The Effect of Multiple Lathyrogenic Agents Upon Wound Healing in Rats[1] (37007)

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Different classes of lathyrogens interfere with inter- and intramolecular covalent cross-bonding at different steps in the maturation of collagen. For example, β-aminopropionitrile, a monoamine oxidase inhibitor, specifically inhibits action of lysyl amine oxidase, which converts lysyl residues near the N-terminal ends of the alpha chains of collagen into corresponding γ-semialdehydes (3, 4, 9, 11, 12). Thus, an early event in covalent cross-bonding, formation of aldehyde groups, is prevented. D-Penicillamine also inhibits covalent cross-bonding of collagen but apparently acts by chelating lysyl derived aldehydes after they have been formed (5, 6, 7, 13). Therefore, in the enzymatic conversion of lysine to aldehyde, BAPN inhibits the action of an important enzyme and D-penicillamine chelates the end product. It is not possible to prevent the formation of all aldehydes by administration of low toxicity doses of BAPN. Addition of D-penicillamine, which blocks covalent cross-bonding at a different step and whose toxicity is not additive with that of BAPN, could be therapeutically useful. Simultaneous administration of BAPN and D-penicillamine theoretically could produce additive inhibition of covalent cross-bonding without increasing toxicity due to either drug alone. To test this hypothesis the following experiments were performed.

Materials and Methods. The first group of experiments were 10-day toxicity studies for BAPN and D-penicillamine in female Sprague-Dawley rats weighing approximately 200 g each. Weight gain and mortality were utilized to measure toxicity. The dose of BAPN ranged from 23 to 300 mg/100 g of body weight/day; D-penicillamine doses ranged from 25 to 250 mg/100 g of body weight/day. Seventy-six rats were divided into 19 groups of four rats each. Nine groups received BAPN, eight groups received D-penicillamine, and two control groups received saline. BAPN was diluted in sterile water and injected intraperitoneally; D-penicillamine was diluted in normal saline and injected subcutaneously. Rats receiving D-penicillamine and control rats were given pyridoxine hydrochloride (20 mg/rat/day). Control animals were pair-fed against experimental groups.

The second group of experiments was designed to test the hypothesis that two lathyrogenic agents acting at different steps in the cross-bonding process could produce an additive effect. Female Sprague-Dawley rats, weighing approximately 200 g, were divided into eight groups of 10 rats. Wounds were created by making 2 cm midline longitudinal incisions through skin and panniculus on the dorsal surface of the neck. A second wound was made 3 cm caudal to the neck wound. Skin edges were coated with No. 40 steel interrupted sutures and allowed to heal for 10 days. Group I received 23 mg BAPN/100 g body weight/day. Group II received 10 mg D-penicillamine/100 g body weight/day; Group III received BAPN and D-penicillamine in the above doses and Group IV received saline during the healing period. Group V received 100 mg BAPN/100 g body weight/day. Group VI received 20 mg D-penicillamine/100 g body weight/day; Group VII received both BAPN and D-penicillamine in the same doses as Groups V and VI; Group VIII received saline.

At the end of 10 days the entire dorsal neck incision, with 2 cm of surrounding nor-

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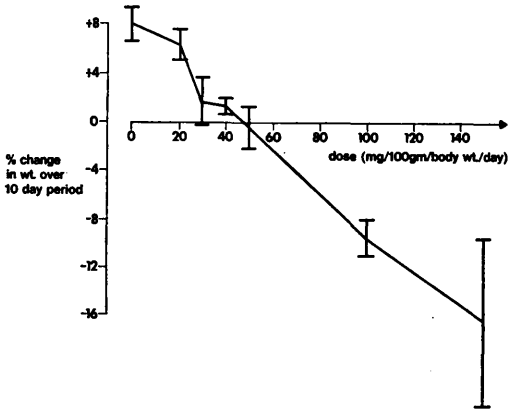

FIG. 1. Dose/toxicity graph of β-aminopropionitrile in rats.

mal skin on either side, was excised. From this sample a 1 cm section of scar grossly free of infection or disruption was selected, excised together with surrounding skin, and the burst strength was measured by the Charney, Williamson, Bernhard method (1). The caudal wound was excised, coarsely minced, placed in 0.45 *M* NaCl (2 ml/0.1 g tissue) and shaken with a wrist-action shaker for 24 hr at 4°. After centrifugation for 1 hr at 37,000*g* and 4°, the supernatant was passed through a Frittered disc filter of medium porosity. An equal volume of concentrated HCl was added to the supernatant and 6 *N* HCl (1 ml/16 mg tissue) was added to the residue. Both samples were hydrolyzed overnight at 120°, evaporated to dryness, and resuspended in distilled water. The samples were neutralized, diluted, and analyzed for hydroxyproline by the Woessner method (14).

Results. Toxic signs in rats receiving BAPN included alopecia, diarrhea, failure to gain weight, and death. Toxic signs were dose related. All animals receiving greater than 150 mg BAPN/100 g body weight/day died within 24 hr. One animal died in the group receiving 150 mg BAPN/100 g body weight/day. These data have been recorded in Fig. 1 as percentage weight change over a 10-day period. Rats receiving BAPN failed to gain weight when the dose was increased above 40 mg BAPN/100 g body weight/day; below 40 mg/100 g body weight no toxic signs were present.

The only toxic sign in rats receiving D-penicillamine was weight loss; all animals survived when doses up to 250 mg D-penicillamine/100 g body weight/day were administered. These data are recorded in Fig. 2. Failure to gain weight was noted when doses greater than 150 mg D-penicillamine/100 g body weight/day were administered.

Data for the low dose groups have been tabulated in Table I. The saline soluble fractions of Groups I and II, while not differing significantly from each other, are significantly different from Group III ($p < 0.01$). Likewise, the burst strengths of Groups I and II do not differ significantly but both are significantly different from Group III ($p < 0.01$). The saline soluble fraction and burst strength of Group IV differ significantly from all other groups ($p < 0.01$). The insoluble collagen fractions in all groups did not differ significantly. Toxicity, as measured by failure to gain weight and mortality, was not observed in Group III.

Results for high dose groups are tabulated in Table II. The saline soluble fractions of Groups VI and VII while not differing significantly from each other are significantly different from Group V ($p < 0.01$). By contrast the burst strengths of Groups V and VII do not differ significantly but both are significantly different from Group VI ($p < 0.01$). The saline soluble fraction and burst strength of Group VIII differ significantly from all other groups ($p < 0.01$). The apparent difference in burst strength between saline controls (Group IV) in the low dose

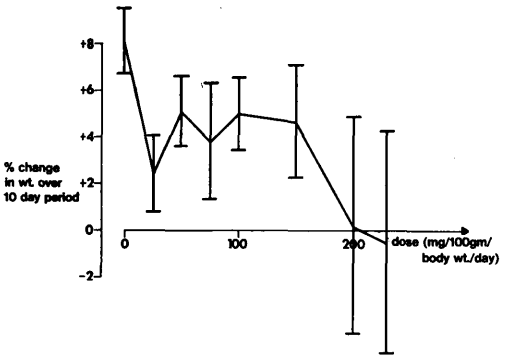

FIG. 2. Dose/toxicity graph of D-penicillamine in rats.

TABLE I. The Effect of Low Doses of BAPN and Penicillamine upon Wound Collagen and Burst Strength of 10-Day-Old Skin Wounds in Rats.[a]

	I BAPN (23 mg/100 g/day)	II Pen (10 mg/100 g/day)	III BAPN + pen (23 + 10)	IV Control (saline)
Soluble collagen (μg/mg)	0.8 ± 0.09	1.0 ± 0.08	1.6 ± 0.20	0.5 ± 0.09
Insoluble collagen (μg/mg)	19.7 ± 0.47	21.9 ± 0.57	20.0 ± 0.73	20.7 ± 0.57
Burst strength (g/cm)	299 ± 20.7	264 ± 17.3	215 ± 15.7	365 ± 12.02

[a] All values are means of 10 animals ± standard errors.

experiment and saline controls (Group VIII) in the high dose experiment is not significant ($p > 0.5$). Although burst strength measurements performed by the Charney–Williamson method have wide variations, the difference between significant ($p < 0.01$) differences and nonsignificant differences as reported in this paper are real and reproducible. The insoluble fractions of Groups V and VII are not significantly different from each other but both differ from Groups VI and VIII ($p < 0.01$). Toxicity was not increased in Group VII as compared to Group V, but toxic signs in both groups were greater than in other groups. All wounds healed without complications.

Discussion. Results of the low dose experiment show that two agents acting at different stages during maturation of newly synthesized connective tissue produce an additive effect upon solubility of wound collagen and burst strength of scar tissue. Relatively small doses of both agents were selected in this experiment to maximize any additive effect. Apparently, aldehydes which formed in spite of lysyl oxidase inhibition were chelated by D-penicillamine and prevented from participating in covalent cross-bonding. Toxicity did not appear to be increased by the use of two agents simultaneously.

In the high dose experiment, effect of two agents upon solubility of newly synthesized collagen and burst strength were not additive. Theoretically, if formation of all aldehydes was inhibited by BAPN, the addition of the chelating agent, D-penicillamine, would have no effect. Our data add support to the concept that BAPN and D-penicillamine act at different stages in the maturation of collagen.

The insoluble fractions in two groups receiving high doses of BAPN (Groups V and VII) were greater than other groups. This finding may be related to technical difficulties excising a wound with very little scar tissue. Wounds in these rats appeared to heal primarily by epithelization.

An interesting result in the high dose experiment was apparent lack of correlation between solubility of newly synthesized collagen and burst strength. Soluble collagen in the D-penicillamine group (Group VI) was significantly greater than the corresponding BAPN group (Group V); yet, burst strengths in the BAPN group were significantly less. These results could be explained either by an effect of BAPN on burst strength unrelated to collagen solubility or an effect of D-penicillamine on collagen solubility unrelated to burst strength. There is evidence to suggest that D-penicillamine solubilizes previously existing insoluble collagen (2, 8). Therefore, increased soluble fraction in Group VI could be the result of solubilization of old collagen not contributing to the burst strength of the experimentally produced scar. Interestingly, in Groups I and V a fourfold increase in the dose of BAPN produced only a 0.2 μg/mg (25%) increase in the soluble fraction whereas the burst strength decreased from 299 to 86.2. This finding could be due to an effect of BAPN not measurable by collagen solubility or a general toxic effect of BAPN. Before

TABLE II. The Effect of High Doses of BAPN and Penicillamine upon Wound Collagen and Burst Strength of 10-Day-Old Skin Wounds in Rats.[a]

	V BAPN (100 mg/100 g/day)	VI Pen (20 mg/100 g/day)	VII BAPN + pen (100 + 20)	VIII Control (saline)
Soluble collagen (μg/mg)	1.0 ± 0.08	1.5 ± 0.1	1.6 ± 0.07	0.4 ± 0.01
Insoluble collagen (μg/mg)	25.3 ± 0.9	17.0 ± 0.6	24.2 ± 1.2	19.4 ± 0.6
Burst strength (g/cm)	86.2 ± 7.4	217.8 ± 18.0	92.5 ± 9.2	437.9 ± 93.8

[a] All values are means of 10 animals ± standard errors.

postulating a general toxic effect, however, a dose–response curve for BAPN should be determined with strict quantitative measurements of burst strength and soluble fractions. The fact that the soluble fraction was never greater than 1.6 μg/mg in any of the groups suggests that the number of cross-links available to the agents was limited.

Therapeutic utilization of such data will require demonstration of a similar additive effect following simultaneous administration of multiple lathyrogenic agents to human beings and further study of signs and symptoms previously reported following administration of BAPN. Malaise, nausea, vomiting, dermatitis, and a mild transient hepatitis similar to Dilantin or Compazine hypersensitivity have been reported (10). Such extramesenchymal signs and symptoms are not dose related and appear more characteristic of hypersensitivity rather than drug toxicity. Connective tissue effects, however, are dose related and probably represent the major pharmacological effect of lathyrogens.

Summary. 1. Simultaneous administration of BAPN and D-penicillamine in low nontoxic doses produced a significant additive inhibition of cross-bonding in collagen of healing wounds. Additive effect was not observed following administration of high doses of β-aminopropionitrile and D-penicillamine.

2. Toxic signs in rats, including partial alopecia, diarrhea, failure to gain weight, began to appear when the dose of BAPN exceeded 40 mg/100 g body weight/day.

3. Toxicity in rats treated with D-penicillamine was limited to weight loss and was noted when doses greater than 150 mg of D-penicillamine/100 g of body weight/day were administered.

4. These results support the hypothesis that D-penicillamine and BAPN act at different stages in the maturation of collagen and suggest that simultaneous administration of low nontoxic doses of several lathyrogenic agents may be more useful in treating human beings than large doses of a single agent.

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