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Research Article

A comparative study of oral diacerein and transdermal diacerein as Novasomal gel in a model of MIA induced Osteoarthritis in rats

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Abstract

Background: Osteoarthritis is a chronic pathology of the joints causing disability and morbidity. Diacerein is a disease-modifying agent indicated for osteoarthritis management with enhanced performance and have much lower side effects profile than conventional non-steroidal anti-inflammatory drugs. Oral administration of Diacerein is associated with a laxative effect, thus causing treatment discontinuation.

Aim: This study aimed to evaluate the activity of Diacerein novasome-based transdermal gel compared with standard oral treatment in the management of induced osteoarthritis in a rat model.

Materials and methods: A single intra-articular injection of monosodium iodoacetate was administered to the left knee joint, resulting in the development of Osteoarthritis. Disease progression and the effect of both routes of Diacerein treatment were evaluated by morphological, biochemical, histological, and radiological studies.

Results: Osteoarthritis was successfully induced in rats' knee joints. Morphological studies revealed that both Diacerein treatments significantly reduced joint swelling as compared to the untreated group. The serum TNF- α and IL-1 β levels were significantly lower in both Diacerein treatment groups as compared to the untreated group throughout the course of the study. Histological and radiological findings confirmed that transdermal Diacerein treatment protects against cartilage degradation like oral treatment.

Conclusion: Novasomal technology proved its efficacy as a carrier for the transdermal delivery of Diacerein. The in-vivo study on an animal model of osteoarthritis showed that Diacerein transdermal gels provided sufficient pharmacological activity for the attenuation of the disease. This finding could support its use as an alternative to the standard oral treatment to avoid side effects.

Keywords

Diacerein, novasome, Osteoarthritis, transdermal delivery, monosodium iodioacetate

Introduction

Osteoarthritis (OA) is a chronic pathology that is prevalent in society as a major cause of disability and morbidity (Saleh 2011). Hip and knee joints, being weight-bearing, are mostly affected especially in individuals with obesity and /or advanced age (Omar et al 2022). OA is considered a degenerative disease that involves inflammation and destruction of the joint and surrounding tissues includ-

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ing the cartilage, synovium, and bone (Saleh et al. 2009; Mohammed et al. 2014).

Paracetamol and NSAIDs are the common agents prescribed for the alleviation of OA symptoms without any contribution to limiting disease progression (Weng et al. 2023). Given that OA is a chronic illness, extended use of these drugs is linked to a number of adverse effects, some of which involve the kidney and the gastrointestinal tract in the case of nonselective COX inhibitors and the cardiovascular system in the case of selective COX-2 inhibitors (Cooper et al. 2019).

Diacerein (DCN) is a semi-synthetic anthraquinone derivative indicated for the management of OA (Pavelka et al. 2016). It is completely converted into rhein following oral administration (de Oliveira et al. 2020). The basic mechanism is related to the ability of DCN and its active metabolite rhein to inhibit the IL-1 β system and subsequent events that result from their activation including the production of pro-inflammatory cytokines involved in cartilage degeneration (Almezgagi et al. 2020).

In comparison to that of NSIAD drugs, the action of DCN dose does not involve any interference with prostaglandin inhibition; therefore, unwanted gastrointestinal or cardiac side effects are avoided (Patel et al. 2022). However, the limited solubility of DCN in GIT fluids restricts its absorption resulting in poor bioavailability between 35% and 50% (Nicolas et al. 1998). Furthermore, undissolved rhein is oxidized in the colon by bacteria into rhein-9-anthrone which induces a laxative effect, associated with diarrhea or softness leading to poor compliance and treatment discontinuation (Pelletier and Martel-Pelletier 2018). Due to concerns regarding diarrhea frequency and severity, the European Medications Agency (EMA) prohibited DCN-containing medications in senior patients with OA in 2015 (Panova and Jones 2015).

Several attempts were made to overcome the side effects of oral DCN administration by using intra-articular injections or transdermal administration routes (Siddiqui Bet al. 2020; Eladawy et al. 2021).

The pharmacokinetic and physicochemical properties of DCN encouraged its transdermal delivery, including its molecular weight of 358.294g/mol, lipophilicity (log P = 1.7), and half-life of 4.25 hours (Aziz et al. 2019).

Transdermal drug delivery is considered a non-invasive and patient-friendly approach to administering medications (Noor and Ghareeb 2021). However, drug delivery through the human skin is limited by the barrier function of the stratum corneum (Abdulbaqi and Rajab 2020). Therefore, vesicular carriers were used to enhance the permeation (Mirtaleb et al. 2021). However, previous generation systems such as liposomes and niosomes showed limited ability to perform this purpose (Ahmed et al. 2023). Novasomes (NSs) are thought to be a recently created type of vesicular structure that is made up of non-ionic surfactants, and free fatty acids, with or without cholesterol (Illastria Rosalina et al. 2023). When mixed with nonionic surfactants, free fatty acids were hypothesized to increase transdermal penetration, because they work by fluidizing the lipids of the stratum corneum and

enhancing the flexibility of the vesicular structure (Zakaria et al. 2023).

Monosodium iodoacetate (MIA)is a chemical substance that acts by inhibiting glycolysis and promoting chondrocyte death (Xu et al. 2020). This chemical model was used due to its ability to produce OA in closely related features to human disease (de Sousa Valente 2019).

This study aimed to evaluate the efficacy of a transdermal gel based on DCN NS compared with the standard oral treatment in managing knee OA induced by MIA injection in rats.

Experiments

Materials

DCN (purity of >98%, CAS no. 13739-02-1) and MIA were acquired from Hangzhou Hyper Chemicals Limited, Hangzhou, China. Sodium carboxymethylcellulose (Na-CMC) was provided as a gift from Pioneer, Sulaymaniyah, Iraq. Cholesterol (CH) and Span 60 were from Xi'an Sonwu Biotech Co., Ltd, Shaanxi, China. Stearic acid was purchased from Himedia, Mumbai, India. Methanol and chloroform were obtained from Alpha Chemicals, Maharashtra, India. All solvents and chemicals used were of analytical grade.

Determination and preparation of DCN doses

The DCN dose in rats was calculated based on its recommended dose in humans (100 mg per day) (Jacob et al. 2022). It was calculated using the following equation (Shabbir et al. 2023):

$$AED_{(mg/kg)} = HED_{(mg/kg)} \times K_m ratio_{(H/A)}$$
 Eq. 1

Where K_m is a correction factor determined by dividing the average body weight (kg) of a species by its body surface area (m²). The values of human K_m and animal K_m are 37 and 6, respectively (Shabbir et al. 2023). The K_m ratio can be expressed as the ratio between human and rat K_m factor (37/6 = 6.2).

$$AED_{(mg/kg)} = 8.85 \text{ mg/kg} \text{ and } AED_{(mg)} = 1.77 \text{ mg}$$

For oral administration of DCN, an oral suspension was prepared using 0.5% Na-CMC as a suspending agent. The concentration of the suspension was (6 mg / mL).

DCN NS-based gel was used for transdermal application. Firstly, DCN NSs were prepared by thin film hydration method using Span 60, cholesterol, and stearic acid as vesicle-forming agents. The preparation method is described in a previous research (Fareed and Kassab 2023). Then, an amount equivalent to 1 gm of DCN NSs was obtained by cold centrifugation at 16,000 rpm at 4 °C. The gel was prepared by the hot-cold method in which the required weight of hydroxy propyl methylcellulose K15 M (HPMCK15M) to produce 3% concentration was slowly added to 30 mL of water heated to 70 °C. Then, the collected vesicles were redispersed in 60 mL of cold water and added to the polymeric dispersion. The weight of the gel was then adjusted using distilled water to 100 gm. The final concentration of DCN in the gel was 1% w/w.

Animals used in the study

Sixty male Swiss albino rats aged three months with an average weight of 200 ± 15 gm, were used in this study. The animals were housed at room temperature (25 ± 1 °C) and a 12-h light/dark cycle in the animal house at the Research Center for Cancer Research and Medical Genetics, Baghdad, Iraq. The rats had free access to both food and water. The protocols of the *in-vivo* studies in rats were approved (**RECAUBCP262022A**) by the Research Ethics Committee for Experimental Studies, College of Pharmacy, Baghdad University, Iraq.

Experimental design

After acclimating for 2 weeks, these rats were divided at randomly into four groups of the same size (n = 15 for each group). An electric clipper was used to shave off the hair that was growing around the knee joint before the experiment began. Before the injection, ketamine at a dose of 80 milligrams per kilogram of body weight and xylazine at a dose of 10 milligrams per kilogram were given to each animal (Bhatia et al. 2022). With the use of a micro-syringe with a 26-gauge needle, OA was produced in groups B, C, and D by administering a single intra-articular injection of MIA solution mixed in normal saline to the left knee joint of each animal. Then, fifty microliters of normal saline were injected into the right knee joint of each animal in the control group by intra-articular injection. The experimental groups consisted of the following participants:

- Group A is the normal control group, which was kept under normal laboratory conditions.
- Group B is the OA induction group. This group received oral 0.5% Na-CMC suspension by an oral gavage for 21 days.
- Group C is the standard oral DCN-treated group. This group was treated with 0.3 mL oral DCN suspension in 0.5% Na-CMC (6 mg/mL). The daily dose was 8.85 mg/kg given by an oral gavage for 21 days.
- Group D is the transdermal DCN NS gel-treated group. This group was treated with 0.2 gm of 1% transdermal DCN NS gel for 21 days at a gel weight equivalent to 8.85 mg/kg of animal weight.

Assessment of joint swelling (Edema)

Knee joint swelling after MIA intra-articular injection is regarded as the earliest change noticed. Knee joint swelling was recorded as a difference in diameter between both knees in three rats (n=3) randomly selected from each group on days 0,4,10 and 21 by using a calibrated digital caliper (Nija et al. 2021).

Assessment of serum biochemical parameters

The serum levels of TNF- α and IL-1 β for all of the experimental groups (A–D) were measured on days 0,4,10 and 21 of the experiment. Commercially available ELISA kits were used in accordance with the manufacturer's instructions (Bioassay Technology Laboratory, China). After animals were anesthetized, blood samples were collected by direct heart puncture using 5 mL plastic syringes. Blood was then withdrawn into gel tubes before centrifugation at 3000 rpm for 10 minutes. After complete blood separation, serum was withdrawn, transferred into 2 mL plain plastic tubes, and stored at -20 °C for further analysis (Al-Kenany and Al-Shawi 2023). The manufacturer's procedure was followed for conducting the analysis to measure absorbance by using a microplate reader spectrophotometer.

Assessment of histopathological changes

Knee joints were collected on days 0, 4,10, and 21 of the study by randomly selecting three rats from each group. After the animals received anesthesia using ketamine and xylazine, the knee joints were removed and fixed using 10% formalin for histological inspection. Following the decalcification of the samples with nitric acid at a concentration of 5%, the samples were subsequently embedded in paraffin. Afterward, slices of 5 µm in thickness were produced and stained with hematoxylin and eosin (H&E). The histological slides were examined by routine light microscopy (GENEX Laboratories, USA). The examination was carried out by a senior pathologist without knowledge of the treatment group and one representative slide for each group was used (Ghanim 2020). The semi-quantitative score was used to assess changes in the synovium and sub-synovium between the experimental groups on day 21 for comparison purposes (Jean et al. 2007).

Radiological assessment of the knee joint

The lateral and anteroposterior views of the knee joints were taken on day 21 of the experiment for three animals in each group to evaluate the disease progression and the effect of the treatment interventions on the degree of bone and joint changes. The rats received anesthesia by using ketamine and xylazine to induce relaxation. An X-ray machine (Mobilett XP, Siemens) with an 11-second exposure time and 45 kV voltage was used to optimize the resultant image while preserving the least possible hazard (Chattopadhyay et al. 2020).

Statistical analysis

The Graph Pad Prism version 9 was utilized for statistical analysis. For all data, the findings were expressed as the mean along with the standard deviation, with the exception of the histopathological scoring system, which used the median value along with the interquartile ranges. Analytical statistics in the form of an ANOVA test and post hoc Tukey's multiple-comparisons test were utilized to investigate the significance of the relationships between the various groups. The P-value needed to be lower than 0.05 to be considered significant. The non-parametric Kruskal-Wallis test was followed by Dunn's multiple comparisons test for the analysis of the scores for various groups that were included in the histopathological section.

Results

Effect of DCN treatment on edema profile

Swelling of the rat's knee joint was the first sign noted a few hours following the MIA injection as shown in Fig. 1A. It was recorded on days 0, 4,10, and 21 of the experiment as shown in Fig. 1B. All the OA-induced groups demonstrated swelling and an increase in diameter in their left joint as compared with the normal right joint at day 0, with no significant difference among groups according to ANOVA test and post hoc Tukey's multiple comparisons test. A noticeable reduction in joint swelling started on day 4 in all groups and it continued to subside during the entire study period. A statistically significant (p-value<0.05) difference was found in joint diameter reported on days 4,10, and 21 between the disease control group and the treatment groups (both oral and transdermal DCN treatments). However, the animals that received transdermal DCN NS gel showed more reduction in joint diameter than those who received the standard oral treatment on days 10 and 21, without statistical insignificance.

Effect of DCN treatment on serum of TNF- α and IL-1 β Levels

The serum levels of TNF- α and IL-1 β for control, OA, and both treatment groups reported on days 0,4,10, and 21 of the experiment are shown in Table 1. At day 0, a significant increase in the serum levels of by TNF- α and IL-1 β was evident in all MIA-injected groups as compared with the control group (p-value<0.05). On days 4, 10, and 21 serum of TNF- α and IL-1 β levels of the OA group B were significantly higher (p-value<0.05) than those of the control group and treatment groups C and D (p-value<0.05). Groups C and D still displayed significantly higher(p-value<0.05) levels than the control group but their serum levels were significantly lower(p-value<0.05) than the control group at days 10 and 21. Group D showed numerically lower levels of TNF- α and IL-1 β on days 10 and 21 as compared to group C which received oral treatment. However, the difference was statistically insignificant (p-value>0.05).





Figure 1. A. Swelling of the Rat's knee joint a few hours after injection of MIA; **B.** Edema size was reported on days 0, 4, 10, and 21 for control Group (A); OA Group (B); Oral Treatment Group (C); and Transdermal Treated Group (D).

Table 1. Serum levels of TNF- α (A) and IL-1	β (B) for all experimental β	groups reported on days	s 0,4,10, and 21 of the study.
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Days of the		Experimen	ntal groups		ANOVA Tukey's multiple comparisons test			st			
study					test						
	Group A	Group B	Group C	Group D	P- value	Adjusted P- value					
						C vs. D	B vs. D	B vs. C	A vs. D	A vs. C	A vs. B
Serum level of TNF-a											
0.672641	0.419814	0.96301	0.000085^{*}	0.000228*	0.000353*	0.000070*	112.3 ± 1.747	180.5 ± 5.617	184.9 ± 0.7489	195.5 ± 21.84	0
0.059996	0.033009	0.97234	0.002375*	0.000116^{*}	0.000082^{*}	0.000061*	117.8 ± 4.494	168.2 ± 6.491	165.8 ± 11.11	148.7 ± 0.2496	4
0.160513	0.000495	0.006988	0.000533*	0.000066*	0.000003*	0.000005*	113.1 ± 5.867	$158.8{\pm}\ 2.372$	143.6 ± 2.621	135.9 ± 4.119	10
0.595532	0.002719	0.01346	0.014282^{*}	0.002865*	0.000055*	0.000093*	107.5 ± 8.488	143.1 ± 0.6241	127.6 ± 0.8737	122.8 ± 3.141	21
Serum level of IL-1 β											
0.990167	0.398619	0.549877	0.000007*	0.000006^{*}	0.000003*	0.000002*	3.372 ± 0.9288	10.14 ± 0.6620	9.488 ± 0.1285	9.346 ± 0.2113	0
0.005937*	0.001126*	0.511926	0.000043*	0.000002^{*}	0.000001^{*}	$< 0.000001^{*}$	8.925 ± 0.7608	$8.421 {\pm}~ 0.01976$	6.741 ± 0.3854	3.283 ± 0.08893	4
0.050568	0.000095*	0.002081*	0.000638*	0.00004^{*}	0.000001^{*}	0.000002*	$4.123{\pm}~0.5138$	8.045 ± 0.2273	$6.623 {\pm}~ 0.06917$	5.822 ± 0.2371	10
0.091185	0.006128*	0.265698	0.025616*	0.00089*	0.000135*	0.000157*	3.480 ± 0.5830	6.830 ± 0.4941	6.040 ± 0.5929	4.939 ± 0.07987	21

Data were expressed as mean±SD, *P <0.05 for the ANOVA test and Tukey's multiple comparisons test, n= 3 animal/time period.

Effect of DCN treatment on the histological findings of the experimental groups

The histological photomicrographs obtained by processing knee joints from all experiment groups are shown in Fig. 2. DCN NS transdermal gel was tested to heal the knee joint's injured surface and compared with oral treatment.

A normal, smooth cartilage surface together with an ordered organization of chondrocytes was observed under the microscope in the slices of rat knee joints taken from the control group's left knees (Fig. 2A). The chondrocytes were well organized in columns, with smooth and unbroken articular cartilage on the surface.

The histopathological changes in the OA group at day 4, represented by only a slight thickening of the synovium membrane, were evident as shown in Fig. 2B. No therapeutic effects were noticed at day 4 for both DCN treatments (Fig. 2E, F).

Structural impairment of the cartilage and synovium was recorded on days10 and 21 for the OA group where damaged chondrocytes can be observed in Fig. 2C, D. The changes can be summarized by hyperplasia and hypertrophy of the synovium lining cells that started at day 10. The histopathological changes extended to inflammation, new blood vessel formation, and bone lysis evident by abrasions of the rough edges around the femur and tibia (Fig. 2D).

Both DCN treatments slowly reverted the damage caused by MIA injection. Therapeutic effects were noticed on day 10 (Fig. 2G, H). Therapeutic DCN treatment by both routes started to attenuate the disease.

The maximum therapeutic benefits for groups C and D were recorded on day 21, as illustrated in Fig. 2I, J. The histological findings were further interpreted on the basis of semiquantitative synovitis scores. The scores for groups B, C, and D are shown in Table 2. The results of the non-parametric Kruskal-Wallis test indicated a significant difference (P-value<0.05) in the synovitis scores among the three groups. Furthermore, the post-hoc Dunn's multiple comparisons tests indicated that both DCN treatment groups had a significantly lower synovitis score than group B. Despite group D showing numerically lower synovitis scores than group C, the difference was statistically insignificant.

Effect of DCN Treatment on the radiological findings of the experimental groups

Analysis of the radiographic images obtained from X-ray studies on day 21 revealed a normal joint struc-

ture with no cartilage damage nor bone destruction for the control group, as shown in Fig. 3A. However, MIA injection caused an evident deterioration of the trabecular bone architecture in the OA group as compared with the normal histological features (Fig. 3B). The oral DCN-treated animals exhibited less damage represented by irregular joint surfaces with minor joint space narrowing and cartilage erosions as illustrated in Fig. 3C. The last group of rats treated with DCN NS transdermal gel showed modest joint surface unevenness and the lowest articular cartilage erosion as shown in Fig. 3D.

Discussion

The occurrence of joint swelling in MIA-injected knee joints of rats is a typical pattern of inflammation produced by this chemical model as reported by another study (Chattopadhyay et al. 2016). The swelling exhibited a decreasing pattern throughout the study indicating a single episode of inflammatory reaction which is in contrast with the human OA characteristic of recurrent episodes of inflammation (Chattopadhyay et al. 2016). However, it is a useful model to study the efficacy of drugs in attenuating of inflammation (Ahmed et al. 2012).

The elevated levels of serum biochemical markers represented by TNF- α and IL-1 β at the initial phase in the MIA-injected groups is attributable to pro-inflammatory enzyme activation during inflammatory factor precursor production (Jaleel et al. 2020). DCN therapy, whether oral or transdermal, efficiently reduces cytokines by inhibiting inflammatory responses and reducing pro-inflammatory cytokines, including TNF- α and IL-1 β (Abdel-Aziz et al. 2021).

The histopathological sections confirmed the results previously obtained from morphological and biochemical studies. The actions of DCN started at day 10 for both oral and transdermal treatments indicating a delay in its action. This finding may be may explained by the chemical nature of DCN, being a prodrug, and its requirement of conversion into its active metabolite rhein prior to exerting its effect (Jung et al. 2020). Previous studies revealed that sufficient concentration of rhein in the synovial cavity is essential to start its effect, which requires time to develop (Jain et al. 2014). Accordingly, the results of the present study showed that oral and transdermal treatments require the same time to produce the required therapeutic concentrations of rhein at the synovial sites.

Table 2. Histological evaluation of rat left knee joint on day 21 of the study.

Group	Synovitis score	Kruskal-Wallis test	Dunn's multiple comparisons test	
		p-value	Group	p-value
B (OA Group)	14 (13-15)	0.000189*	B vs C	0.011870*
C (Oral DCN Suspension)	9 (8-10)		B vs D	0.004711*
D (Transdermal DCN NS Gel)	9 (8-10)		C vs D	>0.9999999

Data were expressed as median with interquartile ranges, synovitis score was used to address pathological changes (1–18),*P <0.05 for Kruskal-Wallis test and Dunn's Multiple Comparisons Test, n= 6 animal/group.



Figure 2. Photomicrograph of histological section from rats left knee joints stained with H&E for control Group (**A**); OA Group at Day 4 (**B**); OA Group at Day 10 (**C**); OA Group at Day 21 (**D**); Oral Treatment Group at Day 4 (**E**); Oral Treatment Group at Day 10 (**F**); Oral Treatment Group at Day 21 (**G**); Transdermal Treatment Group at Day 4 (**H**); Transdermal Treatment Group at Day 10 (**I**); Transdermal Treatment Group at Day 21(**J**). Blue triangles indicate hypertrophy, black triangles indicate hyperplasia, orange triangles indicate inflammation and green arrows indicate blood vessel formation.



Figure 3. Radiographic Images on day 21 for control Group (**A**); OA Group (**B**); Oral Treatment Group (**C**); Transdermal Treated Group (**D**).

Despite no significant difference being found in the synovitis score between the oral and transdermal treatments, the transdermal gel of DCN based on novasomal carriers achieved histopathological features closely related to oral treatment. This finding indicated the success of the novasomal carriers in enhancing DCN permeation through the skin and eliminating the events associated with the formation of laxative species associated with oral treatments.

Radiological examination of different groups confirmed the efficacy of the investigated DCN treatment routes in preserving knee joint structure as compared with the OA group. Fig. 3B shows obvious damage in bone architecture brought by MIA injection in the OA group. This finding is attributed to the high dose administrated i.e. 3mg which is capable of producing an OA feature similar to late OA changes in humans throughout the course of the experiment indicating that OA development by MIA injection is time, dose, and species-dependent (Udo et al. 2016).

Good control of the pathological changes associated with OA development as a result of MIA injection was obtained after DCN NS-based transdermal gel treatment. This finding is related to its ability to provide sufficient serum levels of DCN and eventually in the targeted knee joint (Jung et al. 2020). Novasomal carriers enhance the skin permeation of DCN through the skin and stratum barrier due to its surfactant and lipid content, which disturb the barrier function of the stratum corneum (Tawfik et al. 2021). DCN NS-based transdermal gel is clearly an effective alternative to oral treatment due to its protective in vivo anti-inflammatory effects in vivo. It prevents damage from MIA injection by blocking the catabolic pathways of pro-inflammatory cytokines, such as TNF- α and IL-1 β , in OA.

Conclusions

In this study, vesicular carriers of DCN were developed into a transdermal gel and investigated as an alternative to conventional oral treatment. The DCN transdermal gels showed promising pharmacological effects against the MAI-induced OA in rats, as confirmed by morphological, biochemical, histological, and radiological investigations. This finding could support the use of DCN transdermal gel as an alternative for oral treatment to avoid side effects, thereby enhancing patient compliance and therapeutic outcomes.

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