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Impaired wound healing due to Cyclophosphamide (CLP) alleviated by supplemental *Ginkgo biloba* (GB)

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Abstract

Objective: To evaluate whether the extract of *Ginkgo biloba* (GB) could reverse the healing suppressant effect of Cyclophosphamide(CLP). Methods: The wound healing effects of CLP (12.6 mg/kg/ IP single dose on 3'd post wounding day) and GB (50 mg/kg IP) daily were tested on dead space and excision wound models in male rats. Results: CLP has significantly ($p<0.05$) reduced and GB has significantly($p<0.05$) increased the breaking strength and hydroxyproline content of granulation tissue in dead space wounds. GB has significantly ($p<0.01$) reversed the suppressant effect of CLP. In case of excision wounds there was no change in wound contraction and epithelization period by CLP, GB or GB+CLP when compared to control. Conclusion: The healing suppressant effect of CLP was reversed by co-administration of GB. This effect of GB may due to its antioxidant property.

Key words: Dead space wounds, excision wounds, granulation tissue, hydroxyproline.

1. Introduction

Antineoplastic agent such as mitomycin C, cyclophosphamide (CLP) ect. inhibit wound healing at all stages [1-3]. This effect, therefore would delay their usage for at least 7-10 days [4,5] after surgical operation. Chemotherapy can achieve most beneficial effect when antitumor agent is given immediately after operation, when the decrease in residual tumor is maximal. In view of this, a method to overcome the suppressant effect of CLP without compromising its antitumor activity is very essential. It is reported that GB promotes wound healing in rats [6]. Hence the present study was undertaken to observe the influence of GB on healing suppressant effect of CLP.

2. Materials and methods

Singly housed male Wistar rats (150-200 g) were used in this study. The study protocol was approved by Institutional Animal Ethics Committee. Following an overnight starving, animals were anesthetized with pentobarbitone and suitably wounded after shaving the area to be operated to bear either dead space or

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excision wounds. The wounds were not dressed and no systemic or local antimicrobial agents were used.

The dried extract of GB (courtesy from M/s. Ranbaxy, Mumbai) standardized to *Gingko flavane* 24% was dissolved in distilled water and administered intraperiotoneally at a dose of 50 mg/kg. Cyclophosphamide injection(200 mg/ 2 ml) was obtained from M/s Biochem Pharmaceutical Industries, Mumbai and administered IP at a dose of 12.6 mg/kg.

2.1 Wound models

2.1.1 Dead space wounds

Dead space wounds were created by implantation of a polypropylene tube (2.5x0.5 cm) beneath the dorsal paravertebral lumbar skin. On day -10 the harvested granulation tissue was subjected to physical as well as biochemical evaluation. Hydroxyproline (measure of collagen) was estimated calorimetrically [7] and breaking strength of the granulation tissue was measured by continuous water flow technique [8].

2.1.2 Excision wounds

Excision wounds were created by excising a circular piece (500 mm^2 in area) of full thickness skin from the dorsal interscapular region [9]. Wound contraction was monitored by measuring wound area, plannimetrically, on alternate days till the wounds were completely healed. This was expressed as percentage of wound concentration. Time taken for complete epithelization was noted by recording the days required for fall of scab leaving no raw wound behind.

2.2 Drug administration

Animals bearing a given wound were divided into four groups. First group of animals received distilled water (l ml/day/ animal) and served as control, second group of animals received a single dose CLP 12.6 mg/kg IP on 3rd post wounding day, third group received GB 50 mg/kg/IP daily for 10 days in case of dead space wounds and for 21 days in case of excision wounds.

2.3 Statistical analysis

Results were expressed as mean ± SE. The data were analyzed by one way ANOVA by Scheffe's post-hoc test.

3. Results

3.1 Dead space wounds

Table 1 shows the effect of CLP, GB and CLP+GB on dead space wounds. The breaking strength of granulation tissue was significantly ($p<0.05$) reduced by CLP. GB not only increased the breaking strength of granulation tissue by itself but also reversed significantly ($p<0.05$) the suppressant effect of CLP.The hydroxyproline (OHP) content of granulation tissue was significantly ($p<0.05$) reduced by CLP. GB increased the OHP content of granulation tissue significantly ($p<0.05$) when compared to control. Co administration of GB resulted in reversal of the suppressant effect of CLP on OHP which was statistically significant (Table I).

Table 1.
Effect of Cyclophosphamide(CLP) and *Gingko biloba* (GB)on dead space wounds

Groups n=8	GTTS(g)	OHP(mg/g of tissue
Control	308±10.46	15.83±1.30
Cyclophosphamde	220.0±11.18[a]	10.76±0.82[a]
Gingko biloba	426.15±15.67[a]	20.94±0.85[a]
CLP+GB	292.90±9.92[b]	24.64±1.54[b]
A-value	51	5

a.$p< 0.05$ *vs* control; b.$p<0.05$ *vs* cyclophosphamide;
Values represent mean± SE.; GTTS=Granulation tissue tensile strength; OHP= Hydroxyproline

Table 2.
Effect of Cyclophosphamide(CLP) and *Gingko biloba* (GB)on excision wounds

Groups	Wound contraction(%) mean±SE							Epithelization
n=8	4th	6th	8th	10th	12th	14th	16th	period(days)
Control	24.03± 4.07	53.02± 5.11	65.47± 4.37	89.90± 1.04	89.05± 1.72	92.82± 1.33	96.85± 1.34	17.5± 0.62
CLP	12.55± 2.03a	34.75± 3.17a	45.31± 3.28a	74.60± 3.57a	86.55± 1.95	92.50± 1.07	93.07± 1.61	17.75± 0.72
GB	13.60± 3.68a	49.52± 6.64	61.15± 7.31	80.52± 2.89	84.99± 2.69	90.80± 2.0	95.0± 1.34	18.38± 0.45
CLP+GB	17.67± 2.08	40.80± 4.10	60.83± 4.02b	83.53± 1.70b	89.30± 1.53	92.73± 1.18	96.28± 0.92	18.75± 0.38

a.p<0.05 *vs* control; b.p<0.05 *vs* cyclophosphamide; Values represent mean± SE.

3.2 Excision wounds

The rate of wound contraction in control animals was 24.03%, 65.47%, 89.05% and 96.85% on 4th, 8th, 12th, and 16th days respectively. In CLP treated rats there was significant ($p<0.05$) delay in wound contraction on 8th and 10th days. GB did not alter the pattern of wound contraction from that of control animals. Further, GB did not modify the suppressant effect of CLP on wound contraction except on 8th and 10th day (Table 2). The period of epithelization in control group was 17.5±0.63 days. GB treated group, CLP treated group and GB+CLP treated group did not show any significant changes in period of epithelization when compared to control (Table 2).

4. Discussion

Cyclophosphamide was found to suppress the healing of dead space wounds but no effect on excision wounds. Healing suppressant effect encountered in this study agrees with similar observation by other workers [1-3]. So, it could be safely inferred that, CLP like other antineoplastics suppress wound healing. Some authors suggest that these agents be withheld in perisurgical period till the healing of wounds is well advanced [10].

But often it may be necessary to use antineoplastics either to attack seedling occurring during surgery and to treat metastatic growth or immunosuppression. In such a situation it is desirable to have an agent that would beneficially modify wound suppressant property of these agents. GB has been reported to promote wound healing [6]. The present study was planned to see if GB could produce positive profiles on CLP depressed healing.

Data generated clearly shows that GB antagonizes healing suppressant effect of CLP. Thus the findings of the study hold bright prospects for combined use of GB and antineoplastics. However before advocating such combination one has to answer an important question. Will concurrent supplementation of GB antagonize antitumor activity of CLP?

The present study cannot answer this. Further study on this aspect of GB is worthwhile before advocating the use of GB to reverse antihealing effect of CLP.

Our data suggest that supplemental GB might be useful in the immediate post operative period to prevent impairment of wound healing likely to be induced by CLP.

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