

Augmentation of Wound Healing by Royal Jelly (RJ) in Streptozotocin-Diabetic Rats

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Abstract—Chronically diabetic rats prepared by a single i.v. injection of streptozotocin were used to study whether royal jelly (RJ) possesses a hypoglycemic reaction and whether it can augment wound healing. Oral RJ administration of 10, 100 and 1000 mg/kg/day did not show any insulin-like activity (the hypoglycemic reaction). RJ, however, showed some anti-inflammatory activity by decreasing exudation and collagen formation in granulation tissue formation in the cotton pellet method. RJ also shortened the healing period of desquamated skin lesions. Thus, RJ possesses an anti-inflammatory action and is able to augment wound healing, but does not have an insulin-like action in streptozotocin-diabetic rats.

Royal jelly (RJ) has been demonstrated to possess several pharmacological activities in experimental animals such as vasodilative and hypotensive activities by cholinergic substances (1), cholinesterase and phosphatase activities (2), increase of growth rate (3), disinfectant action (4–6), weak DNA damaging property (7), antitumor activity (8–11), antihypercholesterolemic activity (12) and a protective action against radiation damage (13). RJ has also been reported to possess a protective effect against radiation therapy and cancer chemotherapy (14–16) and has effects on the growth of children (17, 18), dysautonomia (19, 20), and chronic diseases (21–24) in clinical studies.

Takasu and Oota (21) reported a diabetic patient whose malaise and decline in the body weight were improved by RJ. Mizuno (22) reported three cases of improvement of malaise and prostration in diabetic patients. Takasu and Oota (23) also reported in a double blind trial that malaise and prostration were eliminated by the treatment of RJ in two out of two cases. Although, the number of cases is limited, RJ seemed to be effective on malaise

and prostration in diabetic patients. Because RJ is effective on dysautonomia, it might be reasonable that RJ is effective on malaise and prostration because these are closely related. Moreover, as mentioned above, RJ might have an additional action against diabetic mellitus.

The present investigation was undertaken to study the hypoglycemic reaction and augmentation of wound healing by RJ in streptozotocin-induced diabetic rats.

Materials and Methods

Animals: Slc Sprague-Dawley strain male rats, 5 weeks old, were purchased from Japan SLC, Inc. and kept under the following conditions: room temperature of $23 \pm 1^\circ\text{C}$, humidity of $60 \pm 10\%$, and lights on for 12 hr a day (7:00–19:00). Rats were maintained on commercial rat chow (MF, Oriental Yeast Co., Japan) and purified water (distilled and deionized water) ad libitum for the duration of the study.

Drugs and chemicals: Royal Jelly (RJ, pure raw royal jelly, Lot No. B830615) was a gift from Yamamoto Apiary (8). RJ was dissolved

in purified water to the final concentrations of 0.1% (1 mg/ml), 1.0% (10 mg/ml) and 10.0% (100 mg/ml). Insulin (Ultralente MC, Lot No. A951003 and Rapitard, Lot No. A651414) was purchased from Kodama Co., Ltd. Insulin preparations of Ultralente MC and Rapitard were mixed in an equivalent volume, and 5 units/100 g body wt. was injected s.c. Streptozotocin (Lot No. 16F-0227) was obtained from Sigma Chemical Company. Streptozotocin was dissolved in 1% citrate buffer (pH 4.5) to a concentration of 20 mg/ml (25, 26). All the other chemicals and solvents were purchased from Wako Pure Chemical Industries, Ltd., except for where indicated.

Streptozotocin-diabetic rats: Streptozotocin-induced diabetic rats were prepared by the modified method of Junod et al. (25, 26). Thus rats with a body wt. of more than 400 g, which were starved for 18 hr but allowed free access to water, were administered streptozotocin solution i.v. through the jugular vein at a dose of 20 mg/kg (0.1 ml/100g body wt.), and then 10% sucrose solution was given ad libitum to prevent a precipitous fall of blood glucose level. A blood sample (0.3 ml) was obtained from the jugular vein 2 days after administration of streptozotocin, and blood glucose level was measured by the glucose-oxidase method (27, 28) using a Monocard Chemistry System (Amco, Inc.). Urine glucose level was measured with Diastix (Miles-Sankyo). Blood glucose level was measured periodically for the following 6 months, and the rats with a continuous blood glucose level of more than 400 mg/dl were considered to be chronically streptozotocin-diabetic rats.

Influence of RJ on blood glucose level and histopathological examination of the pancreas and kidney in streptozotocin-diabetic rats: Chronically streptozotocin-diabetic rats were grouped into 4 groups (20 rats/group). The first group (G1) to the third group (G3) were given RJ at the doses of 1, 10 and 100 mg/100 g body wt./day by forced oral administration, respectively, for 4 weeks. The fourth group (G4, control) was given purified water in the same manner as above. Blood glucose level was measured 2 and 4 weeks after the initiation of RJ administration. The water consumption during the experimental period of 4 weeks was 160–200 ml/rat/day. Rats were

sacrificed by decapitation, and the pancreas and kidney were extracted and immediately kept in 10% neutralized formalin. They were then cut into approximately 3 μ m thick slices after being embedded in paraffin wax and stained with hematoxylin and eosin stain, and also other special stains such as Masson trichrome stain, Gomori's aldehyde-fuchsin stain, triple staining of cell types in the pancreatic islets, and Azan-Mallory stain. The stained sections were examined by a light microscopy.

Effect of RJ on the formation of granulation tissue by the cotton pellet method: A cotton pellet method (29) was applied to investigate the effect of RJ on the formation of granulation tissue. Chronically streptozotocin-diabetic rats were divided into 3 groups (8 rats/group) and an additional 8 intact rats (intact control) were used in the following experiment: A cotton ball (15 mg) was implanted s.c. into the right side of the rat's back and similarly, one was implanted into the left side. Chronically streptozotocin-diabetic rats were divided into 3 groups (G5 to G7), which were given one of the following: RJ at 10 mg/100 g body wt./day, p.o.; an insulin mixture of 5 units/100 g/day, s.c.; or purified water at 1 ml/100 g/day, p.o., for 2 weeks. Intact rats (G8) were also given purified water in the same manner as G7. Rats were sacrificed by decapitation and the cotton pellet surrounded with granulation tissue was extracted, weighed to determine the wet wt., and then dried at 80°C for several days until a constant dry wt. was obtained. The cotton pellet was then cut into small pieces, which were placed in 2 ml of 6N HCl and then hydrolyzed at 105°C for 18 hr. The resulting solution was analyzed for hydroxyproline using the method of Woessner (30).

Effect of RJ on experimental skin desquamation in streptozotocin-diabetic rats: Chronically streptozotocin-diabetic rats were divided into 3 groups (7 rats/group) and additional 7 intact rats (intact control) were used. The capilli of each rat was removed by means of a hair remover, and then rats showing no inflammation in the head skin 24 hr after the treatment were subjected to the experiment. The head skin, 10 \times 10 mm², was desquamated, and the lesion was carefully

examined every day until complete healing was obtained. Rats (G9 to G11) were given RJ at 10 mg/100 g body wt./day, p.o., an insulin mixture of 5 units/100 g/day, s.c., or purified water of 1 ml/100 g/day, p.o., for 2 weeks. Intact rats (G12) were also given purified water in the same manner as G11.

Results

Influence of RJ on body weight: The influence of RJ on the body weight of chronically streptozotocin-diabetic rats during the 4-week experimental period is shown in Fig. 1. G1 to G3 showed no significant differences from G4 (control).

Influence of RJ on blood glucose level: The influence of RJ on the blood glucose level for 4 weeks is shown in Fig. 2. The blood glucose level decreased by 0.3%, 10.3%, 7.4%, and 3.4% in G1 (RJ, 1 mg/100 g body wt.), G2 (RJ, 10 mg/100 g body wt.), G3 (RJ, 100 mg/100 g body wt.), and G4 (control), respectively. Although RJ produced a slight decrease of blood glucose level, the final blood glucose level was still high, more than 400 mg/dl.

Influence of RJ on pancreas and kidney in chronically streptozotocin-diabetic rats: The results of histopathological observation of the pancreas and kidney are summarized in Table

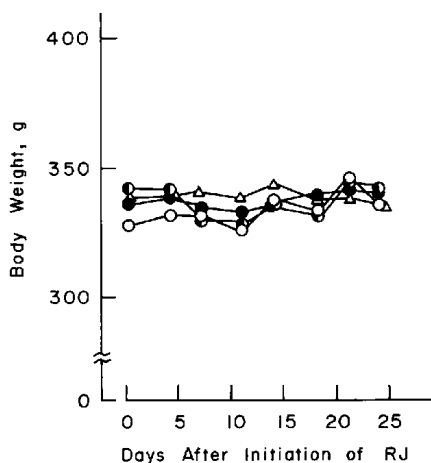


Fig. 1. Influence of RJ on body weight of streptozotocin-diabetic rats. ○: G1 (RJ, 1 mg/100 g body wt.), ◐: G2 (RJ, 10 mg/100 g body wt.), ◑: G3 (RJ, 100 mg/100 g body wt.), △: G4 (control, purified water). n=20.

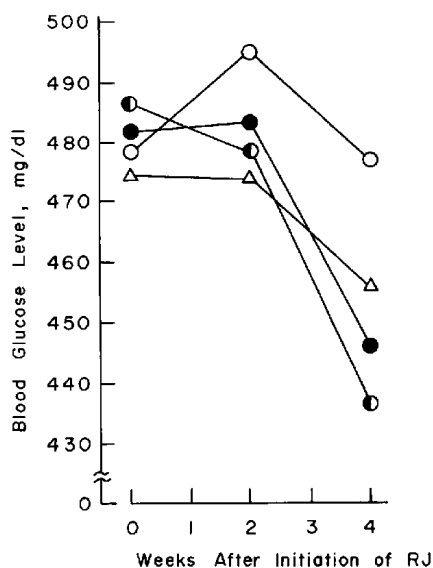


Fig. 2. Influence of RJ on blood glucose level of streptozotocin-diabetic rats. ○: G1 (RJ, 1 mg/100 g body wt.), ◐: G2 (RJ, 10 mg/100 g body wt.), ◑: G3 (RJ, 100 mg/100 g body wt.), △: G4 (control, purified water). n=20.

1. The severity of the abnormality was evaluated by the following scoring system: marked (+++, score 4), moderate (++, score 3), slight (+, score 2), very slight (\pm , score 1), and no change (-, score 0); and the mean values of the severity score \pm S.D. are shown in the Table. In all rats, G1 to G4, pancreas islets showed a decrease in B cells, degranulation, and vacuolization. Slight vacuolization and lipomatosis were also in the acinus of all the groups. All rats also showed the following: diffuse glomerular degeneration and nodular lesions, focal glomerular sclerosis, and exudative lesions; the tubuli showed hyaline droplets, thickening of the basal membrane, the presence of calcium; and interstitial small round cell infiltration was observed in the kidney. There were no significant differences among G1 to G4.

Effect of RJ on the formation of granulation tissue by the cotton pellet method in streptozotocin-diabetic rats: Wet weight, dry weight, and moisture content of the cotton pellet are summarized in Fig. 3. Among the streptozotocin-diabetic rats, there was no significant difference in the wet and dry

Table 1. Histopathological observations of the pancreas and kidney in streptozotocin-diabetic rats influenced by RJ

Histopathological observations	G1	G2	G3	G4
Pancreas				
Islet				
Decreased B cell	4.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00
Degranulation	3.4±0.55	3.0±0.00	3.0±0.00	3.0±0.00
Vacuolization	2.2±0.45	2.0±0.00	2.2±0.45	2.0±0.00
Acinus				
Vacuolization	1.8±1.10	1.6±1.52	0.2±0.45	0.6±0.89
Lipomatosis	0.4±0.55	0.6±0.55	0.8±0.84	0.8±0.84
Bleeding, necrosis, fibrosis	0.0	0.0	0.0	0.0
Ductal hyperplasia	0.0	0.0	0.0	0.6±1.34
Kidney				
Glomerulus				
Nodular lesion	0.8±1.10	0.6±0.55	0.8±0.84	0.6±0.55
Diffuse lesion	1.6±0.89	2.0±0.71	2.0±0.71	1.6±0.55
Exudative lesion	1.0±0.00	0.8±0.45	1.0±0.00	1.0±0.00
Arteriolar thickening	0.6±0.89	0.2±0.45	0.6±0.89	0.2±0.45
Tubule				
Hyaline droplet	2.0±1.00	1.8±0.45	1.8±1.10	1.2±0.84
Basal membrane thickening	0.2±0.45	0.4±0.89	0.0	0.0
Calcium	0.6±1.34	0.2±0.45	0.0	0.2±0.45
Stroma				
Round cell	0.0	0.4±0.89	0.0	1.0±1.00

Severity of abnormality was scored as follows: marked (score 4), moderate (score 3), slight (score 2), very slight (score 1) and no change (score 0). The mean of the score±S.D. is shown. G1: RJ, 1 mg/100 g body wt.; G2: RJ, 10 mg/100 g body wt.; G3: RJ, 100 mg/100 g body wt. G4: Control (purified water). n=20.

weights. However, in the moisture content, G5 (RJ, 10 mg/100 g body wt.) had a significantly lower value ($P<0.05$) compared to G7 (control). Compared to G8 (intact control), the moisture content of G5 (RJ, 10 mg/100 g body wt.) showed a tendency to decrease ($P<0.10$), and the dry weight of G6 (insulin, 5 units/100 g body wt.) significantly decreased ($P<0.05$). The hydroxyproline content in the cotton pellet is shown in Fig. 4. A trend for a lower hydroxyproline content ($P<0.10$) was indicated in G5 (RJ, 10 mg/100 g body wt.); however, this was not the case in G6 (insulin, 5 units/100 g body wt.).

Effect of RJ on experimental skin desquamation in streptozotocin-diabetic rats: Effect of RJ on complete healing of desquamated lesion is shown in Fig. 5. Days required for complete healing for G6 to G12 were 18.29 ± 5.28 , 19.71 ± 5.59 , 20.57 ± 4.31 , and $15.10\pm$

3.88 days, respectively. Compared to G11 (control), there tended to be a shorter period ($P<0.10$) required for complete healing in G9 (RJ, 10 mg/100 g body wt.).

Discussion

The present data demonstrated that in streptozotocin-induced diabetic rats, RJ decreased the moisture content and collagen content in the cotton pellet, indicating the inhibition of exudation and reduction of granulation tissue formation.

In the process of inflammation, it has been well-recognized that capillary permeability increases, resulting in the exudation to form edema, in the acute phase of inflammation. Thus, the decrease of moisture content indicates that RJ might inhibit capillary permeability, acting as an anti-inflammatory drug. In the later stage of inflammation, the

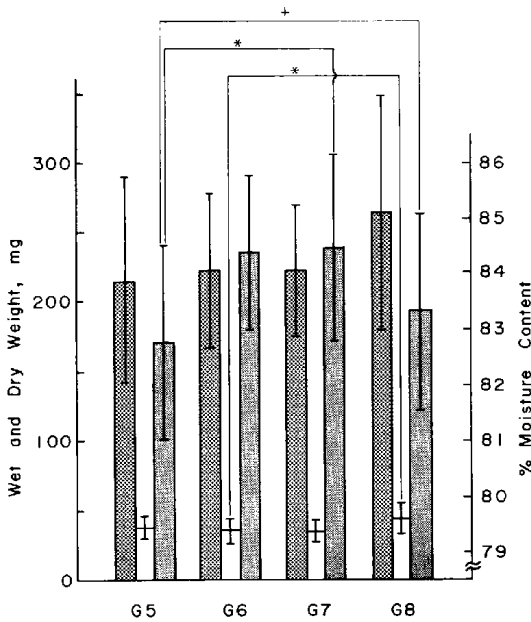


Fig. 3. Effect of RJ on the formation of granulation tissue by the cotton pellet method in streptozotocin-diabetic rats. [stippled]: wet weight; [white]: dry weight; [checkered]: moisture content; G5: RJ, 10 mg/100 g body wt.; G6: insulin, 5U/100 g body wt.; G7: control (diabetic rat); G8: control (intact rat). * $P < 0.05$, + $P < 0.10$, $n = 8$.

formation of granulation tissue is also found. The inhibition of granulation tissue formation in the later stage of inflammation is well-established as a characteristic of anti-inflammatory drugs. Hydroxyproline is one of the major constituents of collagen, which comprises the major portion of granulation tissue, so the decreased hydroxyproline content indicates a decreased amount of granulation tissue. Because RJ possessed the activity to reduce granulation tissue formation, RJ might also be effective in the later stage of inflammation.

These two facts indicate that RJ acts like an anti-inflammatory drug in streptozotocin-diabetic rats in both the acute and chronic phases of inflammation. However, insulin did not possess any such the effects.

The present study also demonstrated that the time required for complete healing of the desquamated lesion, 18.3 days, was shorter than the control, 20.6 days, although it was longer than the one for the intact control rat,

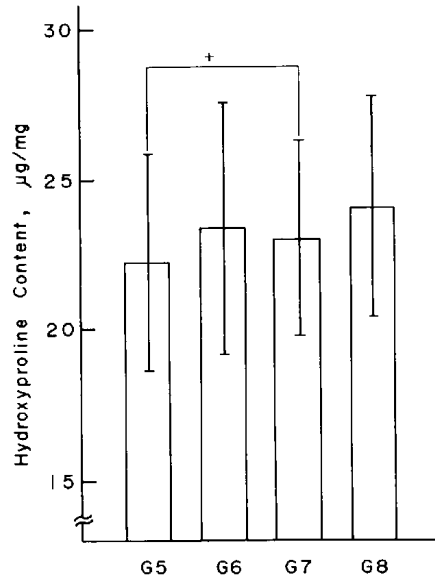


Fig. 4. Effect of RJ on collagen formation of granulation tissues by the cotton pellet method in streptozotocin-diabetic rats. G5: RJ, 10 mg/100 g body wt.; G6: insulin, 5U/100 g body wt.; G7: control (diabetic rat); G8: control (intact rat). * $P < 0.10$, $n = 8$.

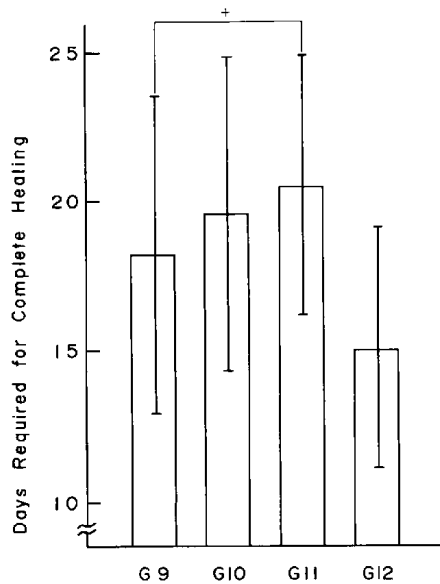


Fig. 5. Effect of RJ on complete healing of skin desquamated lesion in streptozotocin-diabetic rats. G9: RJ, 10 mg/100 g body wt.; G10: insulin, 5U/100 g body wt.; G11: control (diabetic rat); G12: control (intact rat). * $P < 0.10$, $n = 7$.

15.1 days. Thus, RJ might be effective for enhancing the healing activity in a certain manner. Generally, wound healing takes a longer period of time in diabetic patients. This phenomenon was also confirmed in the present investigation by comparing the time required for healing of G11 (control, streptozotocin-diabetic rat) and G12 (intact control). The administration of insulin did not have any shortening effect on healing.

In general, an anti-inflammatory drug retards wound healing by depressing responsible cell proliferation, which is a disadvantageous effect of these drugs. As described above, RJ acted like an anti-inflammatory drug, inhibiting capillary permeability and reducing granulation tissue formation. Despite these activities, RJ shortened the period required for complete healing. This property may be attributable to one of the many components in RJ, such as skin respiratory factor (31), but this additional factor has not yet been identified.

The formation of granulation tissue was the highest and also the healing period was the shortest in intact rats. The formation of granulation tissue was lower and the healing period was the longest in diabetic rats. In case of RJ, the formation of granulation tissue was the lowest; however, the healing period was shorter. The complexity of the disease, such as depressed metabolism, as well as the complexity of the active components of RJ might be the reason for these differences.

In the present investigation, streptozotocin was administered i.v. to produce diabetic rats. Streptozotocin has been known to break down the islet cells of the pancreas and to reduce blood insulin level, producing experimental type I diabetic mellitus (25). Since the blood glucose level was not lowered by RJ, the present data also indicate that RJ does not possess any insulin-like effect in streptozotocin-diabetic rats, which do not produce insulin.

The wound healing activity of RJ has not yet been reported in diabetic or normal subjects. Shinoda et al. (1) reported that the water-soluble fraction of RJ contained a factor, possibly acetylcholine, which had a vasodilating activity, resulting in a transient increment of blood flow in canine femoral

artery. This activity might play some role in the enhancement of wound-healing activity.

In conclusion, streptozotocin-induced diabetic rats were prepared, and these chronically diabetic rats were subjected to the experiments to determine whether RJ possesses anti-inflammatory and healing effects. RJ was found to reduce capillary permeability, production of granulation tissue in the cotton pellet method, and also to shorten the healing period in desquamated skin lesions in streptozotocin-diabetic rats. Thus, RJ possesses anti-inflammatory-like action, but not insulin-like action, in streptozotocin-induced chronically diabetic rats. The mechanism of these actions must be further investigated.

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