Rhein lysinate suppresses the growth of breast cancer cells and potentiates the inhibitory effect of Taxol in athymic mice

Ya-Jun Lin and Yong-Su Zhen

Earlier studies have shown that rhein, one of the major bioactive constituents of the rhizome of rhubarb, inhibits the proliferation of various human cancer cells. However, because of its water insolubility, the antitumor efficacy of rhein is limited *in vivo*. In this study, we studied the antitumor activity of rhein lysinate (the salt of rhein and lysine and easily dissolving in water) and its mechanism. Inhibition of breast cancer cell proliferation was determined by MTT assay and the mechanism of action of rhein lysinate was investigated by western blot analysis. The therapeutic efficacy of rhein lysinate was evaluated by human cancer xenografts in athymic nude mice. Rhein lysinate inhibited the proliferation of breast cancer cells (MCF-7, SK-Br-3, and MDA-MB-231). The IC_{50} values were 95, 80, and 110 μmol/l, respectively. Rhein lysinate inhibited the phosphorylation of epidermal growth factor receptor, MEK, and ERK with or without EGF stimulation. It also inhibited tumor growth and enhanced the therapeutic effect of Taxol on MCF-7 xenografts in athymic mice. Rhein lysinate inhibited the phosphorylation of epidermal growth factor receptor and MAPK signal pathway. These results suggest that rhein lysinate might be useful as a modulation agent in cancer chemotherapy. *Anti-Cancer Drugs* 20:65–72 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Keywords: epidermal growth factor receptor, human breast cancer, MAPK signal pathway, rhein lysinate, xenograft

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Introduction

Rhein (4,5-dihydroxy-anthraquinone-2-carboxylic acid) is one of the major bioactive constituents of the rhizome of rhubarb (*R. palmatum L.* or *R. tanguticum Maxim*) [1,2]. In earlier studies, rhein was found to have a variety of bioactivities, such as inhibiting IL-1-induced chondrocyte activation [3,4], decreasing cellular hypertrophy of mesangial cells [5], inhibiting tumor cell proliferation, inducing tumor cell apoptosis, and showing synergy with mitomycin [6–8]. Furthermore, it inhibits 12-*O*-tetradecanoylphorbol-13-acetate-induced cell transformation. One of the major barriers to rhein clinical application is its insolubility in water. Rhein only dissolves in dimethyl sulfoxide (DMSO) [1], which is harmful to cells. Even though it dissolves in DMSO, if diluted by water, it will precipitate. Thus it impedes the in-vivo uses of rhein. In this study, we prepared rhein lysinate (RHL), which does dissolve in water. The structure of RHL is shown in Fig. 1. The molecular weight of RHL is 430 and molecular formula is $C_{21}H_{22}N_2O_8$. RHL not only can be given through oral administration but can also be given through parenteral administration. No report about the manufacture of RHL and its antitumor activity has been found yet. In this study, we investigated the antitumor activity of RHL, especially its combination with Taxol and its mechanism of action.

Materials and methods

Chemicals

Rhein (98%) was purchased from Nanjing Qingze Medicine Ltd (Nanjing, Jiangsu, China). Lysine was purchased from Beijing Solarbio Science and Technology Co. (Beijing, China). RHL was made in our department. Taxol was purchased from Beijing Union Pharmaceutical Factory (Beijing, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) and DMSO were obtained from Sigma Aldrich (Shanghai, China). EGF was obtained from Roche (Shanghai, China). Antibodies against HER-2, p-EGFR (Tyr1068), k-Ras, p-c-Raf (Ser338), c-Raf, p-ERK1/2 (Thr202/Tyr204), ERK1/2, p-p38 (Thr180/Tyr182), p38, p-JNK (Thr183/Tyr185), JNK, p-MEK1 (Ser221), and MEK1 were purchased from Cell Signaling Technology (Beverly, Massachusetts, USA). Antibodies against epidermal growth factor receptor (EGFR) and β-actin were purchased from Santa Cruz Technology (California, USA). Secondary antibodies were purchased from Cell Signaling Technology.

Cell lines and cell culture

Human breast cancer cell lines (MCF-7, SK-Br-3, and MDA-MB-231) were used in this study. MCF-7 and MDA-MB-231 cells grow quickly with EGFR moderate expression. SK-Br-3 cells grow slowly with EGFR

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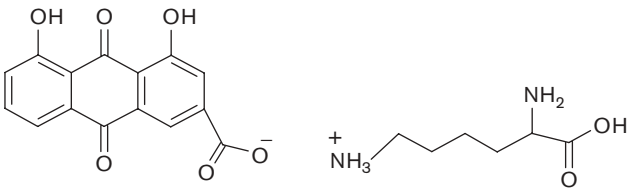
Fig. 1



The structure of rhein lysinate.

moderate expression and HER-2 overexpression (Fig. 3a). MCF-7 cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (Gibco, Grand Island, New York, USA). MDA-MB-231 cells were maintained in Dulbecco's modified Eagle's medium (DMEM)/F12 supplemented with 10% fetal bovine serum. SK-Br-3 cells were maintained in DMEM supplemented with 15% fetal bovine serum. All cells were grown in a humidified atmosphere of 5% CO_2 at 37°C.

Animals

Female BALB/c athymic mice, 6–8 weeks old, weighing 20–22 g, were purchased from Vital River Experimental Animal Technology, Co. Ltd (Beijing, China). These animals were housed under sterile conditions, with food and water provided under sterile conditions. These experiments were performed with these protocols approved by the Animal Care and Use Committee, Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences.

Cell viability assays

Cell viability was examined by MTT assays [9], according to the instructions of the manufacturer. MCF-7, MDA-MB-231, and SK-Br-3 cells were plated onto 96-well plates (MCF-7 3000 cells/well, MDA-MB-231 5000 cells/well, and SK-Br-3 10 000 cells/well, respectively). Twenty-four hours later, breast cancer cells were treated with the indicated concentrations of RHL or rhein (in DMSO solution), respectively, for 48 h. All assays were done in triplicate. The IC_{50} was defined as the drug concentration that resulted in a 50% reduction in the number of cells compared with untreated control. The IC_{50} values were determined directly from the dose–response curves. For drug combination the Chou–Talalay [10] combination index (CI) method was used to analyze the data obtained.

Stimulation of cells

MCF-7 cells were grown to 80% confluence in medium containing 10% fetal bovine serum then serum deprived for 24 h before addition of drug. Cells were then treated with vehicle (PBS) or 80 μmol/l RHL. Twenty-four hours after drug treatment, vehicle (PBS) or 10 μg/l EGF was added and cells were harvested 10 min later [11].

Protein extraction and western blot analysis

Total cellular protein was extracted using a lysis buffer (50 mmol/l Tris–HCl, pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 150 mmol/l NaCl, 1 mmol/l EGTA, 1 mmol/l phenylmethylsulfonyl fluoride, 1 mmol/l Na_3O_4, 1 mmol/l NaF) with protease inhibitor cocktail (Roche, Basel, Switzerland) [12]. The proteins were quantified by using a BCA protein assay kit (Pierce, Rockford, Illinois, USA). Equivalent amounts of protein were loaded onto 10% SDS–PAGE gel and transferred to nitrocellulose membrane. First, the membrane was inoculated in a blocking buffer containing BSA (1%) and Tween 20 (0.1%, v/v) in PBS (PBS/Tween 20) at room temperature for 1 h. Second, it was inoculated overnight at 4°C with proper primary antibodies. Then it was inoculated with proper secondary antibodies, at room temperature for 2 h. Each membrane was developed using an enhanced Chemi-Imager5500 chemiluminescence system (Alpha Innotech Corporation, Miami, Florida, USA).

Effects of rhein lysinate, Taxol, and the combination on tumor growth in athymic mice

MCF-7 cells (5×10^6 cells per animal) were injected subcutaneously into the armpit of 6–8-week-old BALB/c female athymic mice. When the tumor volume (TV) was $1 \times 1 \times 1\,cm^3$, the mice were killed. After removing the necrotic portion of the tumor mass, the tumor was cut into $2 \times 2 \times 2\,mm^3$ blocks, and the blocks were inoculated subcutaneously into the armpit of female athymic mice (seven groups, six mice for each group). One week later, when the solid tumors were palpable, the mice were treated by intraperitoneal injection with saline, lysine (100 mg/kg), RHL (50 mg/kg), RHL (100 mg/kg), Taxol (10 mg/kg), RHL (50 mg/kg) plus Taxol (10 mg/kg), and RHL (100 mg/kg) plus Taxol (10 mg/kg), respectively. RHL and lysine were given twice a week for a total of 4 weeks and Taxol was given once a week for 2 weeks. The TV was measured every 4 days. At the end of the experiment, the mice were killed and tumor weight was measured. Tumors in each group were removed and the level of EGFR phosphorylation was determined by immunohistochemical staining.

Immunohistochemical analysis

Tumor specimens taken from tumor-bearing mice were routinely dehydrated and paraffin-embedded. The sections (5 μm) were incubated in 3% hydrogen peroxide and then blocked by incubation in 2.5% BSA in PBS. The primary antibody for p-EGFR was applied to the slides at a dilution of 1 : 50 and incubated at 4°C overnight. After being washed in PBS, the samples were treated with biotin-labeled secondary antibody at a dilution of 1 : 200 for 1 h at room temperature. Detection was performed with an SABC immunohistochemical kit (Boster Biotechnology Co. Ltd, Wuhan, China). The slides were stained by using a diaminobenzidine kit (Boster Biotechnology Co. Ltd), then washed, counterstained

with hematoxylin, dehydrated, treated with xylene, and mounted. The intensity of immunohistochemical staining was evaluated as follows: –, negative; +, focal expression in < 5% of cancer tissue; + +, focal expression in 5–20% of cancer tissue; and + + +, diffuse expression > 20% of cancer tissue. The tissues with '+ +' and '+ + +' staining of p-EGFR were classified as the high expression group and the tissues with '+' staining were classified as the low expression group [13].

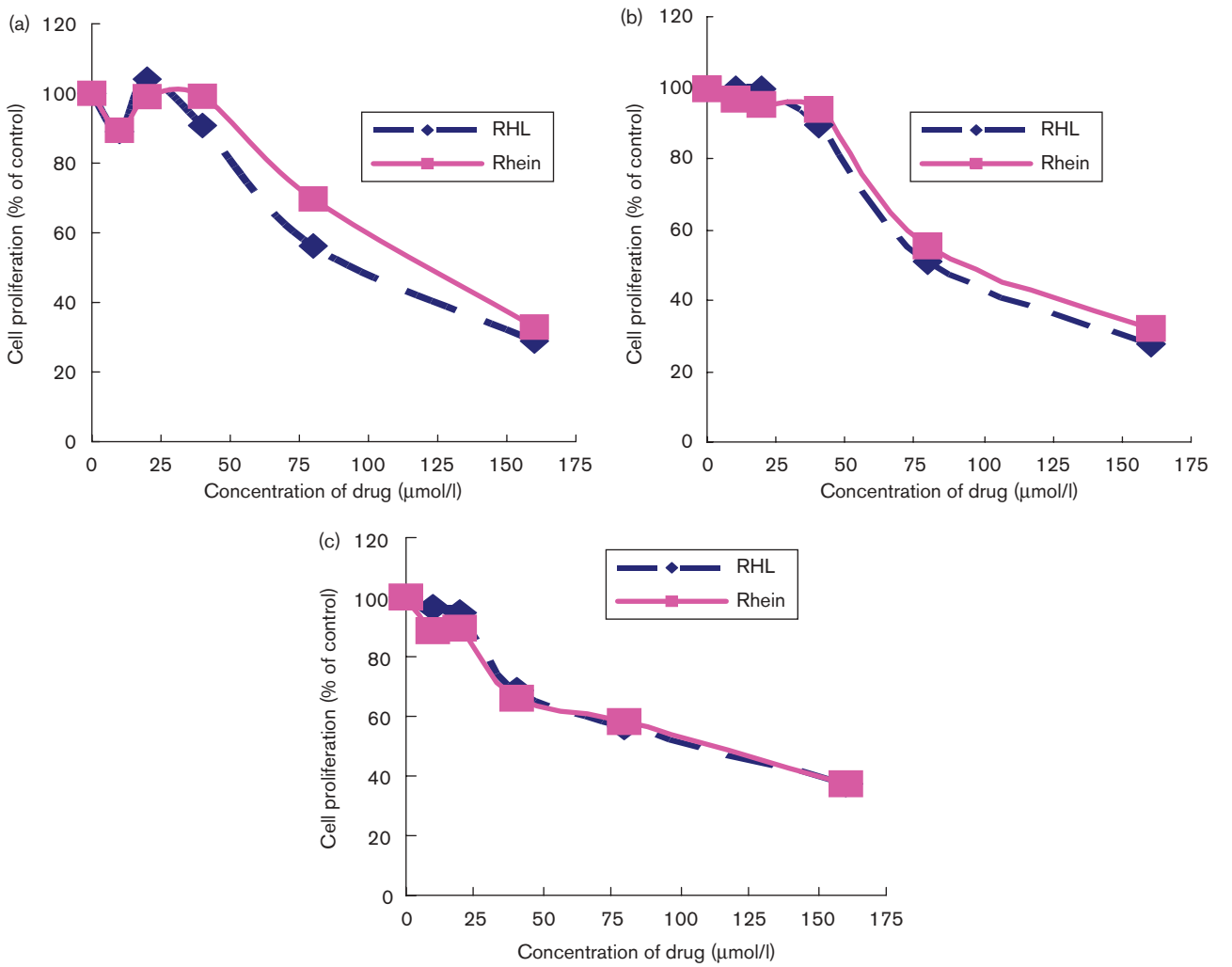
Evaluation of drug efficacy and drug combination

Tumor growth was followed every 4 days by measuring tumor length (L) and width (W) using a caliper. Each set of measurements was converted to TV (mm^3) using the standard formula, $TV = (L \times W^2)/2$. Drug efficacy was assessed as inhibition rate, inhibition rate $\% = (1 - T/C)\ \%$, $T/C =$ (mean TV of treated group)/(mean TV of control group). For evaluating the combined effect of the two drugs, observed values were compared with predicted values (c) calculated from the equation $c = a \times b/100$, where a and b are T/C with single agents. Observed values lower than those predicted were considered a synergism, and values lower than 70% of those predicted were considered a significant synergism [13,14].

Statistical analysis

Statistical analysis was performed between control and the different treated groups. Comparisons of means were carried out by one-way analysis of variance. Differences with a value of $P < 0.05$ were considered to be statistically significant.

Fig. 2



Effect of rhein lysinate (RHL) and rhein on cell proliferation of MCF-7 cells (a), SK-Br-3 cells (b), and MDA-MB-231 cells (c). Cells were treated with various concentrations of RHL at 37°C for 48 h. The effects on cell proliferation were examined by the MTT assay, and cell proliferation was calculated as the percentage of control. All assays were done in triplicate.

Results

Comparable cytotoxicity of rhein lysinate and rhein

RHL moderately inhibited cell proliferation of EGFR moderate expression breast cancer cells (MCF-7, SK-Br-3, and MDA-MB-231). The IC_{50} values were 95, 80, and 110 μmol/l, respectively. RHL showed approximate potency of inhibition to different breast cancer cells, although HER-2 was overexpressed in SK-Br-3 cells. We also observed that RHL and rhein (in DMSO solution) displayed the same inhibition to respective breast cancer cell lines (Fig. 2).

Synergism of rhein lysinate in combination with Taxol inhibited the proliferation of MCF-7 breast cancer cells

MCF-7 cells were treated with different concentrations of RHL or Taxol for 48 h, and then the effect of RHL or Taxol on cell viability was measured by using the MTT assay. Both RHL and Taxol inhibited cell proliferation in a dose-dependent manner and IC_{50} values of RHL or Taxol were 95 μmol/l and 1.2×10^{-7} mol/l, respectively. In this study, we used IC_{25}, IC_{50}, and IC_{75} of RHL and Taxol as the reference combination dose. RHL displayed synergism with Taxol to inhibit the proliferation of breast cancer cells. The CI is shown in Table 1. Taxol at 1.6×10^{-8} μmol/l and RHL at 40 μmol/l inhibited MCF-7 cell proliferation by 50%; the CI value was 0.63. Taxol at 2.7×10^{-9} μmol/l and RHL at 80 μmol/l inhibited MCF-7 cell proliferation by 75%; the CI value was 0.43.

Inhibition of the epidermal growth factor receptor-k-Ras-c-Raf-MEK-ERK signal pathway by rhein lysinate

RHL inhibited the phosphorylation of EGFR in MCF-7 breast cancer cells, but had no influence on the expression of EGFR. RHL also inhibited the expression of k-Ras, c-Raf, MEK, and the phosphorylation of c-Raf, MEK, and ERK. Nevertheless, it had no influence on the expression of ERK, p38, JNK, and the phosphorylation of p38 and JNK (Fig. 3b). In this study, we found that Taxol increased the phosphorylation of EGFR and ERK in MCF-7 cells. When it combined with RHL (80 μmol/l), the increase of the phosphorylation was attenuated (Fig. 3c). Thereby the combination of Taxol and RHL might reduce the occurrence of Taxol-resistant cells.

We next determined the effect of RHL on EGF-induced phosphorylation and expression of EGFR, MEK, and ERK1/2. Pretreatment of MCF-7 cells for 24 h with 80 μmol/l RHL blocked the ability of EGF to induce phosphorylation of EGFR, MEK, and ERK1/2. However, there was no conspicuous effect of drug or EGF treatment on total EGFR, MEK, and ERK1/2 expression (Fig. 3d).

Effect of rhein lysinate on tumor growth of MCF-7 xenografts in athymic mice and the synergism with Taxol

RHL acts synergistically with Taxol to inhibit the proliferation of MCF-7 human breast cancer cells *in vitro* (Table 1); we therefore, examine whether RHL sensitizes MCF-7 xenografts in athymic nude mice to Taxol. The growth of human breast cancer MCF-7 xenografts in athymic mice was moderately inhibited by RHL (100 mg/kg) or Taxol (10 mg/kg) alone. The inhibition rate was 46.7 or 60%, respectively. The combination of 10 mg/kg Taxol and 100 mg/kg RHL suppressed the growth of MCF-7 xenografts by 85.8%. A synergistic effect was observed on MCF-7 xenografts in athymic mice and the observed value was 14.2% (70% of predicted value is 14.9%) (Table 2). The tumor growth curve of BALB/c athymic mice inoculated with MCF-7 breast cancer cells also demonstrated that RHL (100 mg/kg) inhibited tumor growth and improved the efficacy of Taxol (Fig. 4).

Rhein lysinate inhibited the phosphorylation of epidermal growth factor receptor *in vivo*

To investigate whether repression of tyrosine phosphorylation of EGFR was connected with the therapeutic effects of RHL on tumors *in vivo*, tyrosine phosphorylation of EGFR in tumors from the mice in each group (control, RHL alone, Taxol alone, or RHL plus Taxol) was analyzed by immunohistochemical staining (Fig. 5). The control tumor had moderate positive yellow staining (+), which represents the low level phosphorylation of EGFR, but no such staining (−) was detected in RHL-treated tumor tissues. A high positive yellow phosphotyrosine staining (+ +) was also observed in Taxol-treated tumor tissues, but not in combined RHL and Taxol-treated tumor tissues.

Discussion

The major finding of this study was that RHL inhibited breast cancer cell proliferation by blocking the phosphorylation of EGFR and its important downstream

Table 1 The synergistic effect of RHL and Taxol on breast cancer MCF-7 cell proliferation

	IC values (mol/l)		
Group	IC_{25}	IC_{50}	IC_{75}
Taxol	2.8×10^{-9}	1.2×10^{-7}	4.9×10^{-6}
RHL	5.0×10^{-5}	1.0×10^{-4}	2.0×10^{-4}
Taxol + RHL	$1.2 \times 10^{-10} + 4.0 \times 10^{-5}$	$1.6 \times 10^{-8} + 4.0 \times 10^{-5}$	$2.7 \times 10^{-9} + 8.0 \times 10^{-5}$
CI values	1	0.63	0.43

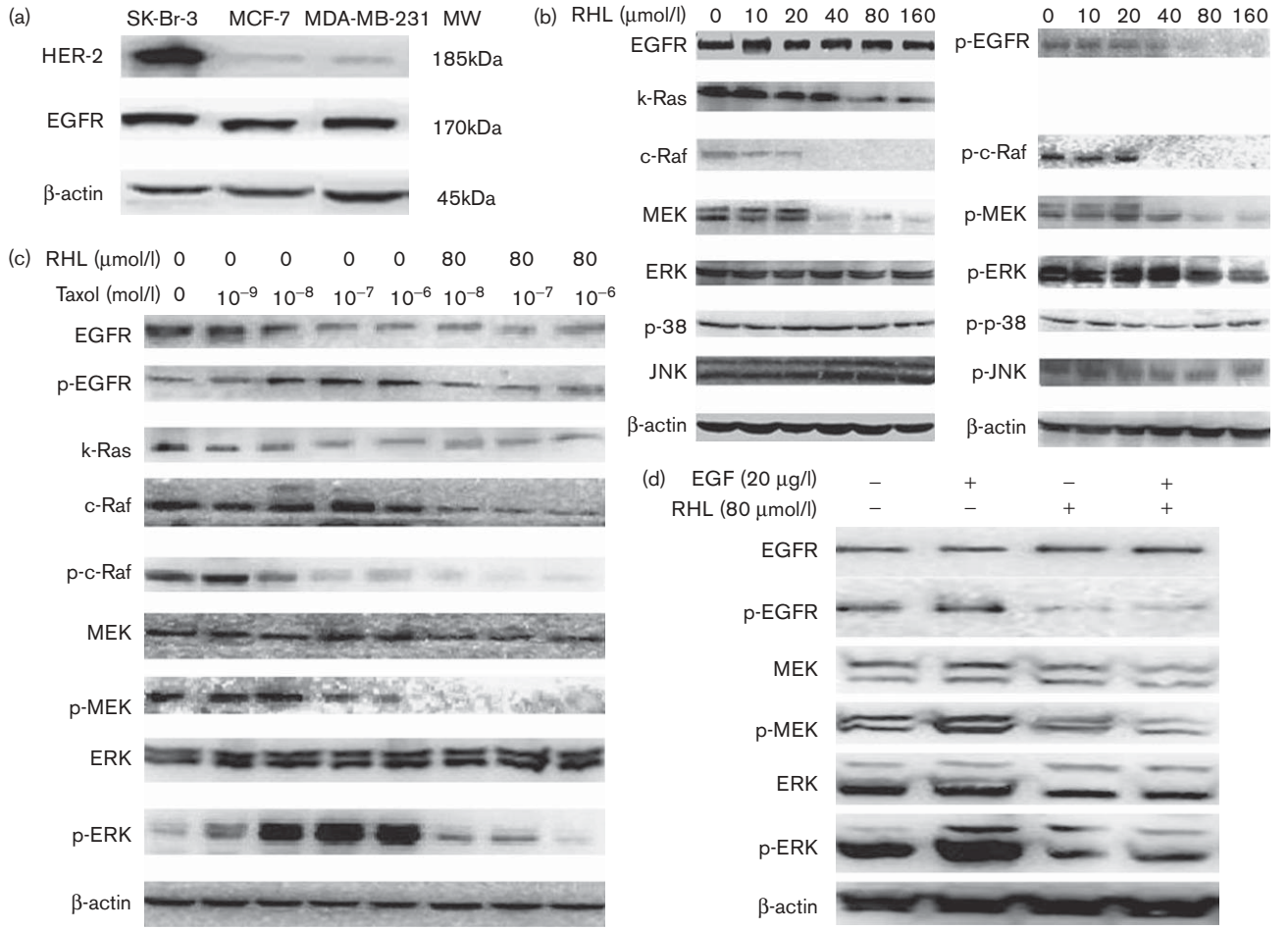
CI > 1 antagonism; CI = 1 addition; CI < 1 synergism.
CI, combination index; RHL, rhein lysinate.

Table 2 **The growth inhibition of human breast cancer MCF-7 xenografts by RHL and Taxol in athymic mice** $\bar{x} \pm s$

Group	Dose (mg/kg)	Body weight after treatment (g)	Tumor weight (g)	Inhibition rate (%)
Control		23.5 ± 1.9	1.2 ± 0.3	
Lysine	100	23.6 ± 1.1	1.1 ± 0.1	8.3
RHL	50	23.9 ± 1.0	0.8 ± 0.3	32.5[a]
RHL	100	24.1 ± 3.2	0.6 ± 0.4	46.7[a]
Taxol	10	24.3 ± 1.4	0.5 ± 0.1	60.0[a]
Taxol + RHL	10 + 50	24.0 ± 1.5	0.4 ± 0.3	65.0[a]
Taxol + RHL	10 + 100	23.9 ± 2.2	0.2 ± 0.1	85.8[a,b]

There are six mice per group. No mice died during the experiment.
RHL, rhein lysinate.
[a]Compared with control $P<0.05$.
[b]Compared with Taxol $P<0.05$. Determined on day 32 after tumor inoculation.

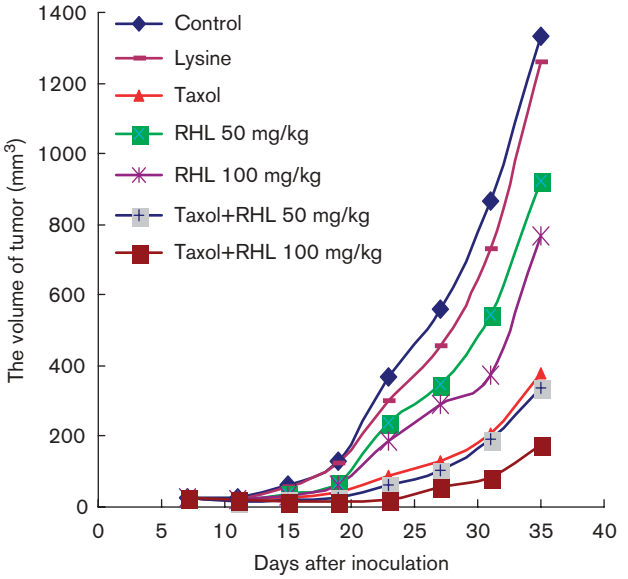
Fig. 3



RHL decreases the phosphorylation of EGFR, MEK, and ERK1/2 in MCF-7 cell line. (a) The expression levels of HER-2 and EGFR in SK-Br-3, MCF-7, and MDA-MB-231 cell lines. MCF-7 cells were grown to 80% confluence in medium containing 10% FBS. (b) Cells were then treated with vehicle (PBS), 10, 20, 40, 80, and 160 μmol/l RHL. (c) Cells were then treated with vehicle (PBS), 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} mol/l Taxol alone or in combination with 80 μmol/l RHL. Forty-eight hours after treatment, cells were harvested and lysates were subjected to western blot analysis for EGFR, c-Raf, MEK, ERK1/2, p38, and p-JNK phosphorylation and expression levels using site-specific antibodies as indicated. (d) MCF-7 cells were grown to 80% confluence in medium containing 10% FBS then serum deprived for 24 h before treatment with vehicle (PBS) or 80 μmol/l RHL. After 24 h RHL treatment, vehicle (PBS) or 20 μg/l EGF were added to medium. Cells were harvested 10 min after EGF treatment and lysates prepared for western blot analysis of EGFR, MEK, and ERK phosphorylation and expression level using site-specific antibodies as indicated. EGFR, epidermal growth factor receptor; FBS, fetal bovine serum; RHL, rhein lysinate.

mitogen-activated protein kinases (MAPK) signal pathways *in vitro*. Furthermore, RHL also inhibited tumor growth by blocking the phosphorylation of EGFR *in vivo*. The phosphorylation of proteins on tyrosine residues by protein tyrosine kinases (PTKs) is an important event in the control of cell proliferation, differentiation, mitosis,

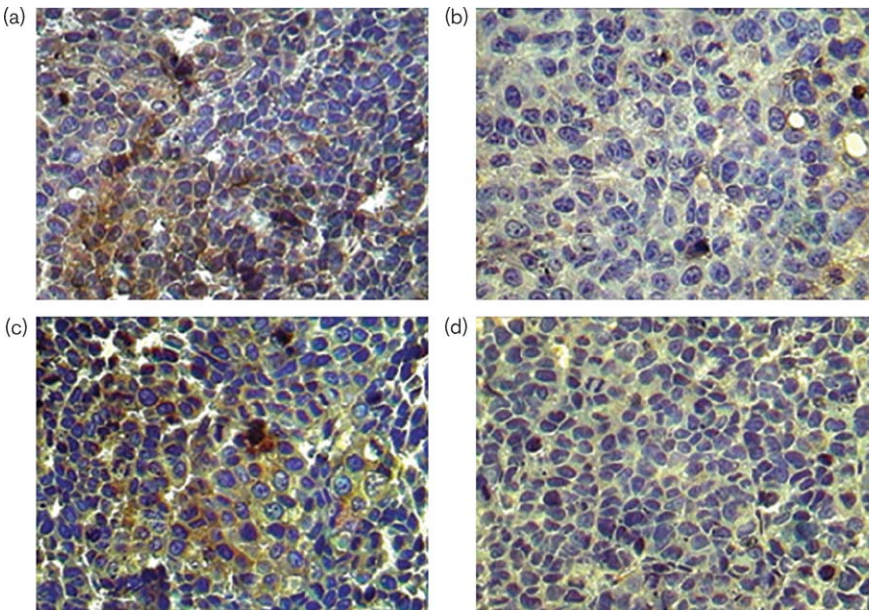
Fig. 4



Effect of rhein lysinate (RHL) on tumor growth in athymic nude mice bearing human breast cancer MCF-7 xenografts. Tumors were inoculated into the armpit of BALB/c female athymic mice. One week later, when solid tumors were palpable, the mice were given saline, lysine, RHL by intraperitoneal injection twice a week for 4 weeks and Taxol (10 mg/kg) by intraperitoneal injection once a week for 2 weeks. Tumor volume was monitored every 4 days for 4 weeks. Statistical determinations were performed using one-way analysis of variance.

and apoptosis. More than 50% of the known receptor PTKs have been repeatedly found to be either mutated or overexpressed in association with human malignancies. EGFR is an important member of receptor PTKs and has a close relationship with breast cancer [15–17]. The deregulation of the EGFR seems to be most prevalent in various carcinomas [18]. EGFR and its ligands (EGF, TGF-α) have been implicated in various tumors of epithelial origin (e.g. breast [19,20], colon [21], ovarian [9], and nonsmall cell lung cancer [22]). It is well known that rhein, together with emodin and aloe-emodin, is the main anthraquinone derivatives of rhubarb. It was reported that emodin suppressed the tyrosine kinase activity of HER-2 and preferentially inhibited cell growth and the transformation phenotype *in vitro* for human breast cancer cells that overexpress the HER-2 oncogene [23]. In this study, we selected three human breast cancer cell lines with moderate expression of EGFR to examine the inhibition of RHL to cell proliferation. The results showed that RHL had the same inhibition to human breast cancer cells (MCF-7, SK-Br-3, and MDA-MB-231), although HER-2 was overexpressed in SK-Br-3 cells. It was probable that HER-2 was not involved in the effect of RHL on cell proliferation. We also observed that RHL at concentrations of 100 mg/kg inhibited the growth of MCF-7 xenografts in athymic mice. Evidently, RHL had antitumor activities *in vitro* and *in vivo*.

Fig. 5



Immunohistochemical staining of representative tumor tissue sections taken from control (a), 100 mg/kg rhein lysinate (RHL)-treated (b), 10 mg/kg Taxol-treated (c), and 10 mg/kg Taxol plus 100 mg/kg RHL-treated (d) mice inoculated with MCF-7 cells. Positive (yellow) staining indicates the level of tyrosine phosphorylation.

MAPK signal family is one of the downstream of EGFR signal pathway. It is a family of intracellular enzymes that constitute important mediators of signal transduction pathways and coordinate the cellular response to a variety of extracellular stimuli [24]. This large superfamily of serine/threonine protein kinases consists of three major MAPK families: extracellular signal-regulated kinases (ERK1 and ERK2), c-Jun NH_2-terminal kinases (JNK1, JNK2, and JNK3), and p38MAPK [25]. Of these, only the Ras-MEK-ERK pathway is associated with suppression of apoptosis [26]. ERK1/2 lies downstream of a group of kinases Ras, Raf, and MEK1. On stimulation by extracellular signals such as the EGFR family, they are successively activated by phosphorylation [27,28]. Constitutive activation of Ras/Raf/MEK/ERK signal is a hallmark of many human cancers, such as breast, lung, and colorectal cancers as well as melanoma [29–31]. RHL inhibited cell proliferation in human breast cancer cells. After 48-h treatment with RHL (10–160 μmol/l), evidence for the inhibition of cell proliferation was clearly documented by the decreasing phosphorylation of EGFR, c-Raf, MEK, ERK, and the decreasing expression of k-Ras, c-Raf, and MEK (Fig. 3b). In this study, we had seen increased phosphorylation of EGFR, MEK, and ERK1/2 in MCF-7 cells after EGF stimulation. We also observed a significant decrease EGF-induced EGFR, MEK, and ERK1/2 phosphorylation with 80 μmol/l RHL pretreatment. It suggested that RHL could inhibit the phosphorylation of the EGFR-MAPK signal pathway with or without EGF stimulation.

RHL not only inhibited the proliferation of tumor cells but also improved the therapeutic efficacy of chemotherapeutic drugs. Taxol, which is widely used in the treatment of breast cancer, increases the phosphorylation of EGFR in some tumors. Accordingly, it activates MAPK signal pathways, and attenuates Taxol-induced cell death [32–34]. In this study, RHL significantly reduced the increase of EGFR and ERK phosphorylation induced by Taxol *in vitro* (Fig. 3c). Furthermore, the growth of human breast cancer MCF-7 xenografts in athymic mice was moderately inhibited by RHL (100 mg/kg) or Taxol (10 mg/kg) alone. The inhibition rate was 46.7 or 60%, respectively. The combination of 10 mg/kg Taxol and 100 mg/kg RHL suppressed the growth of MCF-7 xenografts by 85.8%. A synergistic effect was observed between RHL and Taxol on MCF-7 xenografts in athymic mice. To investigate whether the repression of tyrosine phosphorylation of EGFR is connected with the therapeutic effects of RHL on tumors *in vivo*, tyrosine phosphorylation of EGFR in tumors from the mice of each group was analyzed by immunohistochemical staining (Fig. 5). The results showed that RHL inhibited the growth of MCF-7 xeongrafts in athymic mice in association with the reduction of EGFR phosphorylation. In the case of combination RHL could inhibit the increase of EGFR phosphorylation induced by Taxol. Notably, there was synergism in the combination of Taxol and RHL in the treatment of MCF-7 xenografts. Previously, emodin, an analog of rhein, was reported to inhibit the growth of the HER-2 overexpressing MDA-MB-361 breast cancer xenografts and sensitized the tumor to Taxol in athymic mice model [14]. The results of that study were in concordance with ours. It would be interesting to further study whether RHL also inhibits the growth of the HER-2 overexpressing breast cancer cells and sensitizes them to Taxol.

The RHL used in this study is highly soluble in water. Thus it may facilitate the use of RHL *in vitro* and particularly in in-vivo therapeutic evaluation. By contrast, the main naturally occurring anthraquinone derivatives such as emodin, aloe-emodin, and rhein are limited for in-vivo application because of their poor water solubility [35]. In addition, the rhein anthraquinone could be converted to labile rhein anthrone by cecal microflora and some intestinal bacterial strains [36]. It is disadvantageous our for rhein to be given orally. In summary, results of this study indicate that RHL, as an active inhibitor, is capable of repressing the tyrosine kinase activity of EGFR, accordingly inhibiting the proliferation of EGFR moderate expression breast cancer cells. Moreover, RHL may sensitize breast cancer cells to chemotherapeutic drugs such as Taxol. These results may have important implications in chemotherapy.

Acknowledgements

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