WOUND HEALING POTENTIAL OF PYRAZOLE DERIVATIVE

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Summary

Pyrazole derivative: 2-{5-[4-(dimethylamino) phenyl]-4*H*-pyrazol-3-yl} phenol was synthesized using chalcone synthesis and cyclization reactions. Its wound healing potential was evaluated on rodents using incision, excision and dead space wound models at different doses viz. 100, 200 and 300 mg/kg. In incision wound model, it showed significant ($p< 0.05$) increase in breaking strength with and without dexamethasone in mice on all doses. In excision model, it showed significant increase in contraction on 200 mg/kg or higher doses without dexamethasone, while with dexamethasone showed significant contraction on all doses in mice on different time intervals. Also they showed significant decrease in epithelisation period on all doses. In dead space model, it showed increase in granuloma breaking strength, but it was significant only on high dose (300mg/kg) without dexamethasone and significant on all dose levels with dexamethasone. Results indicate the significant pro-healing effect of synthetic pyrazole derivative.

Keywords: Pyrazole derivative,Wound healing Dexamethasone,Breaking strength,Granuloma tissue, Epithelization

Introduction

Wound healing is a complex phenomenon driven by processes, viz., induction of an acute inflammatory process, regeneration of parenchymal cells, migration and proliferation of both parenchymal and connective tissue cells, synthesis of extra cellular matrix proteins, remodeling of connective tissue, and acquisition of wound strength[1]. When tissue is disrupted following injury, collagen is needed to repair the defect and restore anatomic structure and function. The healing process requires a sophisticated interaction among inflammatory cells, biochemical mediators, extra cellular matrix molecules and micro-environmental cell population[2]. Wound healing disorders present a serious clinical problem and are likely to increase since they are associated with diseases such as diabetes, hypertension and obesity. Wound healing is a complex and complicated process. It runs through a number of phases, such as coagulation, inflammation, granulation, fibroplasia, collagenation, wound contraction and epithelialization etc., that occur between injury and healing. These phases either run concurrently or intimately inter-linked through some chemical, biochemical and cellular pathways.

Pyrazoles are used for their analgesic[3], anti-inflammatory[4], antipyretic, antiarrhythmic, tranquilizing, muscle relaxing, psychoanaleptic, anticonvulsant, monoamineoxidase inhibiting, antidiabetic and antibacterial activities. Some derivatives have also proved to show wound healing activity[5]. Pyrazole derivatives were found to possess antioxidant activity[6], hence their use in wound healing may be correlated with it.

Materials and Methods

Chemicals and drugs**:-** Dexamethasone, P-dimethyl aminobenzaldehyde, Ether, sodiumhydroxide, o-hydroxyacetophenone, Hydrochloric acid, Ketamine, Methanol, hydrazine hydrochloride, sutures, thread, needles, surgical instruments.

Synthesis procedure:

Preparation of chalcone:

Equimolar quantities of p-dimethyl aminobenzaladehyde and o-hydroxy acetophenone (0.01mol) were dissolved in minimum amount of alcohol. Sodium hydroxide solution (0.02mol) was added slowly and the mixture stirred for 6 hr until the entire mixture becomes reddish. Then the mixture was poured slowly into 400 ml of water with constant stirring and kept in refrigerator for 24 hours. The precipitate obtained was filtered, washed and recrystallized from ethanol. The completion of the reaction was monitored by TLC. [7]

Preparation of pyrazole derivatives:

Chalcone obtained from first reaction (0.02mol), hydrazine hydrochloride (0.02mol) and sodium acetate in ethanol (25 ml) was refluxed for 6hr. The mixture was concentrated by distilling out the solvent under reduced pressure and poured into ice water. The precipitate obtained was filtered, washed and recrystallized. The completion of the reaction was monitored by TLC[7].

Pyrazole derivative: 2-{5-[4-(dimethylamino) phenyl]-4*H*-pyrazol-3-yl} phenol (precipitate-cyclized product) was obtained. Molecular weight (279.33) and structure was confirmed by GCMS.

Animals

Healthy in bred male rats of Wistar strain weighing between 180g -250g were used for dead space wounds studies and male Swiss albino mice weighing 25-40g were used for incision and excision wound healing studies. They were individually housed and maintained on normal food and water *ad libitium.* Animals were periodically weighed before and after the experiments. The rats were anesthized during infliction of the wounds. The surgical intervention was carried out under ketamine anesthesia (10mg/kg). The surgical material was sterilized and the skin was prepared by depilation of the fur. Animals were closely observed for any infection and the ones showing any signs of infection were separated and excluded from the study.

All the studies were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.(Institutional Animal Ethical Committee, Manipal University, Karnataka, India; Approval No# IAEC/KMC/07/2007-2008)

Toxicity study:-Acute toxicity study was conducted by "up and down or staircase method" in both rat and mice.[8, 9]

Statistical analysis:-The results are expressed as mean ± S.E.M. One-way ANOVA using Tukey's test for post-hoc analysis were applied to the results. Mean values and median values were considered significantly different when $P < 0.05$.

1). Incision wound (for collagenation Phase):-

Two para-vertebral straight incisions of 4 cm each were made of entire thickness of skin on either side, at least one cm lateral to the vertebral column[8]**,** after mopping with alcohol the dry wounds were closed with zero silk threads with the help of curved needle. The interrupted sutures were placed at equidistant points of 1 cm each. Wounds were again mopped with cotton swabs soaked in 70% ethanol. Animals were treated daily with drugs from 0- 9th post wounding day.[9, 10]

2). Excision wound (for wound contraction, Period of epithelialization):-

Accupunch of 10 mm in diameter was impressed on the dorsal thoracic central region, 5cm away from the ears of anaesthetized mice. Full thickness skin was excised to get a wound measuring 79 mm2. After achieving full homeostasis by bloating the wound with cotton swab soaked in warm saline, animals were placed in their individual cages. Animals received drug from 0 day to 21st post wounding day. In this wound model two physical attributes of healing namely wound contraction and epithelization were studied.[11]

3). Dead space wound:-

The dead space wound was created by implanting subcutaneously 2.5×0.5cm polypropylene tube in the lumbar region on the dorsal side[9]. Animals received drugs from 0 day to 9th post

wounding day. On day 10th post wounding day, granulation tissue harvested on the implanted tube was carefully dissected out along with the tube. The tubular granulation was cut along its length to obtain the sheet of the granulation tissue which was further cut into two approximately equal pieces. The breaking strength was measured as described in under the incision wound model. The average of the two readings is taken for group mean. The pieces of granulation tissue collected were dried at 60°C for 24 hours and noting down the dry weight of granulation tissue.[12, 13, 14]

Dexamethasone: 2ml vial of dexamethasone injection (Dexona vials, 8mg dexamethasone/ 2ml) was diluted up to 47 ml with distilled water and administered intramuscularly in a dose of 0.17 mg/kg on alternative day from day 0-9 days in incision and dead space models while for excision study it was from day 0-21st post wounding day.

Results

Toxicity study:- Pyrazole derivative was found safe up to 2000 mg/kg b.w. (oral dose) in mice and rats.

1) Incision wound (for collagenation Phase):-

1a) Skin breaking strength in normal mice treated with Pyrazole derivative

Treatment (dose, mg/kg)	Breaking Strength(g)
Vehicle treatment	103.4±3.4
Pyrazole derivative(100)	174.7±5.1[a]
Pyrazole derivative (200)	193.7±6.3[a]
Pyrazole derivative (300)	201.9±6.9[a]

*Animals were treated with pyrazole derivative for 9days. Animal were sacrificed on day 10th and skin breaking strength was assessed by constant water flow method. All values are Mean± SEM of 5 mice. a denotes $P< 0.05$ compared with vehicle treatment

1b) Skin breaking strength in mice with dexamethasone (0.17 mg/kg) treated with pyrazole derivative

Treatment(dose, mg/kg)	Breaking strength (g)
Vehicle treatment	59.08±1.8
Pyrazole derivative(100)	136.8±4.2[a]
Pyrazole derivative(200)	167.6±4.6[a]
Pyrazole derivative(300)	178.8±6.8[a]

*Animals were treated with pyrazole derivative for 9days, along with dexamethasone on alternate day. Animal were sacrificed on day 10th and skin breaking strength was assessed by constant water flow method. All values are Mean± SEM of 5 mice. a denotes $P< 0.05$ compared with vehicle treatment

2) Excision wound (for wound contraction, Period of epithelialization):-

2a) Wound contraction on day 10th in normal mice treated with Pyrazole derivative (% of original wound size of 79 mm2)

Treatment(mg/kg)	Wound contraction* (% of original wound size of 79 mm^2)			
	Day-4	Day-8	Day-12	Day-14
Vehicle treatment	23.16±1.35	49.46±2.35	76.60±2.95	82.70±0.78
Pyrazolederivative (100)	24.92±2.25	56.81±2.21	83.28±0.96	93.4±2.34
Pyrazolederivative (200)	32.6±2.71[a]	59.54±2.56	86.28±1.10[a]	95.6±4.10[a]
Pyrazolederivative (300)	34±2.37[a]	69.72±2.9[a]	89.91±1.12[a]	97.1±2.68[a]

*Skin from back of animal was excised and their area was measured on alternate day and contraction of the wound was assessed. All the values are mean ± SEM of 5 mice, a denotes P< 0.05 compared with vehicle treatment

2b) Wound contraction on day 10th in mice challenged with dexamethasone, 0.17 mg/kg (% of original wound size of 79mm2)

Group (Dose, mg/kg)	Wound contraction* (% of original wound size of 79 mm^2)			
	Day-4	Day-8	Day-12	Day-14
Vehicle treatment	15.50±1.56	39.76±0.93	66.20±0.32	73.46±2.74
Pyrazolederivative(100)	26.54±1.34[a]	56.23±1.21[a]	78.58±2.21[a]	87.60±3.40[a]
Pyrazolederivative(200)	30.12±2.23[a]	61.12±1.56[a]	82.45±1.90[a]	90.89±2.54[a]
Pyrazolederivative(300)	34.12±2.60[a]	65.35±2.40[a]	86.24±2.60[a]	93.85±1.12[a]

*Skin from back of animal was excised and their area was measured on alternate day and contraction of the wound was assessed. All the values are mean ± SEM of 5 mice, a denotes P< 0.05 compared with vehicle treatment

2c) Period of epithelization (days) on day 10th in normal mice treated with Pyrazole derivative

Treatment(mg/kg)	Epithelization period
Vehicle treatment	15.10±0.80
Pyrazolederivative (100)	11.1±0.56[a]
Pyrazolederivative (200)	10.8±1.3 [a]
Pyrazolederivative (300)	9.5±0.84 [a]

*After inflicting wound the day of fall of Scab was calculated and considered as period of epithelisation.
All the values are Mean±SEM of 5 mice. a denotes P< 0.05 compared with vehicle treatment

2d)Period of epithelisation (days) in mice challenged with dexamethasone, 0.17 mg/kg

Treatment (mg/kg)	Epithelization period
Vehicle treatment	17.38±0.82
Pyrazole derivative(100)	12.50±0.55[a]
Pyrazole derivative(200))	11.9±1.25[a]
Pyrazole derivative(300)	11.2±1.38[a]

*After inflicting wound the day of fall of Scab was calculated and considered as period of epithelisation.
All the values are Mean±SEM of 5 mice. a denotes P< 0.05 compared with vehicle treatment

3) Dead space wound:-

3a)Breaking strength of granuloma tissue on day 10th in normal rat treated with Pyrazole derivative

Treatment (dose, mg/kg)	Granulation breaking strength(g)
Vehicle treatment	139.7±11.1
Pyrazole derivative(100)	168.3±8.3
Pyrazole derivative(200)	172.2±9.7
Pyrazole derivative(300)	190±10.2[a]

a denotes P< 0.05 compared with vehicle treatment

3b) Breaking strength of granuloma tissue on day 10th in rat challenged with dexamethasone, 0.17 mg/kg

Treatment(dose, mg/kg)	Granulation breaking strength(g)
Vehicle treatment	70.6±5.2
Pyrazole derivative(100)	160±8.9[a]
Pyrazole derivative(200)	175.6±10.1[a]
Pyrazole derivative(300)	182±11.4[a]

a denotes P< 0.05 compared with vehicle treatment

3c) Dry weight of granuloma tissue on day 10th in normal rat treated with Pyrazole derivative

Treatment (dose, mg/kg)	Dry Granulation weight(g)
Vehicle treatment	0.10±0.003
Pyrazole derivative(100)	0.12±0.007
Pyrazole derivative(200)	0.12±0.013
Pyrazole derivative(300)	0.15±0.009[a]

a denotes P< 0.05 compared with vehicle treatment

3d) Dry weight of granuloma tissue on day 10th in rat challenged with dexamethasone, 0.17 mg/kg

Treatment(dose, mg/kg)	**Dry Granulation weight (g)**
Vehicle treatment	0.03±0.002
Pyrazole derivative(100)	0.10±0.013[a]
Pyrazole derivative(200)	0.11±0.015[a]
Pyrazole derivative(300)	0.12±0.028[a]

a denotes P< 0.05 compared with vehicle treatment

Discussion

In the present study, wound healing potential of different doses of *Pyrazole derivative* was explored. Healing impact of *Pyrazole derivative* was studied for its influence on normal as well as delayed wound healing processes. The study was conducted in rodents employing three different wound models, viz., incision, dead space and excision involving phases such as collagenation, granulation, wound contraction and epithelization. In all these models the effect of *Pyrazole derivative* was compared to that of dexamethasone and vehicle.

Results indicated that while dexamethasone significantly impaired the healing process (as observed in all three wound models when compared with vehicle treatment), the *Pyrazole derivative* also affect the normal healing. The pyrazole derivative had the abilities to reverse the healing depressant effects of dexamethasone on all the phases of healing viz., collagenation, granulation, and epithelisation that are monitored in the study. In dead space model compound showed same effect as in dexamethasone model. And both of these effects may be having the same uncertain reasons that are contraversial, for which further studies are required.[15-18]

Acknowledgment

Authors are sincerely thankful to Dr. N. Udupa, Dr. Mallikarjun Rao and Manipal University for their support and facilitation for the work.

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