Urological Research 2, 73-77 (1974)

The Effect of Antibiotic Drugs on Wound-Healing

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Received: May 2, 1974

Summary. The effect of various antibiotic drugs on wound-healing was investigated following parenteral administration in rats. The criterion for healing was the tensile strength of an incised and sutured healing skin wound. Therapeutic doses of gentamycin and tetracycline and sub-toxic doses of ampicillin demonstrated a significant inhibition of cicatrisation. The underlying biochemical mechanism involved in delays in the wound-healing process that are not attributable to bacterial causes is assumed to be the inhibition of protein synthesis, particularly that of connective tissue.

Key words: Animal experiments in wound-healing, disturbance in wound-healing by antibiotics.

Post-operative disturbances in wound-healing often indicate serious complications which, in addition to prolonging hospitalization, can jeopardize the result of the operation. On the assumption that any pathogens invading the site of operation will thus be destroyed, chemoprophylaxis is often carried out following aseptic surgery to prevent secondary healing caused by bacterial infection. Under this type of chemoprophylaxis an increase in aseptic disturbances in healing such as sterile haematoma and seroma, aseptic necrosis, tension-induced dehiscence and rupturing has nevertheless been observed. Consequently antibiotics have been suspected of exerting a negative effect on the healing process in the wound area (2, 5).

Because prophylactic administration of antibiotics is frequently practised in urological cases, where resistance to infection is often low, the possibility of a relationship between chemoprophylaxis and delays in the healing process of non-bacterial origin was investigated in experiments with animals.

Methods

86 albino Wistar rats (average weight - 260 g) kept under standard conditions were anaesthetised with Nembutal, and the skin between the scapulae was completely incised for a length of 3 cm under sterile conditions. The wound was immediately closed with 3 interrupted sutures.

The test animals were divided into two groups of 44 (Group I) and 42 (Group II) animals. The sutures were then removed under anaesthesia (Nembutal), those of the animals in Group I on the third post-operative day and those of the animals in Group II on the seventh. The tensile strength of the wounds was measured directly thereafter.

For the purposes of measurement a fold of skin was drawn up on each side of the wound center 1/2 cm from the border of the wound and held with a clamp. The clamp on one side of the wound was fastened securely, while a linearly increasing traction was brought to bear on the clamp on the other side. This effect was achieved by connecting the clamp by means of a string drawn over a cylinder with a container into which water flowed continually. The weight of the container at the time of rupture revealed the tearing point in grams as a gauge for the tensile strength of the wound.

The rats were administered an antibiotic solution by intraperitoneal injection twice daily from the second prae-operative day until the day of the test. Ampicillin, colistin, gentamycin and rolitetracycline- antibiotics with various properties which have proved successful in the clinical treatment of urological disorders- were used in the experiments.

Each of the four antibiotics was administered in two dose ranges, one therapeutic and one subtoxic, as established according to the scientific literature and manufacturers' instructions (Tab. 1). The con-

Table 1. LD_{50} in comparsion to the applicated therapeutic and subtoxic doses of the four used antibiotics (mg/ kg rat/ day i. p.)

Antibiotics	LD_{50}	Applicated	
		Therapeutic dose	Subtoxic dose
Ampicillin	2 000	52	600
Colistin	33	6.7	12.6
Gentamycin	980	8.8	43.6
Rolitetracycline	85 i.v.	24.2	96.8

trol rats were kept under the same test conditions but were given intraperitoneal injections of a physiological saline solution instead of the antibiotic solution.

Results

The tensile strength of the skin wounds of the control rats on the third and seventh post-operative days averaged 94 g and 325 g respectively, values corresponding approximately to those cited by other researchers (3, 15).

In the animals treated with antibiotics, no significant decrease in the tensile strength of the wounds was observed on the third day after operation (Group I; Tab. 2, Fig. 1). In fact, tensile wound strength was increased by ampicillin and tetracycline in both dose ranges and by gentamycin in subtoxic doses. The tearing point remained in the normal range under administration of colistin in both dosages and of gentamycin in therapeutic dosage.

On the seventh day after operation (Group II) the average tear resistance of the wounds of the animals receiving both dosages of colistin did not differ from that of the controls. In all other groups, however, there were decreases of varying degree in tear resistance (Fig. 1). This decrease was significant for the groups given gentamycin and tetracycline in both dose ranges and for the group administered subtoxic doses of ampicillin (Tab. 2).

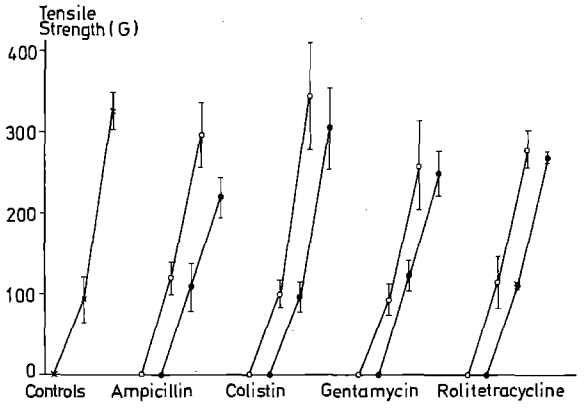

Fig. 1. Various increasing tensile strength of healing wounds with and without application of different antibiotics from operation day to third and seventh postoperative day. o = animals treated with therapeutic doses o = animals treated with subtoxic doses x = untreated controls

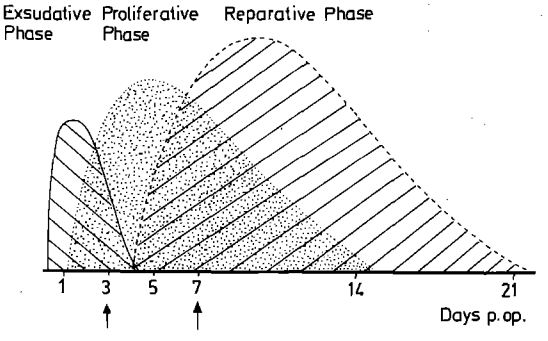

Fig. 2. The three phases of normal wound-healing, (/ = tensile strength of wound tested).

Discussion

In the field of urology, as in other fields of medicine, the rapid increase in the resistance of pathogenic bacteria to nearly all antibiotics represents a constantly growing problem that cannot be solved solely through the development of new chemotherapeutics. It is of primary importance that the use of antibiotics be reduced by restricting the instances in which these drugs are indicated in

Table 2. Tensile strength of healing wounds with and without application of antibiotics (n = number of animals, $\bar{x}$ = average value, $\pm$ s = standard deviation, p = significance)

Antibiotics	Tensile strength (G)							
	3. Day P.O.				7. Day P.O.			
	n	$\bar{x}$	ts	P	n	$\bar{x}$	$\pm$	P
Untreated controls	6	93.6	27.1	-	5	325.4	22.7	-
Ampicillin								
Therapeutic dose	4	121.0	18.9	>0.05	5	296.6	39.7	0.2/0.1
Subtoxic dose	5	108.2	30.1	>0.30	4	217.4	25.7	<0.001
Colistin								
Therapeutic dose	4	99.7	16.5	>0.60	5	342.8	67.0	0.6/0.5
Subtoxic dose	6	97.9	16.5	>0.70	5	304.0	57.6	>0.4
Gentamycin								
Therapeutic dose	5	94.3	16.6	>0.90	5	256.8	56.7	0.05/0.02
Subtoxic dose	4	122.3	17.6	0.05	4	247.4	28.2	<0.001
Rolitetracycline								
Therapeutic dose	6	116.7	28.0	>0.10	5	279.2	22.6	0.02/0.01
Subtoxic dose	4	110.5	2.9	>0.10	4	267.7	10.6	0.02/0.01

order to delay the development of multi-resistant and omni-resistant strains of bacteria (4). In the controversial fields of chemotherapy and chemoprophylaxis, the indications for the administration of antibiotic drugs should be reviewed, particularly those for "antibiotic coverage" following aseptic surgical procedures.

Objections to the routine use of such general chemoprophylaxis after aseptic surgery have recently been increasing, and the ineffectiveness of such measures has been pointed out (6, 14, 16). While it is true that local or general administration of sulfonamides or antibiotics reduces local infection, there is nevertheless a corresponding increase in other wound complications not caused by bacteria (2, 5). Consequently the proportion of delays in healing following aseptic operations currently remains at an average of 6%, just as in the pre-antibiotic aera (2, 12, 14). Several researchers have even reported increased instances of healing by second intention under antibiotic administration (14).

There are various explanations for the failure of chemoprophylaxis. On the one hand there is the so-called "cavity effect" that occurs wherever there are circulatory disturbances or necroses in the wound area. This means that even in very high doses insufficient concentrations of active substance reach the wound area (6, 16). On the other hand the invading pathogens are often microorganisms that are primarily resistant to the antibiotics routinely administered (4, 14). Moreover, there is a danger that should not be underestimated; i.e., that faith in the efficacy of chemoprophylaxis may often result in the neglect of the basic principles of asepsis and tissue preserving surgery (4, 18).

Although local application of chemotherapeutic agents fulfills the requirement of a high concentration of drug at the site of action, this high concentration cannot usually be maintained long enough to exert a reliable bactericidal or bacteriostatic effect due to rapid absorption. In the last analysis such treatment results only in an alteration of the physiological wound environment, accompanied by fluctuations in the pH value and by tissue irritation. This development leads not infrequently to seroma formation, which in turn promotes secondary wound infections (5).

The actual value of post-surgical chemoprophylaxis can be assessed only when, in addition to its efficacy against invading bacteria, the effect of the antibiotic itself on the healing process is taken into consideration. The animal experiments reported here appear to confirm clinical observations which indicate that antibiotics may have an inhibitory effect on the healing process. High concentrations of antibiotics were purposely chosen for the investigation in order to simulate situations that may occur, e. g. an overdose of antibiotics caused by accumulation of the drug due to renal insufficiency.

The test results are distinguished by the fact that on the third post-operative day an increase, on the seventh, however, a decrease in the breaking strength of the wounds was observed under administration of antibiotics.

The interpretation of these observations necessitates a review of the physiology of wound-healing. The healing process can be divided into three characteristic phases (Fig. 2). The exudative phase begins only a few minutes after the onset of the degenerative changes induced by the injury and is characterized by a typical inflammatory reaction accompanied by fibrinogenesis. Approximately 24 h later the proliferative phase sets in, and with it the processes that lead to a final elimination of the injury, primarily through the immigration of fibroblasts. The first collagen fibers formed by the fibroblasts can be detected on the fourth day. Their appearance introduces the last phase of the wound-healing process, the reparative phase, in the course of which the final scar develops (13).

During the first four days the healing strength of the wound can be determined by the degree of adhesion at its edges. This adhesiveness is a function of the amount of fibrin released and the degree of fibrin net formation. Thereafter the healing strength of the wound is increasingly drawn from the newly-generated collagen. Thus on the third day after operation the degree of tensile strength is indicative of the aseptic inflammatory processes and on the seventh post-operative day of the extent of collagen synthesis.

There is no satisfactory explanation for the slight increase in healing strength sometimes observed during the early phase of cicatrisation under prophylactic administration of antibiotics in the above-described experiments, though it may be assumed that this effect is due to the influence of the antibiotic on the inflammatory processes. It is known that, in addition to their specific antibacterial effect, various chemotherapeutic agents also have a non-specific anti-inflammatory effect on inflammatory processes of every origin (11).

On the other hand, the inhibition of healing observed on the seventh post-operative day in the animals treated with ampicillin, gentamycin and tetracycline is probably attributable to a disturbance in collagen synthesis. This view is also supported by the experiments of other researchers, who investigated the effect of antibiotics on animal protein metabolism. Collagen is made up of amino acids and represents the most important protein of the fibers and skeleton of the mammalian organism (8). Consequently an antibiotic-induced disturbance of protein synthesis must also affect collagen formation and thereby the healing process.

Kindler (10) was able by means of autoradiography to demonstrate a significant decrease in the rate of protein and cytoglobulin metabolism in lesions of the abdominal wall in rats given intramuscular injections of tetracycline or a penicillin-streptomycin mixture. In pregnant animals injections of tetracycline also produced an inhibition of calcification and of collagen formation in the bones of the foetus (7). Sulfonamides, penicillin, actinomycin C, neomycin and trimethoprim induced an inhibitory effect on the formation of reparative granulation tissue in the same animal species (11). In addition Aine and Kulonen reported decreased healing strength under parenteral treatment with streptomycin, tetracycline and cycloheximide. In their experiments, the DNA and RNA content of the granulation tissue was decreased by 65 % and 3 % respectively (1).

Struck et al. investigated the effect of chemoprophylaxis by local application (17). They found - again in the rat - that in some cases a significant inhibition of healing was induced in the early post-operative phase by penicillin and chloramphenicol and in the collagen phase by penicillin and tetracycline. Hydroxyproline, an amino acid typical for collagen, was found in the urine in simultaneous assay, and therefore it was assumed that the delay in wound-healing was caused by disturbances in collagen synthesis. The histological findings corresponded with these results. Under treatment with penicillin a large number of fibroblasts with a totally irregular new formation of collagenous fibers was found on the eleventh day after operation.

In addition to these changes in protein metabolism, Jorns (9) described a continuation of fibrinolytic activity of the wound exudation after local application of penicillin and sulfonamide powders and employed this observation to account for an inhibition of granulation tissue formation.

Thus on the basis of these experimental results it can be assumed that in animals various antibiotics, especially after local application, have an inhibitory effect on protein and collagen synthesis and thereby on the healing process which is dependent on this synthesis. In contrast to the view advanced by Hernandez-Richter and Struck (8), this also appears to hold true for the parenteral administration of chemotherapeutical agents, as the above-described experiments have indicated.

Since the basic physiological principles of wound-healing are identical in all mammals, the findings made in experiments with animals may also be assumed without great reservations to be valid for man. These results could thus offer a plausible explanation for at least some of the de-

lays in healing not attributable to bacteria that were clinically observed under antibiotic coverage.

Consequently the investigations described above appear to cast doubt on the value of parenteral and oral chemoprophylaxis following aseptic surgery. In addition to the doubtful efficacy of antibiotics against possible invading pathogens, these drugs may also inhibit cicatrisation. Thus it is possible that the administration of an antibiotic may provoke the very complication it was intended to prevent.

It would nevertheless be completely incorrect to attribute all delays in healing of non-bacterial origin to chemoprophylaxis. The process of cicatrisation is in fact subject to the influence of numerous other endogenous and exogenous factors, among which the extent of iatrogenically-induced trauma continues to play a decisive role. The healing power of wound edges damaged by retractors, surgical hooks, and other blunt surgical instruments is certainly impaired.

On the other hand, it should be emphasized that the experimental results detailed in this paper do not apply to the administration of antibiotics after surgical intervention in cases involving infected tissue, where chemotherapy, rather than the chemoprophylaxis discussed in this paper, is indicated.

Acknowledgement. The authors would like to express their appreciation to Miss Waltraud Schröter for her conscientious technical assistance.

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