Evaluation of Dermal Irritancy Potential of Carboxymethyl-Chitosan Hydrogel and Poly-(Acrylic Acid) Chitin Hydrogel

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Objective: *The wound dressing synthesized from carboxymethylchitosan hydrogel (CM) and chitin-(polyacrylic acid) hydrogel (PAA) were examined for their dermal irritation potential response using the Draize test.*
Material and Method: *Eighteen male Sprague-Dawley rats were divided into three groups (6 rats/each). Rats in group 1 were designed as control, group 2 were treated with CM, and group 3 were treated with PAA. The test materials diameter 1 x 1 cm were topically applied on the skin in group 2 and 3. Two skin sites (1 x 1 cm in size) were located at the back. One site was intact and the other was abraded in such a way the stratum corneum had no bleeding.*
Result: *After 24 and 72 hours of wrappings, the materials were removed and the test sites were evaluated in terms of erythema and edema using adopted Draize scoring system. At the end of the experiment, all rats were anesthesized with intravenous thiopental sodium. Blood samples from descending aorta were collected for liver and kidney function test and all organs were weighed. The results of this experiment showed 1) no irritation of both materials in this animal model; 2) no material-related induced liver and kidney dysfunction and 3) organ weights had no significant difference between the groups.*
Conclusion: *Both CM and PAA should be considered safe to use in the purpose of wound dressing in further clinical trials.*

Keywords: *Carboxymethylchitosan hydrogel, Chitin-(polyacrylic acid) hydrogel, Draize test*

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Being widely distributed in nature, e.g. in crustaceans, insects, cell wall of fungi, and bacteria, chitin, β-(1→4)-poly-*N*-acetyl D-glucosamine is characteristic of a rigid crystalline structure due to intra- and intermolecular hydrogen bonds. Various kinds of derivatives of chitin are synthesized by deacetylation or are introducing different types of substituents at the C6 position of *N*-acetyl-glucosamine[1,2]. The degree of deacetylation imparts water solubility to this insoluble polysaccharide by inhibiting intermolecular association through electrostatic repulsion[1]. Their original structures based on *N*-acetyl glucosanine enhances the performance of carboxymethyl-chitosan (CM) and poly-(acrylic acid)-chitin (PAA)and makes them more biocompatible, more hydrophilic, more biodegradable, and more amenable to various forms than synthetic polymers[2,3]. Non-specific immune enhancement of chitosan and chitin, which could improve wound healing[1,4,5], has recently been proposed in many studies. In addition to the above benefits, and with it most important potential as wound dressing in clinical use, these modified biomaterials should be evaluated in animal models, prior to manufacture, transport, marketing or clinical trial.

Dermal irritancy and corrosion evaluation of topical agents were widely examined using Draize test, described by John Draize[6]. Since then, many committees such as the Federal Hazardous Substance

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Act (FHSA), the Consumer Product Safety Commission (CPSC), the Environmental Protection Agencies (EPA), and the Organization for Economic Cooperation and Development (OECD) have adopted those as a standard procedure[7]. In the present study, Draize test was performed by using the model test in rats. The test sites were evaluated in terms of erythema and edema using adopted scoring system. The objective of the present experiment was to investigate the dermal irritation potency of carboxymethyl-chitosan hydrogel (CM) and poly-(acrylic acid) chitin hydrogel (PAA).

Material and Method

Eighteen Sprague Dawley male rats, weighing between 250 to 300 g were maintained under identical strict hygiene environment with light/dark period of 12 hour-cycle and a room temperature of 23-25°C. Animals were free to access food and water *ad libitum*. The rats were randomly divided into three groups. Each group consisted of six rats, group 1 was designed as control, group 2 was treated with CM, and group 3 was treated with PAA. The test material diameter 1 x 1 cm was topically applied on the skin in group 2 and 3. Two skin sites (1 x 1 cm in size) were located at the back skin. One site was intact and the other was abraded in such a way that the stratum corneum was without bleeding. After 24 and 72 hours of wrappings, the materials were removed and the test sites were evaluated in terms of erythema and edema using adopted Draize scoring system (Table 1). At the end of the experiment, all rats were anesthetized with intraperitoneal thiopental sodium. Blood samples from descending aorta were collected for liver and kidney function test. The heart, liver, and kidney of all rats were separately weighed.

Measurement of liver function

Eighteen rats had serum collected once at 72 hours to detect the level of serum glutamic pyruvic transaminase (SGPT). The greatest amounts of this enzyme were found in hepatocytes. The principle of the test is that enzyme GPT catalyzes the transfer of the α-amino group of alanine to the α-ketoglutaric acid, resulting in the formation of pyruvic and glutamic acids. They will react with the dinitrophenylhydrazine in alkaline solution to become colorized and be detected by spectrophotometer at the wavelength 520 nm.

Measurement of kidney function

Eighteen rats had serum collected once at 72 hours to detect the level of serum creatinine. Serum creatinine is formed in the metabolism of muscle creatine and phosphocreatine, and excreted in the urine. The serum that is not excreted or absorbed by the renal tubules to any degree can be used as a rough index of the glomerular filtration rate (GFR). The interpretation of increased values means that the glomerular filtration rate is reduced. Renal impairment damage may be from obstruction of the urinary system or disease. The principle of the test is that creatinine reacts with picric acid in alkaline conditions to form a yellow complex that absorbs at 500 nm.

Statistical analysis

Data were analyzed with SPSS for window version 7. All data were first analyzed for normal distribution (Kolmogorov-Smirnov-test) and homogeneity of variances (Levene's test) and then subjected to parametric ANOVA plus Scheffe-test. The level of significance was set at 5%.

Table 1. The adopted Draize scoring system was used in the present experiment

Description of erythema or edema	Score assigned
Erythema and eschar formation	
- No erythema	0
- Very slight erythema	1
- Well-defined erythema	2
- Moderate to severe erythema	3
- Severe erythema with slight eschar formation	4
Edema	
- No edema	0
- Very slight edema	1
- Slight edema with raised margin	2
- Moderate edema with raised margin ~1 mm	3
- Severe edema with raised margin > 1 mm and extending beyond the area of exposure	4

Note: The interpretation of scoring; < 2 = slightly irritation, 2-5 = moderately irritation and > 5 = severely irritation

Results

The Draize test was done and detected erythema and edema using adopted Draize scoring system. There was no significant difference between the groups as shown in Table 2. There was no erythema and edema in all groups of the intact skins. Slightly erythema and edema were observed in all groups after 24 hrs of Draize test, but improved at the 72 hrs of experiment. All animals survived and had normal activities. Food and water consumptions were not significantly different between the groups.

Serum GPT and creatinine were measured in all groups of rats for liver function test and kidney function test as shown in Table 3. No significant difference was observed between the groups.

At the end of the experiment, all rats were anesthetized with intravenous thiopental sodium. The heart, liver, and kidney were isolated and weighed immediately. No significant difference was observed between the groups, as shown in Table 4.

Discussion

The duty of dermato-toxicologist is to protect workers and consumers from skin toxicities (irritation and allergy) associated with exposure to products, and the ingredients they contain, and to test toxicity to the skin prior to manufacture, transport, or marketing. Testing for the skin corrosion or irritation has traditionally

Table 2. The score of erythema (Er) and edema (Ed) of rats treated with CM and PAA at 24 and 72 hours

Hrs	Rat no.	Control (group 1) (n = 6)				CM (group 2) (n = 6)				PAA (group 3) (n = 6)			
		Intact		Abraded		Intact		Abraded		Intact		Abraded	
		Er	Ed	Er	Ed	Er	Ed	Er	Ed	Er	Ed	Er	Ed
24	1	0	0	1	1	0	0	1	1	0	0	1	1
	2	0	0	2	1	0	0	1	2	0	0	1	1
	3	0	0	1	2	0	0	1	1	0	0	1	1
	4	0	0	1	1	0	0	2	1	0	0	2	2
	5	0	0	1	1	0	0	2	2	0	0	2	1
	6	0	0	2	2	0	0	1	1	0	0	1	1
	Mean	0	0	1.3	1.3	0	0	1.3	1.3	0	0	1.3	1.2
72	1	0	0	1	0	0	0	0	0	0	0	0	1
	2	0	0	1	0	0	0	1	1	0	0	0	0
	3	0	0	0	1	0	0	0	1	0	0	0	0
	4	0	0	0	1	0	0	1	0	0	0	1	1
	5	0	0	1	0	0	0	1	1	0	0	1	1
	6	0	0	1	1	0	0	0	0	0	0	1	0
	Mean	0	0	0.7	0.5	0	0	0.5	0.5	0	0	0.5	0.5

Table 3. The liver and kidney function test in control rats (group 1), rats treated with CM (group 2). Rats treated with PAA (group 3)

No.	SGPT			Creatinine		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
1	48.96	39.84	33.16	1.20	0.40	0.60
2	36.01	33.60	41.76	0.50	0.40	0.40
3	26.88	48.96	31.60	0.50	0.80	0.60
4	24.06	39.80	36.07	0.60	0.60	0.80
5	44.60	62.03	31.68	0.80	0.90	1.00
6	54.70	41.76	43.78	0.90	0.80	0.60
Mean $\pm$ SD	39.20$\pm$12.30	44.33$\pm$9.97	36.34$\pm$5.27	0.75$\pm$0.27	0.65$\pm$0.22	0.67$\pm$0.21

n = 6 / group

Table 4. The organ weight of rats in control rats (group 1), rats treated with CM (group 2), rats treated with PAA (group 3)

No.	Heart			Liver			Left kidney			Right kidney		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
1	0.81	0.84	0.73	7.22	7.51	7.26	0.92	1.06	0.72	0.93	0.99	0.71
2	0.76	0.76	0.78	7.01	7.48	7.13	0.70	0.98	0.74	0.71	0.99	0.76
3	0.76	0.74	0.70	7.24	6.94	6.71	0.74	0.70	0.70	0.72	0.71	0.71
4	0.72	0.73	0.77	6.69	6.89	7.29	0.69	0.73	0.90	0.71	0.72	0.89
5	0.79	0.73	0.73	7.42	7.29	7.00	0.88	0.74	0.72	0.90	0.72	0.70
6	0.74	0.75	0.75	7.09	7.31	7.16	0.71	0.76	0.91	0.72	0.74	0.89
Mean	0.76	0.76	0.74	7.11	7.24	7.09	0.77	0.83	0.78	0.78	0.81	0.78
SD	0.03	0.04	0.03	0.25	0.26	0.21	0.10	0.15	0.10	0.10	0.14	0.09

No significant difference between groups ($p > 0.05$) for all organ weight of rats

been conducted in animals, particularly in rabbits via the long established Draize test method[6-8]. However, this procedure, among others, has been subjected to criticism, both for its limited predictive capacity for human toxicity, as well as for its use of animals[9,10]. In fact, legislation is pending in the European Union to ban the sale of cosmetic products that have the ingredients tested on animals[8]. Because of these considerations and the advancements in both *in vitro* skin biology and clinical testing, an intensive effort among skin scientists to develop alternative test methods based either on *in vitro* test systems (e.g. using rat, pig or human skin *ex vivo*, or reconstructed human skin models) or ethical clinical approaches (human volunteer studies)[8] have been done. However, some investigators continue to use the Draize test in animal models for testing the new materials before they market it[11-15].

The present study was conducted to test the CM and PAA synthetic biomaterials prior to clinical trials. Both CM and PAA have shown no irritation effects upon either intact or abraded skin of rats during the period of 24-72 hours. The measurements of liver and kidney function as tested through SGPT and serum creatinine respectively showed no effects on both organs. Furthermore, the weight of the heart, the liver, and the kidney in all groups of the experiment were not significantly changed. According to water soluble biomaterials, both may be absorbed from exposed skin. Carboxymethylchitin is eliminated fast from the blood circulation and most portions were excreted into urine[16]. Recently, macrophage activation seems to be attributable to carboxylmethylation of chitin or chitosan[5]. These indicated that CM and PAA did not interfere with the systemic normal function of this vital organ, which was correlated well with the results of Krause's study[17]. Krause has demonstrated that *N-O* carboxymethylchitosan markedly decreases the formation of post sternotomy adhesions in a large-animal model without untoward cardiac side effects. This hydrogel derivative may prove to be of great therapeutic value when used prophylactically in the setting of cardiac surgery[17,18].

Conclusion

In summary, both CM and PAA had no irritation in the rat using the Draize test. Normal liver and kidney function tests and normal organ weights after topical application of these two biomaterials indicate that neither local nor systemic effects from these materials interfered with vital organ functions. Therefore, both CM and PAA should be considered safe to use in the purpose of wound dressing in further clinical trials.

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การตรวจสอบการระคายเคืองของคาร์บอกซีไฮโดรเจลและโพลี(อะคิลิค แอซิด) ไคติน ไฮโดรเจล

วิไล รัตนตยารมณ์, สมเกียรติ วัฒนศิริชัยกุล

นำวัสดุปิดแผลสังเคราะห์คาร์บอกซีไฮโดรเจล(CM) และโพลี(อะคิลิค แอซิด) ไคติน ไฮโดรเจล (PAA) มาตรวจสอบการระคายเคืองต่อผิวหนังโดยใช้วิธี Draize test ในหนูขาวสายพันธุ์ Sprague-Dawley แบ่งหนูเป็น 3 กลุ่ม ๆ ละ 6 ตัว หนูกลุ่มที่ 1 เป็นกลุ่มควบคุม กลุ่มที่ 2 เป็นกลุ่มที่ถูกทดสอบด้วย CM กลุ่มที่ 3 เป็นกลุ่มที่ถูกทดสอบด้วย PAA โดยวัสดุปิดแผลดังกล่าวมีขนาด 1 x 1 เซนติเมตร หนูขาวถูกวางแผ่นทดสอบที่ผิวหนังด้านหลัง 2 ข้าง หลังจากการโกนขน ข้างหนึ่งเป็นผิวหนังปกติ ส่วนอีกข้างหนึ่งเป็นผิวหนังที่ขูดชั้น stratum corneum ออกไป ภายหลังจากการวางแผ่นทดสอบนาน 24 และ 72 ชั่วโมง เปิดแผ่นทดสอบออกและทำการบันทึกผื่นแดง และการบวมโดยใช้ตารางการให้คะแนนตามระบบคะแนนของ Draize test เมื่อสิ้นสุดการทดสอบสัตว์ทดลองทุกตัวได้รับการนำสลบด้วย thiopental sodium เก็บตัวอย่างเลือดจาก descending aorta เพื่อตรวจสภาพการทำงานของตับและไต จากนั้นอวัยวะภายในได้รับการชั่งน้ำหนัก ผลการทดสอบพบว่า 1) ไม่พบการระคายเคืองใด ๆ ในการทดสอบนี้ 2) หน้าที่ของตับไต ยังคงเป็นปกติ 3) น้ำหนักอวัยวะภายในเป็นปกติและไม่แตกต่างจากกลุ่มควบคุม