No of positive animals	EPG (mean±SD)
Before treatment	41/41	8.3±2.7	5/5	19.6±0.4
2 WPT***	0/41(100%)	-	5/5	19.4±0.8
3 WPT	3/41(97.7%)	0.1±0.6	5/5	10.6±0.5
4 WPT	3/41(97.7%)	0.2±0.7	5/5	29.6±0.8

* Female Korean cattle were treated orally with closantel at the dosage level of 5mg/kg body weight. Rectal fecal examinations were performed 2, 3 and 4 weeks post treatment

** Number of eggs per gram of feces

*** Weeks post treatment

Table 2. Efficacy of closantel treatment against experimentally-induced *Fasciola hepatica* infection in Holstein calves

Fecal exam	Treated group*		Untreated control	
	No of positive animals (% efficacy)	EPG** (mean \pm SD)	No of positive animals	EPG (mean \pm SD)
Before treatment	14/14	15.3 \pm 16.0	2/2	19.0 \pm 1.3
2 WPT***	0/14(100%)	0	2/2(0%)	29.5 \pm 20.5
3 WPT	0/14(100%)	0	2/2(0%)	23.5 \pm 7.7
4 WPT	0/14(100%)	0	2/2(0%)	25.5 \pm 7.8

* Holstein calves were treated orally with closantel at the dosage level of 5mg/kg body weight. Rectal fecal examinations were performed 2, 3 and 4 weeks post treatment

** Number of eggs per gram of feces

*** Weeks post treatment

Experimental infection : As shown in Table 2, fecal egg counts performed at 2nd, 3rd and 4th weeks PT did not reveal any *Fasciola* eggs from experimentally-infected Holstein calves. The efficacy of the closantel against *F hepatica* at 18 weeks PI was 100%, based on the three weekly observations of eggs from feces. Calves treated with closantel did not develop any adverse reaction.

Discussion

Results from this study clearly demonstrated that closantel administered once orally at the level of 5mg/kg BW had an excellent chemotherapeutic efficacy against *F hepatica* infection in cattle. The reappearance of *F hepatica* eggs from 3 of the 41 treated cows at 3rd and 4th weeks PT was probably due to some immature flukes which became adult and produce eggs, because previous reports demonstrated that closantel administered subcutaneously at the level of 2.5 or 5mg/kg BW elicited an 100% efficacy against adult *F hepatica*⁴, while 7.5mg/kg BW elicited an 80.8% efficacy against the 6 week-old larval stage of *F hepatica* in cattle⁵. In another study, closantel administered by intramuscular injection at a dose rate of 2.5mg/kg BW was only 60% effective against an 8-week-old *F*

hepatica infection⁶. Similar results were observed in Korean cattle naturally infected with *F hepatica*⁷ in which closantel(5ml/100kg, concentration unlisted) injected via the intramuscular route to 52 cattle resulted in an 84.6% efficacy at 4 weeks post treatment, as determined by fecal examination. The discrepancy of the report with our data is not clear. It may due to the fact that 3 independent test sites and people were involved in the study.

To confirm if closantel administered orally at the level of 5mg/kg BW could clear an adult infection of *F hepatica*, 14 Holstein calves were experimentally infected with 300 metacercariae and treated with closantel at 18 weeks PI. As shown above, closantel cleared *F hepatica* eggs from the feces of all treated calves at 2nd, 3rd or 4th week PT, apparently by killing adult flukes in the bile duct. Although the prepatent period of *F hepatica* infection in these calves was 14 weeks or earlier(data not shown), calves were treated at 18 weeks PI so that any immature fluke that might possibly be in the liver parenchyma could arrived at the bile duct to mature and produce eggs. Therefore, our results indicated that the oral administration of closantel at the level of 5mg/kg BW eliminated all *F hepatica* adults in the treated cows, while some immature flukes survived to become adult and produce eggs later.

Closantel has shown excellent antiparasitic activity against several species of nematodes and trematodes¹ which included *F hepatica*, *F gigantica* and *Fascioloidea magna*. The drug has also been recommended as a treatment for acute paramphistomosis⁸. This study, however, indicated that closantel had no effect against naturally-acquired *Paramphistomum* infections in Korean cattle. A previous report also indicated that moxidectin, a combination of ivermectin/clorsulon and closantel or closantel alone at 7.5mg/kg BW was ineffective against immature stages of paramphistomes in calves⁹. Species identification of adult paramphistomes was not attempted in the present study, but the most frequently isolated paramphistomes from Korean cattle in the studied area is known to be *P explanatum*¹⁰.

The majority of cattle(73%) naturally infected with *F hepatica* in this study was at various stages of pregnancy. It is notable, however, that cows did not display any clinically adverse reaction after treatment with closantel and no calves with congenital defects were born from the treated cows. It has been reported that overdosage of closantel was associated with blindness in kids¹¹ and central nervous system effects and death in laboratory animals¹². However, van Cauteren et al¹² demonstrated that the reproductive system of bulls, rams and ewes was not affected by closantel.

Summary

The efficacy of closantel against naturally-acquired and experimentally-induced *F hepatica* and paramphistomes were administered once orally with closantel at the level of 5mg/kg body weight. In a double-checked fecal examination, cows treated with closantel did not contain *F hepatica* eggs in their feces at second week post-treatment(PT). At the third and fourth weeks PT, however, *Fasciola* eggs were found in the feces of 3 treated cows, resulting in a 97.7% efficacy. Of the 41 treated cattle, 30 were at various stages of gestation. No side effects were observed in any of the treated cows and congenital defects among calves born from the treated cows were not reported.

Closantel was not effective against paramphistomes. In a separate experiment, 16 Holstein calves were experimentally infected with 300 *F hepatica* metacercariae each. *F hepatica* eggs were found in the feces of all infected calves by 14 weeks post-infection(PI). Calves were then treated at 18 weeks PI once orally with 5mg/kg body weight closantel. None of the treated calves contained *F hepatica* eggs in their feces at 2nd, 3rd or 4th week PT. Our results indicate that oral administration of closantel at the level of 5mg/kg body weight eliminated all mature *F hepatica* in the liver, while some of immature flukes survived to become adult and produce eggs.

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