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The effect of resveratrol on surgery-induced peritoneal adhesions in an experimental model†

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Adhesion formation is a common cause of complications following surgery. The aim of this study was to investigate the effect of resveratrol on intra-abdominal adhesion prevention in a rat model. Twenty one Wistar-Albino rats weighing 200–250 g were assigned to three groups, of 7 rats each. After a midline laparotomy was performed, a 1 cm area of the ceacum was abraded in two of the groups. They were then given either resveratrol (Group 1), or saline (Group 2) intraperitoneally. Group 3 rats (sham operation) received no treatment, without the serosal damage. On the 14th day, the rats were killed and the adhesion score was determined according to Mazuji's adhesion grade scale. The tissue levels of malondialdehyde (MDA), nitric oxide (NO), and reduced glutathione (GSH) were measured. The mean Mazuji's adhesion grade in the resveratrol group was 1.0 ± 0.0, in the saline group 2.57 ± 1.51, and zero in the sham operated group ($p < 0.05$ between the resveratrol group and saline group comparison). The levels of MDA and NO in the resveratrol group were significantly lower than those of the saline group ($p < 0.001$). The level of GSH in the resveratrol group was significantly higher than in the saline and sham operated groups ($p < 0.001$ and $p < 0.001$, respectively). Introduction of resveratrol into the peritoneal cavity at the time of surgery reduced adhesion formation effectively in this model. Resveratrol probably acts through reduction of lipid peroxidation products. Copyright © 2006 John Wiley & Sons, Ltd.

KEY WORDS — resveratrol; peritoneal adhesion; rat

INTRODUCTION

Postoperative adhesions are a frequently encountered problem. They are the most common cause of small bowel obstruction in adults[1] and account for 15–20% of female infertility cases.[2] Moreover, they can result in chronic abdominal and pelvic pain. All these adhesion related clinical problems lead to significant morbidity and mortality rates and high financial costs.[3–5] Therefore, the development of modalities to prevent postoperative adhesions would have a major clinical and economic impact.

Adhesion formation is an integral part of wound healing.[6–8] It seems to be a result of inflammatory mechanisms. Numerous mediators of inflammation have been implicated in adhesion formation (including arachidonic acid, cytokines, nitric oxide, and oxygen derived free radicals)[8–10] and previous studies have shown that antioxidants, such as methylene blue and vitamin E are effective in reducing adhesion formation in rats.[9–11]

Resveratrol is a natural phytoalexin present in high concentrations in grapes and red wine and has a variety of biological proprties including antiinflammatory, anticarcinogenic, and antioxidative activities.[12–14] Its effect in preventing adhesion formation has not yet been investigated. Therefore, we used resveratrol administered intraperitoneally to determine whether there is any beneficial effect on the frequency and the degree of experimentally induced intraperitoneal adhesions in rats.

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METHODS

Twenty one male Wistar rats weighing 200–250 g were randomly assigned to 3 groups of 7 rats each. All the rats were fed standard rat diet but food was withheld on the eve of the experiment. Rats were anesthetized by intraperitoneal injection of 75 mg/kg ketamine. The abdomen was shaved and prepared with 1% povidine-iodine solution and alcohol. The peritoneal cavity was accessed via a 3 cm long midline incision. The anterior cecal wall was abraded with 20 strokes of a toothbrush. Moreover, full thickness 4–0 silk sutures were placed in the traumatized anterior cecal wall to increase the adhesion reaction, as previously described.[15] The midline incision was closed in one layer with a running 3–0 silk suture.

Prior to closure of the abdomen, 10 mg resveratrol or 6 ml of 0.9% saline solution was administered intraperitoneally to rats of Group 1 and 2, respectively. The sham operation group (Group 3) underwent midline laparotomy with no treatment and without serosal damage to the cecal wall. The rats were sacrificed 2 weeks after the operations. Complete adhesiolysis was performed and difficulty of adhesiolysis was assessed by a surgeon blind to the experimental protocol using Mazuji's[16] classification. The extent and severity of adhesions in the operation site for each parietal peritoneum were evaluated using this established scoring system. The peritoneal and cecum tissues were quickly removed. In order to perform macroscopic examination, 1 g tissue samples were taken from the cecal wall. They were preserved at -70° until examination. According to this system, the extent of adhesions was evaluated as in Table 1.[16] The mean adhesion grades were compared between the groups.

Biochemical analysis

All solutions were prepared with distilled-deionized water. $ZnSO_4$, NaOH, glycine, $CuSO_4$, sulfanilamide, N-NNDA (N-1- naphthyl ethylenediamine), $NaNO_2$, KNO_3, $Na_2B_4O_7.10H_2O$, KCl, n-butanol, TBA (4,6 dihidroxypyrimidine-2 thiol) , EDTA, GSH, DTNB (5–5′-dithiobis (2-nitrobenzoic acid), and NaN_3, were purchased from Sigma Chemical Co., Germany; KH_2PO_4, Na_2HPO_4, were from Merck Co., Germany, Cadmium (Cd) granules were from Fluka (Chemische Fabrik AG, Bushs, Switzerland).

One hundred milligrams of tissue biopsy specimens were cut into pieces on dry ice, and homogenized in 1.15% KCl-phosphate buffered saline solution (1:9, w/v) using a manual glass homogenizer for approximately 5 min. The supernatant (tissue extract) was used for analysis. As tissue nitrite (NO_2) and nitrate (NO_3) levels can be used to estimate NO production, we measured the concentration of these stable NO oxidative metabolites. Quantification of NO_2 and NO_3) was based on the Griess reaction, in which a chromophore with a strong absorbance at 545 nm is formed by reaction of NO_2 with a mixture of naphthlethylenediamine and sulfanilamide.[17] The results were expressed as μmol/g tissue. Malondialdehyde (MDA) in tissue was determined by the method of Uchiyama and Mihara.[18] Three millilitres of 1% phosphoric acid and 1 ml of 0.6% thiobarbituric acid solution were added to 0.5 ml of tissue extract and the mixture was heated in boiling water for 45 min. After cooling, the color was extracted into 4 ml of n-butanol. The absorbance was measured in a spectrophotometer (Ultraspec Plus, Pharmacia LKB Biochrom, UK) at 532 nm. The amounts of lipid peroxides were calculated as thiobarbituric acid reactive substances of lipid peroxidation and expresed as nmol/g tissue. Glutathione (GSH) was determined by the spectrophotometric method using Ellman's reagent and the results were expressed as nmol/mgwet/ tissue.[19]

Statistical analysis. The statistical package for social sciences (SPSS) version 11.0 was used for statistical analysis. A Kruskal-Wallis test was used to compare the mean values among the groups. Mann–Withney *U*-tests were performed for differences within two groups. The results are given in the text as mean $\pm$ standard deviation (SD) for all comparisons; statistical significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

All animals survived the entire length of the study. No adverse postoperative complications were noted in any animals. Rats in the sham operated group (Group 3) had the lowest adhesion grade (0 ± 0 vs. 2.57 ± 1.51

Table 1. Mazuji's scoring system for adhesion severity and number

Grade	Observation
1	No adhesion
2	Very thin and pathological adhesion
3	Easily detectable moderate adhesion
4	Dense, continuous adhesion (adhesiolysis is not difficult)
5	Dense adhesion (adhesiolysis is difficult)

Table 2. The mean tissue levels of MDA, NO and GSH activity in the resveratrol, saline and sham operated rats

Groups	GSH (nmol/mgwet tisue)	NO (μmol/gwet tissue)	MDA (nmol/gwet tissue)
(1) Resveratrol ($n=7$)	2.27 ± 0.53	117.1 ± 22.9	139.5 ± 10.0
(2) Saline ($n=7$)	1.36 ± 0.27	177.1 ± 57.1	395.0 ± 100.3
(3) Sham ($n=7$)	0.58 ± 0.13	124.2 ± 28.2	211.6 ± 145.0
p values			
1 versus 2	<0.001	0.026	<0.001
1 versus 3	<0.001	0.938	0.443
2 versus 3	0.002	0.052	<0.001

$p < 0.05$ was accepted as statistically significant.

in the saline group and 1 ± 0 in the resveratrol group, $p = 0.003$ and $p < 0.05$, respectively) and rats in the resveratrol group had a significantly lower adhesion grade than rats in the saline group ($p < 0.05$) (Table 1).

The levels of MDA and nitric oxide (NO) in the resveratrol group (Group 1) were significantly lower than those of the saline group (Group 2) ($p < 0.001$). The level of glutathione (GSH) in the resveratrol group was significantly higher than in the saline and sham operated groups ($p < 0.001$ and $p < 0.001$, respectively) (Table 2).

Peritoneal adhesions develop in the majority of patients after abdominal operations. This study shows that resveratrol is a potent inhibitor of adhesion formation and suggests that its action is due to its antiinflammatory and antioxidative effect as shown by the lower tissue levels of NO and MDA in the treatment group.

We need first to understand the pathogenesis of adhesion formation to reveal the potential role of resveratrol as an antioxidative agent in this process. Injuries occurring to the on peritoneum surface play an important role in the development of postoperative adhesions.[6,7] Mediators of inflammation, such as free radicals and NO have been implicated in adhesion development.[20,21] These induce adhesion by damaging cellular membranes.[11]

NO is a potent endogenous vasodilatator. It modulates platelet adhesion and aggregation, leukocytes adhesion, endothelin generation, and plasminogen activator enzyme function.[21] It is a free radical and is therefore highly reactive. Expression of inducible nitric oxide synthase in response to cytokines or endotoxin seems to be part of the inflammatory response and could contribute to vasodilatation, vascular leakage, and tissue damage in some inflammatory condition, such as adhesion formation. In the present study, NO was significantly higher in the saline group than in the resveratrol and sham groups. In addition, we demonstrated that tissue NO levels were clearly decreased by resveratrol. These results show that NO may play an important role in the adhesion formation and decreased adhesion formation in the resveratrol group may be related to its antioxidant and free radical scavenging effect.[22]

The increased concentration of MDA reflects the level of lipid peroxidation in tissues and is considered a marker of tissue injury.[23] We demonstrated that tissue MDA levels were increased in the adhesion group but were decreased by resveratrol. The decreasine in MDA levels in the resveratrol group is attributable to its antioxidant and free radical scavenging effect.[22]

Resveratrol probably reduces the afflicting effects of oxidative stress in living cells, ensuring the integrity of biological membranes as other antioxidants.[10,22,24] Furthermore, resveratrol has been shown to inhibit platelet activating factor (PAF), and thrombus formation.[25] These events may lead to reduction in thromboplastin fibrin generation.[26] Since the role of fibrin in adhesion formation is well documented, this may be another mechanism by which resveretrol reduces postoperative adhesion formation.

The potentially harmful effects of reactive oxygen species are controlled by the cellular antioxidant defense system.[27] GSH is an important constituent of intracellular protective mechanisms against a number of noxious stimuli including oxidative stress.[28] It is often reported as anti-inflammatory because it inhibits the production of several inflammatory cytokines and chemokines and their action.[29] In the present study a reduction in GSH levels occurred after peritoneal adhesion. The increased GSH level in the resveratrol group may be related to its antioxidant and free radical scavenging effect. It is well known that resveratrol may act upon the enzymes involved in glutathione synthesis and maintain levels of glutathione during oxidative stress.[30]

In the literature, as far as we know, there is no report on the use of resveratrol for the preventation of

adhesion formation. In the present study, administration of resveratrol significantly decreased tissue MDA and NO levels and increased GSH levels. This study also shows that the adhesion score in the resveratrol group was significantly lower than that of the saline group, suggesting that resveratrol can reduce the degree and severity of peritoneal adhesion.

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