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STUDY OF WOUND HEALING ACTIVITY OF RIFAMPICIN AND ISONIAZID IN WISTAR RATS

KEY WORDS: Wound healing, Rifampicin, Isoniazid, Incisional model, Excisional model.

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ABSTRACT

Background: Wound healing is a complex physiological process involving inflammation, tissue formation, and remodeling. Rifampicin and Isoniazid, have been reported to exhibit both anti-inflammatory and proinflammatory properties in preclinical models. However, there is a discrepancy in literature regarding their actual effect on wound healing. Hence, this study aims to evaluate and compare the wound healing activity of Rifampicin and Isoniazid using incisional and excisional wound models in Wistar rats. **Methods:** A total of 24 healthy adult albino Wistar rats were randomly divided into four groups. Group I (Control, gum acacia), Group II (Ibuprofen 100 mg/kg), Group III (Rifampicin 54 mg/kg), and Group IV (Isoniazid 27 mg/kg). Incisional wound model and Excisional wound model were used in the study. **Results:** In the incisional model, the mean tensile strength was highest in the Ibuprofen group (479.17 g) compared to other drugs. In the excisional model, Ibuprofen group exhibited significantly enhanced wound contraction from day 6 onwards ($p<0.05$), achieving 97.96% healing by day 22. Rifampicin and Isoniazid showed modest improvement compared to control only on specific days. **Conclusion:** Both Rifampicin and Isoniazid demonstrated limited wound healing potential in comparison to Ibuprofen.

INTRODUCTION

Repair or, healing, refers to the restoration of tissue architecture and function after an injury.[1]

The healing process involves three phases that overlap in time and space: inflammation, tissue formation, and tissue remodeling.[2]

Tissue injury causes the immediate onset of acute inflammation mediated by chemo attractants derived from plasma proteins, resident and recruited hematopoietic cells, extracellular matrix and bacteria. Progression to complete wound healing is accompanied by resolution of the inflammatory response, which is essential for successful repair..[2]

Isoniazid and Rifampicin may aid wound healing through their anti-inflammatory and immunomodulatory effects. However,there appears to be a discrepancy in the reported effects of isoniazid and Rifampicin on wound healing and inflammation. As shown by Priya Gandigawad et al. (2015), both drugs significantly reduced inflammation in experimental models.[3]

However, as noted by David M. Spain (2014), while isoniazid delays early acute inflammation, it does not influence the overall rate of wound healing in mice, suggesting limited direct impact on tissue regeneration.[4]

These conflicting results underscore the need for further research to clarify the role of isoniazid and rifampicin in wound healing.

MATERIALS AND METHODS

Materials:

1) **Animals:** Healthy adult albino rats of either sex weighing between 150-200 grams were used in the experiment. The Study was conducted after approval from the Institutional Animal Ethics Committee. The Study was performed in accordance with the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines.
2) **Drugs And Chemicals:** Drugs (Rifampicin, Isoniazid, Ibuprofen) and chemicals (Gum acacia, Carrageenan, Turpentine oil, Diethyl ether) were obtained from Ozone chemicals, Mumbai, India. Fresh solutions were prepared half an hour before experimenta) Drugs: The standard solution of drugs were taken according to weight of rats and was dissolved in 1% gum acacia suspension. b) Gum acacia: 1% solution
3) The animals were divided into four groups containing six animals in each group:

Group I: Control: Gum acacia in distilled water, 2 ml (p.o.)
Group II: Standard drug: Ibuprofen dissolved in 1% gum acacia [3], 100 mg/kg (p.o.)[5]
Group III: Test drug-1: Rifampicin dissolved in 1% gum acacia , 54 mg/kg (p.o.)[3]
Group IV: Test drug-2: Isoniazid dissolved in 1% gum acacia, 27 mg/kg (p.o.)[3]

4) Equipments:

i) wooden board
ii) cotton thread
iii) saline bottle with infusion set
iv) pulley
v) Urobag
vi) Electronic weighing balance

Experimental Methods:

1) Incision Wound Model:

This method first described by Lee et al also known as constant water method.[6] The rats were anesthetized prior to and during creation of the wounds, with diethyl ether. The dorsal fur of the animals was shaved with depilation cream. A longitudinal paravertebral incision of 6 cm long was made through the skin and cutaneous tissue on the back. After the incision, the parted skin was sutured 1 cm apart using a surgical thread and curved needle. The wounds were left undressed.[7]

The sutures were removed on 8th post wound day and the all the drugs were continued orally.[8]The skin-breaking strength was measured by constant water technique on 10th day.[6]

Each anesthetized animal was secured to the operation table and a line was drawn on either side of wound 3mm away from

the midline. Two allice forceps were firmly applied on line facing each other one of the forceps was fixed while the other was connected to a freely suspended light weight uro bag through a string run over to a pulley. Water was allowed to flow from the reservoir slowly and steadily into the container. A gradual increase in weight was transmitted to the wound site pulling apart the wound edges. As and when the wound just opened up, the water flow was arrested and the weight of water was noted down. [9]

Image 1: Measurement of tensile strength of wound by constant water method

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Image 2A (Right): Measurement of tensile strength by placing allice forceps by wound sides.

Image 2B (Left): Wound dehiscence due to increasing water's weight

Excisional Wound Model:

Described by Morton and Malone [10]. About 300 mm circular area was marked on the back of the rat by a standard ring, and then full thickness of the marked skin was cut carefully.[11] Drugs were administered once daily for 20 days or till the period of complete epithelization in excision wound study.[12] The animal in each group observed on the alternate day and the healing was assessed by using the parameter wound contraction. The wound contraction is calculated by measuring wound area on every alternate day. The wound area in photograph was measured by using the image J software.

Image 3: Excisional wound creation.

Image 4: Healing of wound after 20 days

Percentage Wound Of Healing Were Calculated By Using Walker Formula

Wound area in the day of X Percentage of wound area =----------× 100

Wound area in the first day

Percentage of wound healing = 100 - percentage of wound area.[13]

Statistical Analysis:

Data were entered and analyzed using Microsoft Excel and SPSS version 23. Results are expressed as mean ± standard error of mean (SEM) for continuous variables. The following statistical tests were used:

Descriptive Statistics:

Mean, standard deviation (SD), and standard error of the mean (SEM) were calculated for each group .

Incisional Wound Model (Tensile Strength Analysis):

One-way ANOVA was used to compare mean tensile strength among the four groups: Control, Ibuprofen, Isoniazid, and Rifampicin.

Post hoc pairwise comparisons between groups were performed using the paired t-test.

A P-value < 0.05 was considered statistically significant.

Excisional Wound Model (Wound Area Analysis):

Comparison of wound contraction between groups on alternate days was done using repeated measures ANOVA or paired t-tests, wherever applicable.

RESULTS

Table 1: Measurement Of Tensile Strength In Incisional Wound Model

Group	Mean Tensile strength (gm.)	Std. deviation	Std. error(±)
Control	303.67	30.52	12.46
Ibuprofen	479.17	168.80	68.91
Isoniazid	332.33	112.50	45.93
Rifampicin	339.33	65.49	26.74

Table 2: Comparison Of Tensile Strength Between Two Groups In Incisional Wound Model

Groups	Paired Differences			Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean (±)	
Control Vs Ibuprofen	-175.50	162.37	66.28	0.046*
Control Vs Isoniazid	-28.66	125.98	51.43	0.601
Control Vs Rifampicin	-35.66	77.67	31.70	0.312
Ibuprofen Vs Isoniazid	146.83	232.27	94.82	0.182
Ibuprofen Vs Rifampicin	139.83	198.40	80.99	0.145
Isoniazid Vs Rifampicin	-7.00	161.13	65.78	0.919

*P<0.05 is significant

Table 3: Mean Of Surface Area In Excisional Wound Model Using Image J Software

Day no.	Surface area							
	Control		Ibuprofen		Isoniazid		Rifampicin	
	Mean	SEM(±)	Mean	SEM (±)	Mean	SEM(±)	Mean	SEM(±)
0	300.31	12.41	325.67	9.1	301.95	12.99	300.33	16.58
2	259.99	14.97	249.04	13.41	275.05	12.76	264.01	19.55
4	229.95	19.9	227.33	12.59	229.04	27.61	212.61	18.02
6	221.17	17.1	160.07	21.41	196.77	29.80	182.65	25.30
8	172.18	33.7	109.23	17.23	101.46	16.71	115.53	18.65
10	161.84	34.8	80.97	12.02	71.46	17.06	86.47	21.04
12	149.72	33.8	50.73	11.71	74.02	19.17	55.47	17.32
14	117.85	26.6	38.72	10.3	59.1	19.05	50.01	15.17
16	89.4	20.1	25.44	8.53	53.88	17.82	34.26	8.02
18	66.02	16.62	17.92	7.19	52.58	19.501	29.15	9.24
20	51.79	17.39	11.42	6.16	32.26	17.38	22.78	9.19
22	21.69	6.02	6.5	3.28	24.32	13.85	14.77	6.15

Table 4: Comparison Of % Wound Healing Between

Groups In Excisional Wound Model

Group	Control		Ibuprofen		Isoniazid		Rifampicin	
	Mean	SEM(±)	Mean	SEM(±)	Mean	SEM(±)	Mean	SEM(±)
2	13.408	3.5396	22.290	3.7752	8.9253	.95900	11.338	6.2739
4	22.115	4.1206	30.496	4.787	25.498	7.226	27.781	6.703
6	26.406	4.1168	50.526	6.4345	35.773		38.453	8.236
8	44.340	9.6151	66.953	5.5890	66.786	4.8223	61.758	5.697
10	48.701	10.117	75.077	3.6207	75.746	5.0026	78.030	6.526
12	51.548	10.300	84.103	3.4425	78.596	6.216	80.953	4.826
14	61.531	8.3603	88.270	3.1628	81.156	5.8669	84.071	4.538
16	71.008	6.045	92.111	2.539	82.865	5.493	88.951	2.535
18	78.452	5.369	94.576	2.154	83.288	6.033	90.820	2.640
20	83.381	5.284	96.564	1.847	89.818	5.398	92.586	2.781
22	92.983	1.858	97.968	.9930	92.338	4.307	95.055	1.924

*P<0.05 Is Significant

Table 5: Comparison Of % Wound Healing Between The Groups On Alternate Day For Excisional Wound

Groups	2	4	6	8	10	12	14	16	18	20	22
Control vs Ibuprofen	0.11	0.21	0.01*	0.06*	0.03*	0.01*	0.01*	0.009*	0.01*	0.04*	0.04*
Control Vs Isoniazid	0.25	0.69	0.36	0.06	0.03*	0.04*	0.08	0.17	0.56	0.41	0.89
Control Vs Rifampicin	0.78	0.48	0.22	0.15	0.03*	0.02*	0.03*	0.021*	0.06	0.15	0.45

DISCUSSION

In our study, both Isoniazid and Rifampicin demonstrated poor wound healing potential, likely due to their proinflammatory properties, which may hinder the resolution of inflammation necessary for subsequent healing phases.

For Isoniazid, the mean tensile strength was 332.33 grams, only slightly higher than control (303.67 grams), but significantly less than Ibuprofen (479 grams), indicating weaker collagen formation and mechanical integrity of the healed tissue. In the excisional wound model, the Isoniazid group did not show accelerated healing compared to control, except on day 10 and 12, while the Ibuprofen group demonstrated consistent and faster healing from day 6 to 22. These results suggest that Isoniazid does not significantly support wound repair. This finding aligns with the study by David M. Spain [4], who reported that Isoniazid had no effect on the rate of wound healing . Similarly, Rifampicin showed poor wound healing outcomes. The mean tensile strength in the Rifampicin group was 339.33 grams, much lower than the Ibuprofen group (479.17 grams), indicating weaker connective tissue regeneration. In the excisional wound model, faster healing was observed only on days 10, 12, 14, and 16, whereas Ibuprofen-treated wounds healed more rapidly and consistently across all evaluated days from 6 to 22. These data suggest that Rifampicin, like Isoniazid, does not play a supportive role in wound healing.

Kucharz EJ et al. [14] showed that Isoniazid increases interleukin-2 (IL-2) production and IL-2 receptor expression, suggesting immune activation that may prolong inflammation, thereby delaying healing.

Yael Yuhas and Eva Berent et al. [15]reported that Rifampicin enhances cytokine-induced NF-κB activation and induces iNOS transcription, even in the absence of TNF-α or IL-1β. This leads to increased nitric oxide (NO) production, which, in excess, can damage tissues and impede repair processes.

Yael Yuhas, Inbar Azoulay-Alfaguter et al. [16] also demonstrated that Rifampicin inhibits PGE2 production in IL-1β-stimulated alveolar epithelial cells.

CONCLUSION

In summary, our findings indicate that both Isoniazid and Rifampicin may impair wound healing by sustaining or intensifying the inflammatory response. Further detailed investigations are needed to clarify the inflammatory and wound healing profiles of Isoniazid and Rifampicin across different experimental and clinical settings.

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