Effect of noxythiolin on experimental peritonitis

O. J. A. GILMORE
M.S., F.R.C.S., F.R.C.S. (Ed.)

ELIZABETH T. HOUANG
M.B.B.S., M.R.C.Path.

CLARE REID
B.Sc., A.I.M.L.S.

ELIZABETH J. SHAW
M.D., B.S., F.R.C.Path. (Aust)

*Surgical Professorial Unit and Department of Medical Microbiology,
St Bartholomew's Hospital, London EC1*

Summary

The intraperitoneal instillation of noxythiolin in the treatment of peritonitis is widespread in clinical practice despite contradictory evidence as to its efficacy. In this light the value of noxythiolin was re-appraised by studying its effect in guinea-pigs and mice with induced bacterial peritonitis. Treatment with a 1% solution of noxythiolin reduced the mortality rate of mice by 14% ($P < 0.1$). The guinea-pig model proved unreliable giving inconsistent mortality rates throughout. Further studies are required to determine the optimum dose and concentration of noxythiolin while the search for more effective intraperitoneal antiseptics should continue.

Introduction

Intraperitoneal noxythiolin (Noxyflex) is lethal. This was the conclusion reached by King, Gurry and Brooke (1975a) when they studied rabbits with peritonitis from perforated appendicitis. Tolhurst Cleaver *et al.* (1974) concluded that noxythiolin was no more effective than Hartmann's solution in reducing the mortality of rats with peritonitis.

Despite these animal studies intraperitoneal noxythiolin is often used in clinical practice. Its popularity stems from the work of Stoller (1967) and Browne and Stoller (1970) who showed that noxythiolin significantly reduced the mortality rate of guinea-pigs with bacterial peritonitis. They also reported an impressive series of twenty-three patients with faecal peritonitis: there were only three deaths and two of these were due to pulmonary emboli.

The antimicrobial action of noxythiolin (*N*-hydroxy-methyl-*N'*-methylthiourea) is not fully understood, but various explanations have been forwarded. Pichard (1972) attributed its bactericidal action to the liberation of formaldehyde. Chemically, noxythiolin is similar to methyl mustard oil which has a wide antibacterial spectrum, another possible explanation for its activity (King *et al.*, 1975a). A

recent study showed that it significantly reduces peritoneal adhesion formation (Gilmore and Reid, 1976) while other studies show noxythiolin to have anti-endotoxin (Wright and McAllister, 1967) and anti-mitotic properties (Jamieson, 1972; Desai and Jamieson, 1973). It has been suggested that the bactericidal and anti-adhesive actions of noxythiolin may be non-specific owing to interference with cell division (Gilmore, 1976).

Because the intraperitoneal use of noxythiolin in peritonitis is common and the evidence of its efficacy is contradictory the authors considered a reassessment of its value to be important.

Guinea-pig studies

Using female Dunkin-Hartley guinea-pigs, it was attempted to establish a model for both coliform (*Escherichia coli* 0111B4) and mixed bacterial (*E. coli* 0111B4 and *Bacteroides fragilis* NC9363) peritonitis. However, mortality rates were very inconsistent so it was decided to investigate the possibilities of a mouse model.

Mouse studies

Female, Theiller Original, specific pathogen-free mice weighing 20-25 g were used exclusively. In 1973 Rogers showed that *E. coli* 0141/K85/H4 would multiply in the peritoneal cavity of this mouse. Consequently these same strains were used in the following experiments for the induction of experimental peritonitis.

Study 1: Establishing a model for bacterial peritonitis

Four groups of mice were injected intraperitoneally with *E. coli*, as shown in Table 1. The incidence and time of death of each mouse was recorded.

All mice dying from peritonitis did so within 4-18 hours. The mortality rate increased with the bacterial content rather than the volume of the inoculum. At post-mortem the only abnormality seen was a hyperaemic peritoneum. A bacterial

TABLE 1. Mortality of mice with untreated bacterial peritonitis

Group	<i>Escherichia coli</i> inoculum		No. of mice	
	Volume (ml)	No. of organisms	Total	Deaths
A	0.2	4×10^5	10	0
B	0.4	8×10^5	10	2
C	0.2	4×10^6	10	4
D	0.2	2×10^7	10	6

swab taken from the peritoneal cavity grew a pure culture of *E. coli* in every case. Surviving animals were killed at 1 week and examined. No abnormalities were seen, and again the bacterial swab always grew a pure culture of *E. coli*.

It was concluded from this study that the injection of *E. coli*, 10^7 organisms/ml would give a mortality rate of 50–60%.

Study 2: Intraperitoneal noxythiolin in peritonitis

Preliminary study

Materials and methods. Eighty mice were injected with 0.2 ml *E. coli*, 10^7 organisms/ml. One minute later the mice were injected accordingly:

Group A: Control: nothing.

Group B: Control: 0.2 ml Ringer's solution.

Group C: 0.2 ml noxythiolin, 1% solution.

Group D: 0.2 ml noxythiolin, 0.5% solution.

The mortality rate in each group was recorded and all survivors were killed at 1 week. A post-mortem examination was performed on every animal shortly after death and a bacterial swab was taken from the peritoneal cavity.

Results. As in the previous study, all mice dying from peritonitis perished within 4–18 hours of its induction. The mortality rates are given in Table 2. The survival rate of mice treated with noxythiolin 1% was greater than that of those receiving noxythiolin 0.5%, but this difference was not significant. The mortality rate was identical in the two control groups. The post-mortem findings were identical to those of the previous study.

Definitive study

Materials and methods. This study comprised eight similar experiments in which 220 mice were injected intraperitoneally with *E. coli*, 10^7 organisms/ml.

TABLE 2. Effect of intraperitoneal noxythiolin in mice with bacterial peritonitis—preliminary study

Treatment group	Total	Deaths
A. Control: nothing	20	12
B. Control: 0.2 ml Ringer solution	20	12
C. 0.2 ml noxythiolin 1.0% solution	20	11
D. 0.2 ml noxythiolin 0.5% solution	20	13

ml. One minute later each mouse received a second injection by random allocation:

Group A: Control: 0.2 ml Ringer's solution.

Group B: 0.2 ml noxythiolin 1%.

The mortality rate in each group was recorded and all survivors were killed at 1 week.

Results. The results of this study are given in Table 3. The mortality rate of the control group was

TABLE 3. Effect of intraperitoneal noxythiolin in mice with bacterial peritonitis—definitive study

Treatment group	Total	Deaths	Significance
A. Control: 0.2 ml Ringer solution	110	72	
B. 0.2 ml noxythiolin 1% solution	110	57	$P < 0.1$

Significance: $P < 0.1$.

65.5% and that of the treated group 51.8%. The reduced mortality rate of mice treated with noxythiolin 1% compared with the control group is significant at the 10% level ($\chi^2 = 3.67$, $P < 0.1$). Again as in the previous two studies, all mice dying from peritonitis perished within 4–18 hours, and at post-mortem the only abnormality found was a hyperaemic peritoneum.

Discussion

Theiller Original mice injected with *E. coli* 014: 10^7 organisms/ml give a reliable, reproducible and inexpensive model for the study of peritonitis. The majority of mice dying of peritonitis perished within 4–8 hours of its induction, and all died within 18 hr. This indicates the rapidly progressive nature of the peritonitis induced.

Intraperitoneal noxythiolin 1% in the dose range 80–100 mg/kg body-weight reduced the mortality of mice with peritonitis by 14% (Table 2), but this reduction was not quite significant. Tolhurst Cleaver *et al.* (1974) found Hartmann's solution superior to noxythiolin 0.5% and 0.125% in the treatment of rat faecal peritonitis: in this preliminary mouse study 0.5% noxythiolin was found to be ineffective. King *et al.* (1975a,b) have published papers on peritonitis in rabbits due to perforation of the appendix. They found that significantly more rabbits died which were receiving 5 ml of 2.5% or 10% noxythiolin solutions than those receiving normal saline. Brown and Stoller (1970), on the other hand, found that noxythiolin (500 mg/kg body-weight) significantly reduced the mortality of guinea-pigs with bacterial peritonitis ($P < 0.001$).

There are a number of possible explanations for these varying results. Different animals were studied, different methods of inducing peritonitis were used

the treatment-free interval varied, as did the concentration and dose of noxythiolin given. It may well be that the effectiveness of noxythiolin in peritonitis is not only dose-specific, but also species-specific.

King *et al.* (1975a, b) have described an animal model which simulates perforation of the appendix. Unfortunately any model which mimics the clinical situation produces an uncontrolled peritonitis: furthermore, laparotomy introduces another variable owing to the inevitable loss of intraperitoneal fluid. The only satisfactory method of producing a controlled peritonitis for experimental studies is to inject a known amount of bacteria or a standard suspension of faeces.

In clinical peritonitis all the evidence for the efficacy of noxythiolin comes from uncontrolled studies (Browne and Stoller, 1970; Pickard, 1972; Leger, Moule and de Laitre, 1972). In a controlled trial, Gurrey *et al.* (1976) found 100 ml of noxythiolin 2.5% to be no more effective than 100 ml of normal saline in preventing complications in perforated appendicitis. No adverse reports of noxythiolin in clinical practice have however been published, nor did Gurry *et al.* (1976) notice any in their trial.

A number of conclusions may be drawn from the present studies. Dunkin-Hartley guinea-pigs offer unreliable models for the study of peritonitis. Theiller Original mice injected with *E. coli* 0141 provide a reliable and inexpensive model for peritonitis studies. Intraperitoneal noxythiolin 0.5% solution is ineffective, while a 1% solution (80–100 mg/kg body-weight) reduces the mortality of mice ($P < 0.1$) when given 1 minute after the induction of peritonitis. Intraperitoneal noxythiolin at these concentrations is therefore not lethal, but further studies are required to determine its optimum concentration and dosage, while the search for more effective intraperitoneal antiseptics should continue.

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