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Effect of nifedipine and amlodipine on dead space wound healing in rats

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Effect of two calcium channel blockers (CCBs) nifedipine and amlodipine, was studied on normal and steroid depressed wound healing in albino rats, using the dead space wound model. The drugs enhanced normal healing as evidenced by increase in tensile strength of 10 days old granulation tissue. There was neither a significant change in the hydroxyproline level (or collagen) nor a change in the glycosaminoglycan content in granulation tissue. However, lysyloxidase level was increased significantly. The increase in tensile strength could thus be attributed to better cross-linking and maturation of collagen rather than collagen synthesis *per se*. The drugs were also able to overcome steroid depressed wound healing. It is likely that the prohealing effects may be related to the improved antioxidant status too, since superoxide dismutase levels were observed to be higher in the CCB- treated animals.

Keywords: Amlodipine, Antioxidant, Nifedipine, Wound healing
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Calcium channel blockers (CCBs), nifedipine and amlodipine have been extensively used in various cardiovascular and some non-cardiac conditions[1]. Cellular calcium metabolism appears to regulate extracellular matrix production and wound healing[2, 3]. CCBs also have other properties like inhibition of cell migration caused by chemotactic substance[4], vasodilatory effect[5] and production of collagen matrix proteins[4]. Lupo *et al*[6] reported a dose dependent antioxidant activity of several CCBs comparable to that of α-tocopherol. CCBs have been reported to have a synergistic effect with α-tocopherol in reducing gastric mucosal injury by ischemic reperfusion [7]. Keeping in view the various aspects of CCBs in relation to wound healing (vasodilatation, collagenation, antioxidant activity), the effects of two CCBs, namely nifedipine and amlodipine, on normal and steroid depressed dead space wound healing had been studied in albino rats.

Animal —Healthy Wistar rats (150-250g) of either sex, bred locally were used. They were housed individually in clean polypropylene cages, fed pellated rat chow (Hindustan lever rat pellets) and water *ad libitum*. Animals showing signs of infection were excluded from the study. Ethical clearance was taken from Kasturba Medical College Ethical Committee, Karnataka, India.

Wound model —Dead space wound model was used for the study. All wounding procedures were carried out under light ether anaesthesia. Dead space wounds were created through a small transverse incision in the lumbar region [8]. A polypropylene tube (2.5 × 0.5 cm) was implanted subcutaneously beneath the dorsal Para vertebral lumbar skin. The day the wound was created was considered as day zero. Granulation tissue formed on the polypropylene tube was harvested by careful dissection on the day 10 and used for various investigations.

Drugs —The animals were divided into 6 groups of 8 animals each group. They were treated as described in Table 1.

All the drugs, except dexamethasone, were administered from day 0 to 9. Dexamethasone was

Table 1—Groups of animals given various treatments

Group	Drug	Dose (mg/kg)	Route
1	Control (saline)	0.5	po
2	Nifedipine (NIF)	2	po
3	Amlodipine (AML)	1	po
4	Dexamethsone (DEX)	0.17	im
5	NIF + DEX	2 + 0.17	po + im
6	AML + DEX	1 + 0.17	po + im

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given in the dose of 0.34 mg/kg (im) on day 0 followed by 0.17 mg/kg for the rest of the period.

Parameters —The harvested granulation tissue was cut into two halves and their wet weights noted. One piece was oven dried at 60°C overnight and the dry weight noted. Acid hydrolysate of the dry tissue was used for the determination of hydroxyproline (OH-P)[9], hexosamines (HA)[10] and hexuronicacid (HUA)[11]. Tensile strength of the wet tissue was measured[12]. An extract of the wet tissue 1:10 w/v with 0.02 *M* potassium phosphate buffer (*p*H 7.6) was prepared and protein content[13] and superoxide dismutase (SOD) level[14] were estimated. A part of the wet granulation tissue was homogenized with 0.01*M* potassium phosphate buffer, *p*H 8.2, containing 1.5 *M* urea. The homogenate was centrifuged at 3000 rpm for 10 min and supernatant used as the source of lysyloxidase (LO). Lysyloxidase activity was assayed spectro-fluorimetrically using a monoamine substrate and adaptation of the peroxidase coupled assay [15].

Statistical analysis —The results were analyzed using one way analysis of variance (ANOVA) followed by Post hoc Scheffe's test using SPSS computer package and Student's *t* test. *P* values < 0.05 were considered statistically significant.

Nifedipine and amlodipine *per se* did not have any significant change in the granulation tissue dry weight and hydroxyproline level but tended to increase the tensile strength of the granulation tissue (Table 1). While dexamethasone showed depressed wound healing as evidenced by significant decrease in dry weight of the granulation tissue, hydroxyproline level and tensile strength (Table 2). However both nifedipine and amlodipine reversed the dexamethazone depressed wound healing as evidenced by significant increase in tensile strength and dry weight of granulation tissue but not much change in the hydroxyproline level. (Table 2)

There was no increase in glycosaminoglycon content but the levels of enzymes superoxide dismutase and lysiloxidase were significantly increased. As CCBs indicating better cross-linking of collagen bundles and tensile strength of granulation tissue (Tables 2,3).

Nifedipine and amlodipine belong to the dihydropyridine group of CCBs, having some structural similarity to the antioxidant vitamin α-tocopherol [6]. Several compounds having antioxidant property (vitamins A, E, C, ß-carotene and trolox) are reported to enhance healing by acting on different phases of healing like epithelisation, wound contraction, fibrogenesis, vascularization, collagenation *etc* [16]. They have been shown to improve healing even in infected and diabetic wounds [5, 16].

Table 2 —Effect of nifedipine and amlodipine on dry weight, tensile strength and hydroxyproline (OH-P) of granulation tissue.

[Values are mean ± SE, from 8 animals in each group except control, where 6 animals were used]

Drugs	Dry weight (mg/100g)	Tensile strength (G)	OH-P (mg/g of tissue)
Control	36.4 ± 2.4	325 ± 37	28 ± 3.2
Nifedipine (NIF)	41.8 ± 6	427 ± 18	23 ± 2
Amlodipine (AML)	38 ± 2	407 ± 13	24.3 ± 2.9
Dexamethasone (DEXA)	22 ± 3.6	253 ± 40	18.8 ± 1.7
NIF + DEXA	44 ± 5.7[b]	497 ± 15[a,b]	32 ± 5.3
AML + DEXA	96 ± 15	485 ± 30[a,b]	18.6 ± 2.7

P values: < 0.05,
[a] vs. control and [b] *vs.* DEXA

Table 3— Effects of nifedipine and amlodipine on superoxide dismutase (SOD), lysiloxidase (LO), hexuronic acid (HUA) and hexosamines (HA)

[Values are mean ± SE from 8 animals in each group]

Drugs	SOD (Units/g of wet tissue)	LO (SFU/g of wet tissue)	HUA (mg/g of dry tissue)	HA (mg/g of dry tissue)
Control	900±73	1183±160	14.8±9.6	11.92±2.3
Nifedipine	1116±111	3000±300[a]	15.3±2.1	12.3 ±2.5
Amlodipine	1205±95	1583±217	2.39±1.58	10.9±2.4

[a] $P<0.05$ vs control
SFU- Spectroflurometric units

Nifedipine and amlodipine improved normal healing apparently by promoting collagen maturation (better cross-linking). While in the case of steroid depressed healing, both were able to overcome the suppression by promoting collagenation as evidenced by increase in lysiloxidase and superoxide dismutase.

Overall, the prohealing action of the two CCBs seems to be related to their antioxidant, vasodilatory and collagen maturation properties. Hence it can be concluded that these CCBs can be safely used in patients undergoing surgery; their prohealing effect can be utilized favourably, especially when the patient is on a known suppressor of wound healing like antineoplastic agents.

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