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# Inhibition of chemokines prevents intraperitoneal adhesions in mice

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**BACKGROUND: The present study evaluates the efficacy of a broad-spectrum chemokine inhibitor, NR58-3.14.3, in the prevention of adhesion formation after i.p. surgery in mice. METHODS: A total of 110 eight week old female Balb/c mice underwent laparotomy. Forty animals were randomly assigned to receive daily i.p. injections of either vehicle (control) or NR58-3.14.3. Time-course of adhesion formation was assessed. A titration of NR58-3.14.3 was conducted for i.p. and s.c. administrations. The effectiveness of a single intra-operative dose of NR58-3.14.3 was evaluated. Number, extent, location and type of adhesions were recorded. Immunohistochemistry of adhesions was done with leukocyte common antigen, CD45. RESULTS: Adhesion scores peaked on post-operative days 6–8. On both days 6 and 8, there were smaller adhesion size and lower cumulative adhesion scores in NR58-3.14.3-treated group. Moreover, on day 8, there were significantly fewer adhesions in NR58-3.14.3-treated group compared to controls. The least effective dose for i.p. administration of NR58-3.14.3 was 0.45 mg/animal. Subcutaneous and single intra-operative i.p. administrations were also effective in the prevention of i.p. adhesions. Although NR58-3.14.3 decreased the number of $CD45^+$ inflammatory cells in the adhesions by 22.5% compared to control group, this was not significant. CONCLUSIONS: Our results show that this broad-spectrum chemokine inhibitor prevents post-operative adhesions in mice and may have a potential clinical use.**

*Key words*: adhesion formation/chemokines/cytokines/murine model/peritoneum

## Introduction

Intraperitoneal adhesions are a significant cause of morbidity among women of reproductive age, causing pain and decreased fertility, and increasing health expenses. Although the precise pathophysiological mechanisms of adhesion formation have not been clearly outlined, it is widely accepted that infection, ischaemia and trauma to the peritoneum are initiators of the inflammatory reaction, which is followed by formation of a fibrin matrix. At first, polymorphonuclear leukocytes appear in the healing lesion, and thereafter influx of macrophages occurs (Milligan and Raftery, 1974; Buckman *et al.*, 1976; Ar'Rajab *et al.*, 1996). The infiltrating cells in turn release cytokines and other growth factors that may play a role in the adhesion process. These factors have been shown to be chemotactic for inflammatory cells and fibroblasts, and to promote angiogenesis, cell proliferation and differentiation.

Adhesions may be viewed as an aberration of the normal wound-healing cascade; normal healing of injured peritoneum would result in regeneration without adherence between intra-abdominal structures. The wound healing is initiated by the release of growth factors and cytokines such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor-β, and interleukin-1 (IL-1) from local macrophages, platelets and endothelial cells. After the arrival of neutrophils, macrophages are recruited into the site of injury, where they further secrete cytokines and growth factors that attract more macrophages and fibroblasts. Fibroblasts then proliferate and extracellular proteins such as collagen are secreted (Kovacs and DiPietro, 1994). If the initial fibrin meshwork is not completely dissolved, the entrapped fibroblasts deposit collagen that converts the fibrin meshwork into fibrous adhesion bands.

Inhibition of the factors that play roles in adhesion formation may provide a therapeutic approach in the prevention of these adhesions. We have previously shown that monocyte chemotactic protein-1 (MCP-1) is likely to play a role in adhesion formation (Zeyneloglu *et al.*, 1998a,b). Inhibition of the action of this chemokine may prevent adhesion formation.

Reckless and Grainger (1999) have recently developed a series of oligopeptides that act as functional chemokine inhibitors. One of these oligopeptides is the broad-spectrum chemokine inhibitor NR58-3.14.3 (BIM-58001) that is a powerful anti-inflammatory agent *in vivo* and *in vitro* (Reckless *et al.*, 2001) This oligopeptide inhibits leukocyte migration induced by a

 

range of chemokines including MCP-1, IL-8, macrophage inflammatory protein-1α (MIP-1α) and RANTES (Reckless *et al.*, 2001).

In the present study, we aimed to evaluate the efficacy of the broad-spectrum chemokine inhibitor on prevention of adhesion formation after i.p. surgery in mice.

## Materials and methods

### *Mice and surgical procedure*

Eight week old female Balb/c mice were obtained from NCI Frederick Laboratories (Bethesda, MD, USA). Approval from the Animal Research Committee of Yale University was obtained before the study. Under the supervision of the Yale University Animal Research Committee, animals were treated in accordance with the standards of the National Institutes of Health, as described in the *Guide for the Care and Use of Laboratory Animals.* Mice were weighed, anaesthetized with an i.p. injection of a 0.2 ml anaesthetic cocktail (0.25 mg xylazine and 2.5 mg ketamine) and underwent laparotomy after cleansing the abdomen with alcohol. A vertical 2.5 cm midline incision was made. The caecum was identified and gently grasped with two dry gauze pads, and the ventral and dorsal surfaces were stroked 20 times. The parietal peritoneum 5 mm to the right of the midline incision was then traumatized by clamping with a haemostat three times for 15 s each. The abdomen was closed *in toto* with metal clips.

### *Experimental design*

In all, 110 female Balb/c mice 8 weeks old were used to evaluate the *in vivo* effects of the broad-spectrum chemokine inhibitor NR58-3.14.3 on postsurgical peritoneal adhesion formation in mice (Table I). They all underwent laparotomy. After the closure of the abdominal wall, 40 animals were randomly assigned to receive daily i.p. injections of either phosphate-buffered saline (PBS; control) or the broad-spectrum chemokine inhibitor NR58-3.14.3, 1 mg/day/animal. All treatments were given in 0.1 ml volume. The broad-spectrum chemokine inhibitor NR58-3.14.3 was obtained from Biomeasure, Inc. (Milford, MA, USA). A set of two animals was killed on each post-operative days 2, 4, 6, 8, 10 and 14 to assess the time-course of adhesion formation. On days 6 and 8 after the operation, 16 and 12 additional animals were killed, respectively. The number, extent, location and type (filmy versus fibrous) of adhesions were recorded by an observer blinded to the treatment. The adhesion size was calculated by multiplying the width and the length of the adhesion surface area on the affected organ such as anterior abdominal wall, intestine or other viscera. Subsequently adhesions were scored according to the criteria reported by Saltzman *et al.* (1996). A cumulative adhesion score was derived for each animal.

After this initial experiment, a titration of NR58-3.14.3 was conducted looking only on day 8 with eight animals per group treated daily with i.p. injections of either PBS (control), 0.9 mg (30 mg/kg), 0.45 mg (15 mg/kg) or 0.225 mg (7.5 mg/kg) of NR58-3.14.3. This experimental design was also conducted for s.c. administration of 0.9 mg (30 mg/kg) or 0.45 mg (15 mg/kg) of NR58-3.14.3.

For the last part of the study, 14 animals were divided into two groups and were treated either with PBS or 0.9 mg (30 mg/kg) of NR58-3.14.3 only at the time of surgery. PBS or NR58-3.14.3 was administered directly into the peritoneum with massaging in order to achieve maximal drug exposure to the traumatized areas at time zero. The evaluation of adhesions was also performed on day 8 for these two groups.

**Table I.** Flow chart of the steps of the experimental design

(1) Evaluation for the time course of adhesion formation
A total 12 animals operated
A set of two animals killed on days 2, 4, 6, 8, 10 and 14
One animal of each set received daily i.p. injections of PBS (control) and the other animal received NR58-3.14.3 (1 mg/day/animal)

⇒ Evaluated for the adhesion formation

(2) Evaluation of adhesion formation after 6 and 8 days
Additional 16 mice operated

→ 8 mice received daily i.p. injections of PBS (control)

→ 8 mice received i.p. NR58-3.14.3 (1 mg/day/animal)

⇒ Evaluated for the adhesion formation on day 6

Additional 12 mice operated

→ 6 mice received daily i.p. injections of PBS (control)

→ 6 mice received i.p. NR58-3.14.3 (1 mg/day/animal)

⇒ Evaluated for the adhesion formation on day 8

(3) Evaluation for the titration of NR58-3.14.3 for i.p. and s.c. administrations
Additional 32 mice operated

→ 8 mice received daily i.p. injections of PBS (control)

→ 8 mice received i.p. NR58-3.14.3 (0.9 mg/day/animal)

→ 8 mice received i.p. NR58-3.14.3 (0.45 mg/day/animal)

→ 8 mice received i.p. NR58-3.14.3 (0.225 mg/day/animal)

⇒ Evaluated for the adhesion formation on day 8

Additional 24 mice operated

→ 8 mice received daily s.c. injections of PBS (control)

→ 8 mice received s.c. NR58-3.14.3 (0.9 mg/day/animal)

→ 8 mice received s.c. NR58-3.14.3 (0.45 mg/day/animal)

⇒ Evaluated for the adhesion formation on day 8

(4) Evaluation of single intra-operative i.p. administration of NR58-3.14.3
Additional 14 mice operated

→ 7 mice received single i.p. administration of PBS (control)

→ 7 mice received single i.p. NR58-3.14.3 (0.9 mg/animal)

⇒ Evaluated for the adhesion formation on day 8

PBS = phosphate-buffered saline.

### *Immunohistochemistry for leukocytes*

Samples were obtained from adhesion sites on day 8 and fixed with 4% paraformaldehyde, embedded in paraffin and cut into sections of 5 μm. Sections were stained with a purified rat anti-mouse CD45 (leukocyte common antigen) antibody (diluted at 1:25, BD PharMingen Laboratories, San Diego, CA, USA) for 30 min at room temperature. In negative control slides, normal rat IgG was used instead of a primary antibody. For positive control, mouse tonsil sections were stained. After several rinses in PBS containing 0.1% Tween 20 (PBS-T), biotinylated secondary antibody (biotinylated goat anti-rat IgG; Vector, Burligame, CA, USA) was applied for 30 min. Following several PBS-T rinses, slides were incubated with streptavidin–peroxidase complex for 30 min (Biogenex, San Ramon, CA, USA). Subsequently, slides were rinsed several times in PBS-T and then were incubated with 3-amino-9-ethyl-carbazol (AEC, Biogenex) for 10 min. Slides were lightly counterstained with haematoxylin prior to permanent mounting. CD45 immunoreactive cells were counted in 10 random areas under a light microscope at ×40 magnification by two investigators blinded to the type and source of the tissues. The average count of the two was used (Berkkanoglu *et al*., 2004).

### *Statistical analysis*

Adhesion sizes and scores on day 6 and day 8 were normally distributed (as determined by Kolmogorov–Smirnov test) and Student's *t*-test was used. A non-parametric test (Mann–Whitney rank sum test) was used to compare the number of adhesions on day 6 and day 8, and also for the data of all days pooled, which were not normally distributed (as determined by Kolmogorov–Smirnov test). One-way analysis of variance (ANOVA) followed by *post hoc* Holm–Sidak test was used to compare the number of adhesions, adhesion sizes and adhesion scores of treatment groups with different dosages. Statistical calculations were performed using Sigmastat for Windows, version 3.0 (Jardel Scientific Corporation, San Rafael, CA, USA)

## Results

### *Effect of broad-spectrum chemokine inhibitor NR58-3.14.3 on adhesion formation*

No abnormalities of healing of the anterior abdominal wall incision, such as wound disruption, infection or incisional hernia were noted in animals. We observed in initial experiments that the adhesion score peaked at 6–8 days after the operation. Therefore, we focused the rest of experiments on days 6 and 8 (Figure 1a, b).

When we evaluated the adhesions from all days pooled, there were fewer adhesions in the broad-spectrum chemokine inhibitor-treated group compared to control group ($P < 0.01$). As for adhesion areas, there was a 52% decrease in the adhesion size in the broad-spectrum chemokine inhibitor-treated group compared to control group ($P = 0.02$). When the cumulative scores were compared, scores were lower in the broad-spectrum chemokine inhibitor-treated group compared to the control group ($P = 0.001$).

On day 6, there was no significant difference in the number of adhesions between two groups. On the other hand, the adhesion size was smaller in the broad-spectrum chemokine inhibitor-treated group compared to control group [19.5 ± 4.1 and 6.8 ± 1.6 $mm^2$ (mean ± SEM) for control and broad-spectrum chemokine inhibitor-treated groups, respectively; $P = 0.01$] (Table II). There was a 29.8% decrease in the cumulative score of adhesions in the broad-spectrum chemokine inhibitor-treated group compared to control group [2.4 ± 0.2 and 1.8 ± 0.2 (mean ± SEM) for control and broad-spectrum chemokine inhibitor-treated groups, respectively; $P = 0.03$] (Table II).

On day 8, there were fewer adhesions in the broad-spectrum chemokine inhibitor-treated group compared to control group with a median of 1 (range 0–1) versus a median of 3 (range 1–4), respectively ($P = 0.02$). Adhesion size was smaller in the broad-spectrum chemokine inhibitor-treated group compared to control group [7.5 ± 1.9 and 2.2 ± 0.8 $mm^2$ (mean ± SEM) for control and broad-spectrum chemokine inhibitor-treated groups respectively; $P = 0.02$]. There was a 57.7% decrease in the cumulative score of adhesions in the broad-spectrum chemokine inhibitor-treated group compared to the control group [2.6 ± 0.2 and 1.1 ± 0.3 (mean ± SEM) for control and broad-spectrum chemokine inhibitor-treated groups, respectively; $P < 0.001$] (Table II).

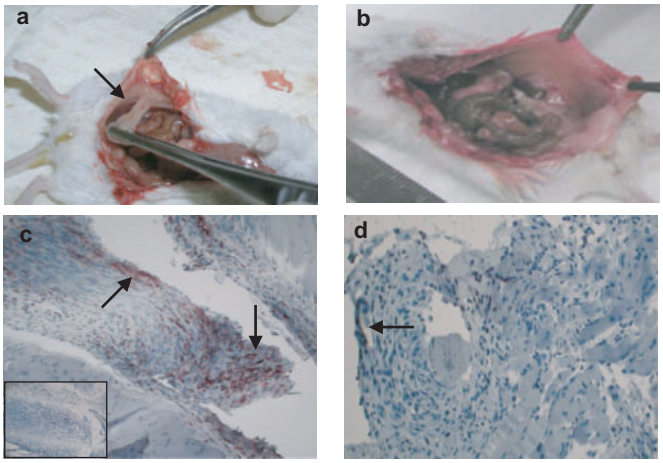


**Figure 1.** (**a–b**) Gross findings of postsurgical adhesions on day 8; (**a**) the arrow points to a single adhesion between caudal abdominal fat and the incision site in a mouse from the control group; (**b**) no adhesion seen in a mouse from the broad-spectrum chemokine inhibitor-treated group. (**c–d**) CD45 immunohistochemistry shows the presence of leukocytes in adhesion sites in day 8 samples; (**c**) an adhesion area from the control group; arrows indicate $CD45^+$ cells; (inset) negative control for antibody; (**d**) an adhesion area from the broad-spectrum chemokine inhibitor-treated group and the arrow indicates $CD45^+$ cells.

**Table II.** Number, size and scores of adhesions observed in mice on post-operative day 6 and 8

| | Control group | Broad-spectrum chemokine inhibitor group | *P* |
|---|---|---|---|
| Adhesions on day 6 | | | |
| Number [median (range)] | 2 (1–4) | 1 (1–2) | 0.16 |
| Size (mean ± SEM) | 19.5 ± 4.1 | 6.8 ± 1.6 | 0.01 |
| Score (mean ± SEM) | 2.4 ± 0.2 | 1.8 ± 0.2 | 0.03 |
| Adhesions on day 8 | | | |
| Number [median (range)] | 3 (1–4) | 1 (0–1) | 0.02 |
| Size (mean ± SEM) | 7.5 ± 1.9 | 2.2 ± 0.8 | 0.02 |
| Score (mean ± SEM) | 2.6 ± 0.2 | 1.1 ± 0.3 | < 0.001 |

When we evaluated the adhesions of four groups of animals having i.p. injections of either PBS (control), 0.9 mg (30 mg/kg), 0.45 mg (15 mg/kg) or 0.225 mg (7.5 mg/kg) per day of NR58-3.14.3, there was no significant difference in the number of adhesions between the groups. On the other hand, the adhesion sizes were significantly smaller in 0.9 and 0.45 mg/day broad-spectrum chemokine inhibitor-treated groups compared to control group and 0.225 mg/day broad-spectrum chemokine inhibitor-treated group [7.7 ± 1.7, 2.1 ± 1.0, 3.0 ± 0.9 and 8.6 ± 3.3 $mm^2$ (mean ± SEM) for control, 0.9, 0.45 and 0.225 mg/day broad-spectrum chemokine inhibitor-treated groups, respectively; $P = 0.01$] (Figure 2a). In addition, there were significant decreases in the cumulative scores of adhesions in 0.9 and 0.45 mg/day broad-spectrum chemokine inhibitor-treated groups compared to control group and 0.225 mg/day broad-spectrum chemokine inhibitor treated group [2.2 ± 0.2, 1.1 ± 0.3, 1.3 ± 0.3, and 1.9 ± 0.5 (mean ± SEM) for control, 0.9, 0.45 and 0.225 mg/day broad-spectrum chemokine inhibitor-treated groups, respectively; $P< 0.05$] (Figure 2b).

When we evaluated the adhesions of three groups of animals having s.c. injections of either PBS (control) or 0.9 mg (30 mg/kg) or 0.45 mg (15 mg/kg) per day of NR58-3.14.3, there was no significant difference in the number of adhesions between the groups, although there was a tendency of decrease in the number of adhesions in groups treated with NR58-3.14.3 compared to the control group ($P = 0.08$). On the other hand, the adhesion sizes were smaller in broad-spectrum chemokine inhibitor-treated groups compared to control group [9 ± 1.9, 3.2 ± 0.8 and 3.6 ± 1.1 $mm^2$ (mean ± SEM) for control, 0.9, and 0.45 mg/day broad-spectrum chemokine inhibitor-treated groups, respectively; $P < 0.05$] (Figure 3). There was no significant change in the cumulative score of adhesions between the groups.

Finally, a single intra-operative dose of NR58-3.14.3 (0.9 mg) did not cause any significant change in the number of adhesions compared to the control group. However, the adhesion sizes were significantly smaller in the NR58-3.14.3-treated group compared to controls [12.6 ± 2.4 and 5.6 ± 1.3 $mm^2$ (mean ± SEM) for control and NR58-3.14.3-treated group, respectively; $P < 0.05$] (Figure 4). However, there was no significant change in the cumulative score of adhesions in the NR58-3.14.3-treated group compared to controls ($P = 0.053$).

### *Immunohistochemistry of the adhesions*

Although the staining of the adhesions revealed that there were 22.5% fewer $CD45^+$ inflammatory cells in the broad-spectrum chemokine inhibitor-treated group compared to control group, this was not statistically different (Figure 1c, d).

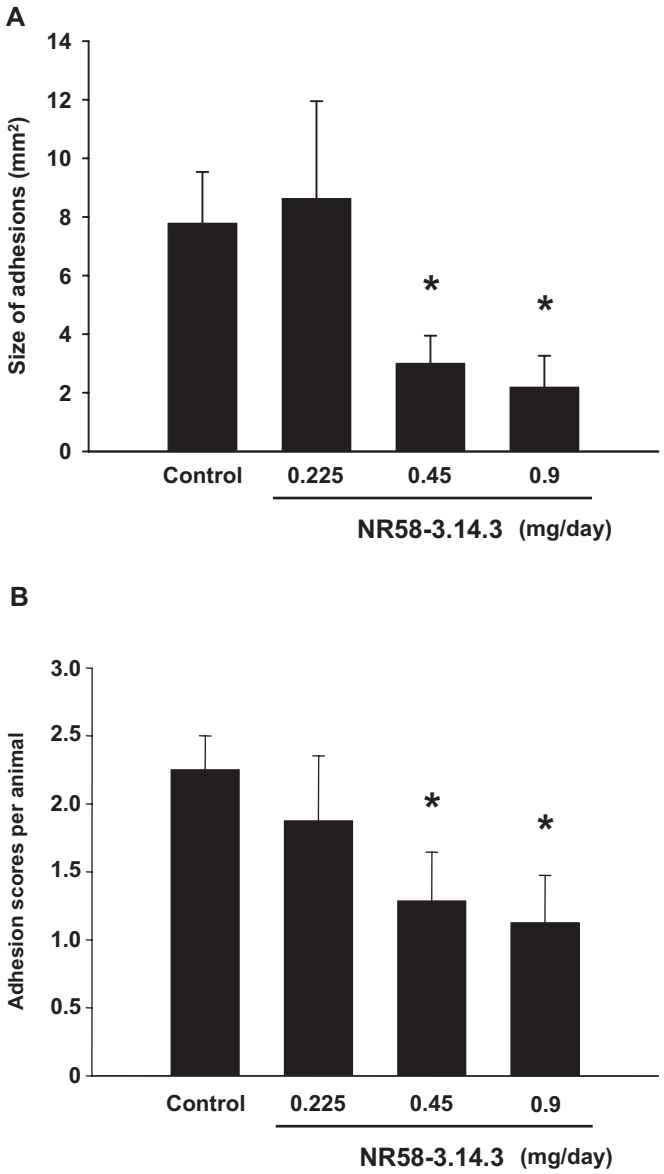


**Figure 2.** Size and score of adhesions observed in mice receiving i.p. different dosages of NR58-3.14.3. (**a**) Adhesion sizes were significantly smaller in 0.9 and 0.45 mg/day broad-spectrum chemokine inhibitor-treated groups compared to control and 0.225 mg/day broad-spectrum chemokine inhibitor-treated groups [7.7 ± 1.7 (mean ± SEM), 8.6 ± 3.3, 3.0 ± 0.9 and 2.1 ± 1.0 $mm^2$ for control, 0.225, 0.45 and 0.9 mg/day broad-spectrum chemokine inhibitor-treated groups, respectively; $*P = 0.01$]. (**b**) There were significant decreases in the cumulative scores of adhesions in 0.9 and 0.45 mg/day broad-spectrum chemokine inhibitor-treated groups compared to control and 0.225 mg/day broad-spectrum chemokine inhibitor-treated groups [2.2 ± 0.2 (mean ± SEM), 1.9 ± 0.5, 1.3 ± 0.3 and 1.1 ± 0.3 for control, 0.225, 0.45 and 0.9 mg/day broad-spectrum chemokine inhibitor-treated groups, respectively; $*P < 0.05$].

## Discussion

Histopathogenesis of inflammation and repair of the mesothelium is relatively well understood. Adhesion formation begins with a fibrin matrix, which typically occurs during coagulation. Between post-injury days 1 and 3, the adhesion is composed of a variety of cellular elements including leukocytes encased in fibrin matrix. This matrix is then gradually replaced by vascular granulation tissue containing macrophages, fibroblasts and giant cells. Early peritoneal polymorphonuclear leukocytes modulate the function of lately recruited macrophages (Kuraoka *et al.*, 1992). By the fourth day, a considerable amount of fibrin matrix disappears and large numbers of fibroblasts with associated collagen are present. The macrophages become the predominant type of leukocytes. If any factor

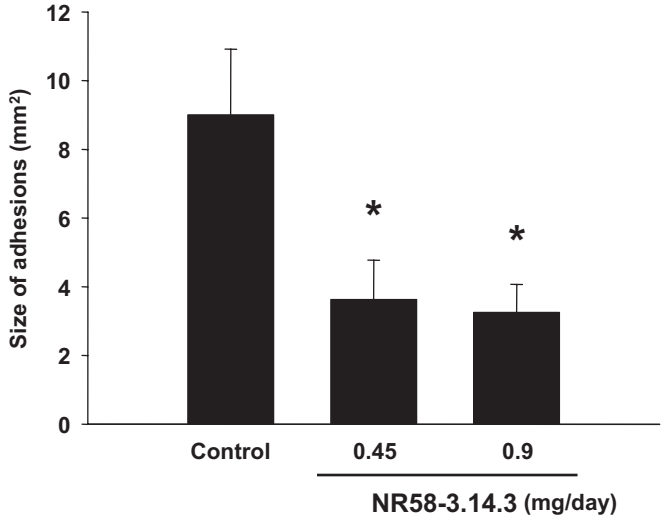


**Figure 3.** Size of adhesions observed in mice receiving different s.c. dosages of NR58-3.14.3. The adhesion sizes were smaller in broad-spectrum chemokine inhibitor-treated groups compared to control group [9 ± 1.9 (mean ± SEM), 3.6 ± 1.1 and 3.2 ± 0.8 $mm^2$ for control, 0.45 and 0.9 mg/day broad-spectrum chemokine inhibitor-treated group, respectively; *$P < 0.05$].

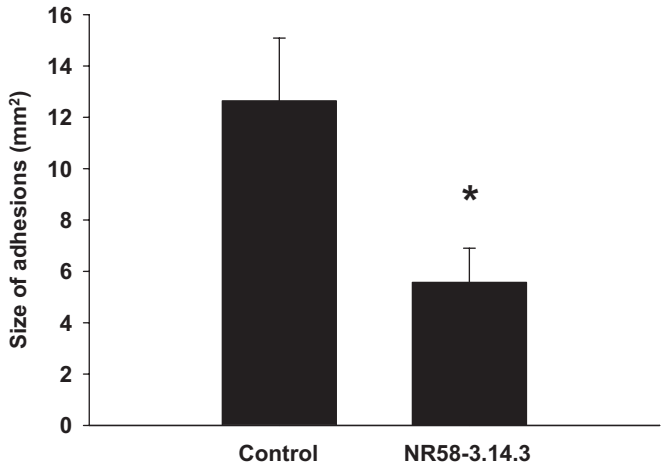


**Figure 4.** Size of adhesions observed in animals given a single intra-operative dose of NR58-3.14.3 (0.9 mg). The mean adhesion size was significantly smaller in the broad-spectrum chemokine inhibitor-treated group compared to control group [12.6 ± 2.4 (mean ± SEM) $mm^2$ and 5.6 ± 1.3 $mm^2$ for control and broad-spectrum chemokine inhibitor-treated group respectively; *$P < 0.05$].

(i.e. ischaemia, infection and excess fibrin formation beyond the clearance capacity of macrophages) interrupts the healing cascade by day 5, adhesions may form.

Chemokines are implicated in a range of inflammatory processes. They can recruit leukocytes into the inflammatory areas including surgical traumas. Several cytokines are continuously produced by macrophages and fibroblasts entrapped within the fibrin meshwork, including tumour necrosis factor-α, IL-1, PDGF, EGF and MCP-1 (Kovacs and DiPietro, 1994; Zeyneloglu *et al.*, 1998a), which in turn recruit leukocytes and increase collagen synthesis (Kovacs, 1991).

Recently, Reckless and Grainger (1999) developed a series of oligopeptides that act as functional chemokine inhibitors. One of these oligopeptides is the broad-spectrum chemokine inhibitor NR58-3.14.3 that is a novel broad-spectrum inhibitor of chemokine function (both CXC and CC types) (Reckless *et al.*, 2001). NR58-3.14.3 has a plasma half-life of <30 min following i.v. injection into mice, largely as a result of renal clearance (Wilbert *et al.*, 2000). It has been shown that the compound is non-toxic. The acute 50% lethal dose ($LD_{50}$) in mice is >500 mg when administered via the i.v. route and therefore it has a wide therapeutic range (Reckless *et al.*, 2001).

This oligopeptide inhibits leukocyte migration induced by a range of chemokines including MCP-1, IL-8, MIP-1α and RANTES (Reckless *et al.*, 2001). It has also been shown that NR58-3.14.3 has a neuroprotective action in a rat model of cerebral ischaemia–reperfusion injury (Beech *et al.*, 2001), which is associated with a developing inflammatory response with pathological contributions from vascular leukocytes and endogenous microglia. In addition, it ameliorates experimental obliterative bronchiolitis in a rat model (Naidu *et al.*, 2003), which is characterized with peribronchial inflammation, respiratory epithelial cell injury and proliferation of fibrovascular connective tissue. It has also been shown that broad-spectrum chemokine inhibition ameliorates reperfusion injury in rat lungs (Naidu *et al.*, 2004).

Animal models are essential and important to determine the success of various methods of adhesion prevention. In our present model, stroking caecum with dry gauze pad and traumatizing parietal peritoneum by clamping with a haemostat were used for adhesion formation. As we previously demonstrated, this method causes adhesion formation in mice (Zeyneloglu *et al.*, 1998a). Furthermore, bleeding is not a significant contributory factor to the adhesion formation in this model, as previously demonstrated by others (Hershlag *et al.*, 1991). In this study, we observed that the adhesion score peaked 6–8 days after the operation. Therefore, we focused the rest of experiments on days 6 and 8. On the other hand, it has been claimed that animal models are not appropriate for determining the adhesion prevention in humans (Diamond, 1997) because the organs are entirely normal before surgery in animal experiments and this is completely different from the situation in humans. However, animal models have the advantage of allowing us to set the same conditions for all experiments.

In our experimental set-up, we have shown the adhesion-preventive effect of NR58-3.14.3. for the first time. On day 6, NR-58–3.14.3 decreased the cumulative adhesion scores and the size of adhesions, but had no significant effect on the number of adhesions. But on day 8, it decreased not only the cumulative adhesion scores but also the number and size of adhesions significantly. It was also noted that the adhesion sizes on day 8 were smaller than the adhesion sizes on day 6 in both control and treated groups. This may be due to contracture of the adhesion areas with time. On the other hand, the immunohistochemical studies with anti-mouse CD45 antibody revealed no significant difference in leukocyte infiltration in adhesion samples. This may be due to the fact that adhesions

are formed if leukocytes can reach the injury sites and thus it is always possible to see leukocyte infiltrations whenever there are adhesions. Moreover, leukocyte subtypes could be more important in adhesion sites than the total leukocyte number. Therefore, it will be more helpful to assess the leukocyte subtypes in these areas.

Furthermore, we have shown that the least effective dose of NR58-3.14.3 is 0.45 mg/day per animal when it is given i.p. for 8 days. This dosage is effective for decreasing both the adhesion size and the cumulative scores of adhesions. We also looked for the effect of the s.c. route of this compound. Although the s.c. administration of NR58-3.14.3 did not result in any significant decrease in the number of adhesions and the cumulative scores of adhesions, it reduced the adhesion sizes significantly. It seems that this route is also effective. However, the optimal dosage and duration of administration need to be determined. Moreover, we also demonstrated the reducing effect of single intra-operative dose of NR58-3.14.3 on adhesion size. In contrast, a single dose of NR58-3.14.3 did not cause any decrease in the number and the cumulative scores of adhesions.

Individual chemokines and cytokines of the inflammatory cascade can have both pro- and anti-inflammatory activities on various cell types. The interaction between these molecules determines the final level and cellular composition of the inflammatory response. With the use of a broad-spectrum chemokine inhibitor, the cumulative impact of the chemokine network to promote inflammation could be inhibited. Our study demonstrates that NR58-3.14.3 can prevent *de novo* surgical adhesion formation in mice. This effect is probably due to its quite distinct property of broad-spectrum chemokine inhibition, but it may also be due to its undefined pharmacological effects. Nevertheless, if its role is verified in human studies, it may prove to be of value as a therapeutic option in adhesion prevention.

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## References

Ar'Rajab A, Mileski W, Sentementes JT, Sikes P, Harris RB and Dawidson IJA (1996) The role of neutrophils in peritoneal adhesion formation. J Surg Res 61,143–146.

Beech JS, Reckless J, Mosedale DE, Grainger DJ, Williams SC and Menon DK (2001) Neuroprotection in ischemia–reperfusion injury: an antiinflammatory approach using a novel broad-spectrum chemokine inhibitor. J Cereb Blood Flow Metab 21,683–689.

Berkkanoglu M, Guzeloglu-Kayisli O, Kayisli UA, Selam BF and Arici A (2004) Regulation of Fas ligand expression by vascular endothelial growth factor in endometrial stromal cells in vitro. Mol Hum Reprod 10,393–398.

Buckman RF Jr, Buckman PD, Hufnagel HV and Gervin AS (1976) A physiologic basis for the adhesion-free healing of deperitonealized surfaces. J Surg Res 21,67–76.

Diamond MP (1997) Animal adhesion model: design, variables, relevance. In Pelvic Surgery. Springer-Verlag, New York, USA, pp 65–70.

Hershlag A, Otterness IG, Bliven ML, Diamond MP and Polan ML (1991) The effect of interleukin-1 on adhesion formation in the rat. Am J Obstet Gynecol 165,771–774.

Kovacs EJ (1991) Fibrogenic cytokines: the role of immune mediators in the development of scar tissue. Immunol Today 12,17–23.

Kovacs EJ and DiPietro LA (1994) Fibrogenic cytokines and connective tisue production. FASEB J 8,854–861.

Kuraoka S, Campeau JD, Nakamura RM and DiZerega GS (1992) Modulation of postsurgical macrophage function by early postsurgical polymorphonuclear leukocytes. J Surg Res 53,245–250.

Milligan DW and Raftery AT (1974) Observations on the pathogenesis of peritoneal adhesions: a light and electron microscopical study. Br J Surg 61,274–280.

Naidu BV, Farivar SA, Krishnadasan B, Woolley SM, Grainger DJ, Verrer ED and Mulligan MS (2003) Broad-spectrum chemokine inhibition ameliorates experimental obliterative bronchiolitis. Ann Thorac Surg 75,1118–1122.

Naidu BV, Farivar AS, Woolley SM, Grainger D, Verrier ED and Mulligan MS (2004) Novel broad-spectrum chemokine inhibitor protects against lung ischemia-reperfusion injury. J Heart Lung Transplant 23,128–134.

Reckless J and Grainger DJ (1999) Identification of oligopeptide sequences which inhibit migration induced by a wide range of chemokines. Biochem J 340,803–811.

Reckless J, Tatalick LM and Grainger DJ (2001) The pan-chemokine inhibitor NR58-3.14.3 abolishes tumor necrosis factor-alpha accumulation and leucocyte recruitment induced by lipopolysaccharide in vivo. Immunology 103,244–254.

Saltzman AK, Olson TA, Mohanraj D, Carson LF and Ramakrishnan S (1996) Prevention of postoperative adhesions by an antibody to vascular permeability factor/vascular endothelial growth factor in a murine model. Am J Obstet Gynecol 174,1502–1506.

Wilbert SM, Engrissei G, Yau EK, Grainger DJ, Tatalick L, Axworthy DB (2000) Quantative analysis of a synthetic peptide, NR58-3.14.3, in serum by LC-MS with inclusion of a diastereomer as internal standard. Anal Biochem 278,14–21.

Zeyneloglu HB, Senturk LM, Seli E, Oral E, Olive DL and Arici A (1998a) The role of monocyte chemotactic protein-1 in intraperitoneal adhesion formation. Hum Reprod 13,1194–1199.

Zeyneloglu HB, Seli E, Senturk LM, Gutierrez LS, Olive DL and Arici A (1998b) The effect of monocyte chemotactic protein-1 in intraperitoneal adhesion formation in a mouse model. Am J Obstet Gynecol 179,438–443.

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