THE THERAPEUTIC EFFECT OF AUREOMYCIN IN EXPERIMENTAL PERITONITIS IN THE DOG

II

PARENTERAL THERAPY*

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IN A PREVIOUS PAPER[1] aureomycin administered orally was shown to be effective for the cure of an otherwise fatal peritonitis due to intestinal bacteria. Data were also given showing the superiority of aureomycin over other antibiotics with respect to both the rate and degree of suppression of the normal intestinal flora. In this report data are given on the comparative effectiveness of the parenteral route.

A. EFFECT OF PARENTERAL AUREOMYCIN ON THE NORMAL INTESTINAL FLORA

Method. Two normal dogs received 400 mg. of aureomycin intramuscularly daily (in two divided doses) for ten days. In ten others, treatment was given intravenously, six receiving the same dose, and four receiving 1000 mg. so as to approximate the 1250 mg. dose administered by the oral route in the previous study. Daily stool cultures were made from the first day and the quantitative change in various bacterial strains determined by the method of Spaulding *et al.*[2]

Results. There was no change in the flora for the first four days of treatment by the intramuscular route. Thereafter the coliform bacteria progressively declined. In one dog none were recovered on the ninth day. Four days after stopping the drug, coliform organisms reappeared in the stools. In the other the minimum count, 1/100 of the original, was reached on the seventh day and remained unchanged for the next three days.

In the six dogs treated with the same dose intravenously the decline in coliform bacteria was not noted until the fifth to the seventh days. The bacteria completely disappeared by the tenth day in only two. In two others the minimum count reached was 1/100 and 1/50 respectively of the original count. In the remaining two the minimum count was one fifth and one third respectively of the original count.

All four dogs treated intravenously with 500 mg. twice daily were obviously sick after two to four days and all died within three to five days, due to the toxic effect of the drug. Of two which survived for five days one showed no coliform bacteria in the stool, the other showed a reduction to 1/150 of the original count. No change in count was observed in the two which died on the third day.

Counts of clostridia and enterococci were not done in any of the above experiments. Rough estimates in the surviving dogs indicated that they were reduced, but

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complete disappearance was not observed in any instance. This observation is consistent with the fact that these bacteria are less sensitive *in vitro* than the coliform bacteria to the drug.

Comment. In our studies oral administration exerted a more rapid and more effective suppression of the intestinal flora than parenteral administration. While this may have been due to the use of a larger oral dose (1250 Gm. daily) than can be tolerated by the intravenous route, it is more likely that it was due to a higher intraintestinal concentration. Nevertheless, since the foregoing data demonstrate that parenteral aureomycin induces a definitive suppression of the intestinal flora, particularly of coliform bacteria, a therapeutic effect upon peritonitis due to the usual intestinal flora by aureomycin given parenterally might be anticipated.

B. EFFECT OF PARENTERAL AUREOMYCIN ON EXPERIMENTAL PERITONITIS IN THE DOG

Peritonitis, produced by trauma to the appendix, as described in previous papers,[1, 3, 4] if untreated, is nearly always fatal, with an average survival time of 42 hours. If treated by aureomycin orally, the recovery rate is 75 per cent. In recovering dogs, serial cultures of the peritoneal fluid show a steady decline in the number of colonies of all bacterial strains except *Pseudomonas aeruginosa* and *B. proteus,* from about the third day onward, with virtually complete disappearance of all except the latter two strains by the tenth day.

The relative effectiveness of various parenteral routes of administration on the contaminating bacteria and on the survival rate was determined as described below.*

I. INTRAMUSCULAR FOR FIVE AND A HALF DAYS. Aureomycin, 200 mg. in 5 cc. saline was injected twice daily for five and a half days into the gluteal muscles, beginning immediately after the induction of the peritonitis. Edema and lameness of the hind limb developed, whether the glycinate (pH 8.2) or the hydrochloride (pH 2.8) was used. These effects, the severity of which precluded use of a larger dose, subsided a few days after the drug was stopped, and were evidently due to its tissue-irritating properties, for cultures of the fluid at site of injection were sterile. Of ten dogs so treated, three died of peritonitis, on the second, sixth and seventh days respectively. Peritoneal cultures yielded the same findings as in untreated dogs.[1] The decrease in coliform bacteria observed in surviving animals which are on the way to recovery, did not occur in these dogs. Two dogs which died on the eighth and twelfth days respectively did show such a decrease, and at autopsy were found free of peritonitis, but with a large pericecal abscess.

Five dogs survived after being ill for two to three days. The bacteriologic findings during convalescence, and the findings at autopsy after sacrifice on the twenty-first day, were quite like those described in dogs which survived after oral drug therapy.[1]

Comment. The rate of recovery from peritonitis was 70 per cent as compared to 80 per cent when the drug was given by the oral route (Table I). Since this difference is not significant, it appears that enough of the drug is absorbed from the muscles to provide a good antibacterial concentration either within the peritoneal cavity or the intestinal lumen, or both.

The survival rate, however, was 50 per cent as compared to 75 per cent for the oral route. The lower survival rate might be explained on the assumption that in some instances the inflammatory reaction in the injected tissues slows absorption so that the depleting effects of the peritonitis are ex-

* The data which follow do not allow a comparison of effectiveness of various routes of administration on a dose per kilogram basis when limitation of the dosage was necessitated by the irritating or toxic effects of the drug. All dogs used weighed from 10 to 12 Kg.

erted before the necessary concentration of the drug becomes available.

II. Intravenous for Five and a Half Days. Ten dogs were treated as above except that the drug was given intravenously. Five received 200 mg. b.i.d. in 50 cc. normal saline solution and five received 200 mg. b.i.d. in 400 cc. of one sixth molar lactate solution (pH 6.6) given in slow drip.

cause of too rapid excretion.* That the drug did have some therapeutic effect is suggested by the average survival time, which was somewhat longer than in untreated dogs. Since the larger intravenous dose was so toxic as to be lethal even in the normal dog, it is possible that the smaller intravenous dose also excited a toxic effect, not evident in the normal dog, but such as to

Table I.—*Efficacy of Aureomycin Administered by Various Routes in the Treatment of Experimental Appendiceal Peritonitis in Dogs.*

Route	Dose (Gm./day)	No. of Dogs	Dead			Cure of Peritonitis	Survival Rate
			Total	Peritonitis	No Peritonitis		
No drug	.....	10	9	9 (1–2)†	....	10%	10%
Oral preoperative*	1.25 —4–10 days	12	3	1 (8)	2 (12, 18)	92%	75%
Oral postoperative*	1.00 —5½ days	10	3	2 (2, 18)	1 (12)	80%	70%
Oral postoperative	1.00 —2 days	5	2	1 (17)	1 (15)	80%	60%
Intramuscular postoperative	0.4–0.8—5½ days	10	5	3 (2, 6, 7)	2 (8, 12)	70%	50%
Intravenous postoperative	0.4–1.2—5½ days	15	13	12 (1–4)	1 (15)	20%	12.5%
Intravenous and	0.4–1.0—2 days	10	9	9 (2–4)	....	10%	10%
Intramuscular postoperative	0.4–0.8—3½ days						
Intraperitoneal postoperative	0.2–0.5—5½ days	10	10	8 (1–11)	2 (9, 14)	20%	0%
Intraperitoneal and	0.4—2 days	10	2	2 (11, 15)	....	80%	80%
Intramuscular postoperative	0.4—3½ days						
Interperitoneal postoperative	0.4—2 days	5	1	1 (1)	....	80%	80%

* "Preoperative" refers to administration before inducing peritonitis.
"Postoperative" refers to administration after inducing peritonitis.
† Numbers in brackets indicate the day after operation on which the dog died.

Only one dog in each group of five survived. The others died in from one to four days with diffuse peritonitis. The bacteriologic findings in those which died were the same as in corresponding dogs which were untreated.[1] In the two which survived, the bacteriologic findings were the same as in all other surviving dogs.

Because the therapeutic effect and the mortality rate were the same as in untreated dogs, the dose was increased to 600 mg. b.i.d. in five additional dogs, all of which died with diffuse peritonitis within 72 hours. Again the bacteriologic findings in the peritoneal exudate were the same as in the untreated controls.

Comment. The failure to influence the intraperitoneal bacterial flora in all except the two surviving dogs of this series, regardless of drug dosage, suggests that the intraperitoneal or intra-intestinal concentration of the drug was inadequate, presumably because of too rapid excretion.* That the drug did have some therapeutic effect is suggested by the average survival time, which was somewhat longer than in untreated dogs. Since the larger intravenous dose was so toxic as to be lethal even in the normal dog, it is possible that the smaller intravenous dose also excited a toxic effect, not evident in the normal dog, but such as to impair the defense reaction to infection. The bacteriostatic effect of the intramuscular route could hardly equal, let alone exceed that of the intravenous route, hence the superiority of the intramuscular route would appear to be due to a lower degree of toxicity attributable to a lower concentration in the circulation.

Since such a toxic effect may be the cumulative result of repeated dosage, the experiment was repeated in ten dogs, limiting the intravenous administration of the smaller dose (200 mg. b.i.d.) to 48 hours and continuing thereafter with the same dose intramuscularly for several days, or until death. The mortality was 90 per cent. Four dogs died within 48 hours and five on the third or fourth days. The pattern of the

* Owing to the presence of many bacteria in the peritoneal fluid, aureomycin levels in this fluid could not be determined.

intraperitoneal flora from day to day was precisely the same as if no drug had been given. The one dog which survived showed the usual flora pattern in a surviving animal.

Comment. Since the drug given intravenously in this dose for 48 hours was, *per se,* not responsible for death, it must be concluded that the toxic effect of the drug on the defense reaction nullifies the benefits of its antibacterial action, even in the first 48 hours.

III. Intraperitoneal for Five and a Half Days. In order to test the safety and effectiveness of the same dose of the drug applied at the site of contamination, ten dogs were treated by introducing into the peritoneal cavity twice daily 200 mg. of the glycinate in 30 cc. saline solution (pH 8.2), or the hydrochloride in 250 cc. buffered saline solution (pH 6.6), beginning just after inducing the peritonitis.

All ten dogs died—five within 36 hours, two on the seventh day, one each on the ninth, eleventh and fourteenth days. At autopsy all but two dogs, which did not have peritonitis, showed gangrenous appendicitis, with one to two liters of purulent, hemorrhagic and yellow-colored fluid containing leukocytes, red cells and endothelial cells. The tissues were matted together and showed a heavy deposit of aureomycin.

In no instance were the cultures at any stage like those of other dogs dying of peritonitis. There were none or very few *E. coli,* clostridia or enterococci, while *B. Proteus* and Pseudomonas predominated after the first or second intraperitoneal injection. In all orally treated dogs cured of peritonitis the latter two organisms were frequently present in the peritoneal cavity and in the residual pericecal abscess. Although resistant to aureomycin, they appeared not to interfere with recovery.[1] Hence their presence in the dogs treated intraperitoneally may be assumed not to have caused the peritoneal reaction or the death.

Because of the fact that the treatment promptly suppressed the usual pathogenic bacteria, the severe reaction and death are attributable to the chemical peritonitis arising from the severe tissue-irritating properties of aureomycin. That this is a likely explanation was shown in two normal dogs treated with intraperitoneal aureomycin in the same way. One dog sacrificed on the third day and the other, which died on the fourth day, showed, except for the absence of appendicitis, the same gross findings at autopsy as were observed in the dogs of this series, while cultures taken twice during treatment, and again at autopsy, were sterile.

Death without therapy occurs with an average survival time of 42 hours; this period may therefore be regarded as the critical period for the application of effective treatment. Treatment with the antibiotic thereafter may be unnecessary or may serve merely as an adjuvant to the defense reaction. To test this assumption the drug was administered after induction of peritonitis as follows: intraperitoneally for two days, followed by intramuscularly for three and a half days; intraperitoneally for two days; orally, for two days.

IV. Intraperitoneally for Two Days Followed by Intramuscularly for Three and a Half Days. In the series of ten dogs treated intraperitoneally for as long as they survived or up to a maximum of five and a half days, five survived no longer than untreated animals, in spite of effective bacterial suppression. Since the death was considered due to chemical peritonitis, it seemed doubtful if shortening the time of intraperitoneal administration to 48 hours would lower the mortality. Nevertheless, ten dogs were so treated with the glycinate preparation, but intramuscular administration, using the hydrochloride, was continued for three and a half more days.

Eight of these dogs survived after a moderately severe illness of two to three days duration. In these dogs definite depression of all sensitive bacterial species was evident from the first cultures. Coliform

bacteria and clostridia disappeared by the third day and only a scanty growth of *B. Proteus* and Pseudomonas persisted until about the tenth day, when all taps were dry. On the twenty-first day the dogs were sacrificed and autopsy showed the findings usual in healed cases. The two dogs which died (eleventh and fifteenth days respectively) showed the same bacterial findings, except that *B. Proteus* and Pseudomonas persisted until death. The pericecal abscesses were large and the peritoneum showed a resolving inflammation instead of a smooth glossy peritoneum.

V. Intraperitoneal for Two Days. Since it was not clear how much the intramuscular therapy contributed to recovery, five dogs were treated in the same way except that the intramuscular therapy was not given.

Four survived with the usual rapid suppression of coliform bacteria and clostridia. The fifth died within 20 hours with gangrenous appendicitis and fibrinopurulent peritonitis. Thus it appears that an adequate local concentration of the drug is best achieved by the intraperitoneal route. This route, however, is dangerous because of the chemical peritonitis induced by the drug.

It may be concluded that effective therapy during the first 48 hours is decisive and that continued therapy thereafter is not essential. This conclusion was investigated further as follows:

VI. Oral for Two Days. In the previous study[1] the results of oral therapy for five and a half days were about equal to those obtained by intraperitoneal therapy for two days. A more appropriate comparison of these two routes was made by treating five dogs orally for only two days, using the same dose as before, *i.e.*, 500 mg. b.i.d. Three dogs survived after a short illness. Cultures of the peritoneal fluid showed no coliform bacteria or clostridia after the fifth day and were sterile by the tenth day. Autopsy after sacrifice on the twenty-first day showed complete healing except for a very small sterile pericecal abscess. A fourth dog, which died on the fifteenth day, showed the same cultural and autopsy findings except that the abscess was large and contained *B. Proteus* and Pseudomonas. The fifth dog died on the seventeenth day with a resolving fibrinous peritonitis and a large pericecal abscess. In this dog cultures were positive for coliform bacteria, clostridia, *B. Proteus* and Pseudomonas up to the tenth day. The latter two also were present in the abscess.

Thus oral therapy for only 48 hours yielded a survival rate of 60 per cent, and a peritonitis cure rate of 80 per cent, while oral therapy for five and a half days yielded a survival rate of 75 per cent and a peritonitis cure rate of 80 per cent. These results are substantially the same as with treatment by the intraperitoneal route.

Comment. Although the dose by the oral route is higher than can be tolerated parenterally, it is not likely that the result is a higher intraperitoneal concentration of the drug than can be achieved by either the intravenous or the intramuscular route. Experience has shown that to achieve a given intravenous concentration the dose given orally must be much larger than a dose given intravenously.[6] The ineffectiveness of the intravenous route suggests that most of the effectiveness of the drug given orally is due to its ability to suppress the intra-intestinal flora. The virtue of suppressing the intra-intestinal flora is that the defense reaction is relieved of having to deal with much more than the initial contaminating load.

One may, therefore, conclude that the oral route is superior to all others, including the intraperitoneal route, which, though bacteriologically the most effective, is too irritating to be therapeutically safe.

DISCUSSION

Since death occurs within 42 hours if antibiotics are not used, it is obvious that

effective antibacterial action must be made available within this period. The pathogenic potential of the initial contaminating load is the same whatever mode of therapy is applied thereafter. Since there is no reason to assume that that fraction of the drug given orally which is absorbed into the circulation is of any greater therapeutic value than that given intravenously or intramuscularly, the superiority of the oral route is due to a very rapid suppression of intra-intestinal bacteria which continue to contaminate the peritoneal cavity. The speed of such action cannot be accurately determined from the results of serial stool cultures, for obvious reasons. The therapeutic importance of the suppression of the intra-intestinal flora is further emphasized by the fact that the recovery rate was 75 per cent when the appendicitis was produced immediately after effective degermation of the gut had been achieved, without subsequent antibiotic therapy.

The therapeutic superiority of parenteral penicillin to parenteral aureomycin in appendiceal peritonitis in the dog can be explained on the basis of its lack of toxicity rather on its antibacterial qualities.[1] In man, in contrast to the dog, parenteral penicillin is less effective than aureomycin by any route for the treatment of diffuse peritonitis.[5]

It is not intended to draw a parallel from these findings in the dog to the therapeutics of aureomycin in man. There are no data as to the relative effectiveness in man of oral and parenteral aureomycin in the treatment of peritonitis due to intestinal bacteria. In man the tolerance to intravenous aureomycin is such that as much can be given by this as by the oral route. Wide differences in the results between the two routes seem not to have been demonstrated in man.

But the data indicate what should be valid for man as well as the dog: (1) that aureomycin has special value in its capacity for rapid suppression of intra-intestinal bacteria, and thus provides maximum protection against continuing contamination of the peritoneal cavity and (2) that the oral route for this purpose is optimal.

SUMMARY AND CONCLUSIONS

1. Aureomycin administered by the intravenous or intramuscular route has a definite antibacterial effect on the intestinal flora of normal dogs. This action is weaker and slower than that produced by the drug given orally. An intravenous dose comparable to an effective oral dose is toxic and causes death. An intramuscular dose comparable to an effective oral dose cannot be given owing to the severity of the local reaction.

2. The survival rate from an otherwise fatal peritonitis induced by trauma to the appendix averaged 75 per cent when the drug was given orally in various ways. When the drug was given intramuscularly in tolerable doses the survival rate was 50 per cent. When the drug was given intravenously in any dose, the survival rate was only 12.5 per cent. The failure of intravenous therapy might be explained on the basis of too rapid excretion, but it is more likely due to a toxic effect of the drug given by this route which interferes with the defense reaction.

3. Intraperitoneal administration induces a chemical peritonitis, and if continued for more than 48 hours is likely to cause death. When this route was used for not over 48 hours, the survival rate was 80 per cent. In contrast to all other routes of administration, effective antibacterial action was evident in the very first cultures of peritoneal fluid taken immediately after the induction of the peritonitis. This action was sustained after the drug was discontinued. However, this route of administration is too dangerous to be practicable.

4. The cure rate of the peritonitis was 80 per cent or better by the oral route, 80 per cent by the intraperitoneal route, 70 per cent by the intramuscular route and ten to 20 per cent by the intravenous route. The superiority of the oral route is due not to the

action of the absorbed fraction upon the intraperitoneal process, but upon the rapid suppression of the pathogenic potential of the intra-intestinal bacteria which continue to contaminate the peritoneum after the initial trauma to the appendix has been produced.

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