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Journal of Pharmaceutics and Pharmacology Research

Prasad Neerati[1*], J Pharmaceutics and Pharmacology Research
http://dx.doi.org/ 10.31579/1.10004

Research Article Open Access

# Influence of Resveratrol on the Pharmacokinetics and Pharmacodynamics of Naproxen: Involvement of CYP1A2 Inhibition

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**Received date: December 05 , 2018; Accepted date : January 19, 2018; Published date: January 30, 2018.**

**Citation this Article** : Prasad Neerati, Maheshwari K, Influence Of Resveratrol on The Pharmacokinetics and Pharmacodynamics of Naproxen: Involvement of CYP1A2 Inhibition . J Pharmaceutics and Pharmacology Research, **Doi:** http://dx.doi.org/ 10.31579/1.10004.

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## Abstract

The purpose of the present study was to assess the effect of resveratrol (RSV) on the pharmacokinetics of naproxen (NAP) in rats. A single dose of RSV 30mg/kg was administered once during treatment phase. A single dose of NAP 25mg/kg was administered after RSV treatment. The blood samples were collected after NAP dosing at predetermined time intervals and analyzed by HPLC. In comparison with the control, RSV pretreatment significantly enhanced maximum plasma concentration (Cmax), area under the curve (AUC), and half life (t1/2) and significantly decreased apparent oral clearance (CL/F) and apparent volume of distribution (Vd/F), while there was no significant change observed in time to reach maximum concentration (tmax) of NAP. The results suggest that the altered pharmacokinetics of NAP might be attributed to RSV-mediated inhibition of CYP1A2 enzyme. Therefore, combination therapy of NAP along with RSV may represent a novel approach to reduce dosage and results in reduced gastrointestinal side effects of NAP.

## Keywords

Naproxen, Resveratrol, Carrageenan, CYP1A2 enzyme

## Introduction

Naproxen (NAP) (6-methoxy-α-methyl-2-napthalene acetic acid) is a non –steriodal anti-inflammatory drug effective in rheumatoid arthritis (Bowers et al.1975) and as analgesic (Ruedy and Mcullongh 1973). The efficacy of naproxen is related to its plasma concentrations (Dayet al.1982). Naproxen is well absorbed orally in doses as high as 900mg (Runkel et al.1974) and is concentrated largely in plasma (Runkel et al. 1972) because of extensive binding to plasma albumin (Brogden et al. 1984) Naproxen is a stereochemically pure nonsteroidal anti-inflammatory drug of the 2-arylpropionic acid class. The absorption of naproxen is rapid and complete when given orally. Naproxen is eliminated following biotransformation to glucuroconjugated and sulphate metabolites which are excreted in urine, with only a small amount of the drug being eliminated unchanged. The excretion of the 6-0-desmethylnaproxen metabolite conjugate may be tied to renal function, as accumulation occurs in end-stage renal disease. Naproxen undergoes phase I dealkylation by cytochrome P450 (CYP) to the O-demethylated metabolite, followed by phase II acylglucuronidation. Hence, naproxen is oxidised to 6-0-desmethylnaproxen (6-DMN) and conjugated to naproxen acyl glucuronide and 6-0-desmethylnaproxen acylglucuronide (6-DMNG).

A preliminary report demonstrated that human liver microsomal O-demethylation of S-naproxen was decreased by the CYP2C9-specific inhibitor Sulfaphenazole .A subsequent investigation has shown that sulfaphenazole reduced microsomal demethylation of S-naproxen by 47%, and the CYPIA2 inhibitor furafylline decreased O-demethylation of S-naproxen by 28%, suggesting that CYP2C9 and lA2 together account for the majority of human liver demethylation of naproxen.

Resveratrol (RSV) (3, 4′, 5-trihydroxystilbene) is a naturally occurring polyphenolic phytoalexin is present in fruits, vegetables, grape skins and especially in redwine. RSV possesses diverse biochemical and physiological properties including anti-inflammatory, immune modulatory activities as well as wide range of health benefits

ranging from chemoprevention to cardio protection (Kalantari and Das, 2010; Brisdelli et al., 2009). RSV has recently been shown to exert genoprotective, cytotoxic, antiproliferative and proapoptotic actions in different tumoural cell lines (Romano et al., 2013). RSV exhibits anti-inflammatory activity through the modulation of enzymes and pathways that produce mediators of inflammation.RSV possesses good potential to be used as an adjunctive or alterative therapy for inflammatory diseases (Das and Das, 2007; Udenigwe et al., 2008). In addition,RSV has been shown to produce a low profile of side effects (Cottart et al., 2010). Thus, the combination of NAP along with RSV could be an alternative in the treatment of inflammatory diseases.

NAP is an important anti-inflammatory drug with widespread use, and its administration receiving long-term therapy with herbal compounds or dietary supplements containing RSV may occur. An extensive study of the literature did not revealany report of an interaction between NAP and RSV. It is therefore relevant to consider the interaction between NAP and RSV. The aim of this study was to evaluate the effect of RSV treatment on the pharmacokinetics of NAP in rats.

## Materials and Methods

### Materials

Naproxen was a gift sample from Aurobindo Pharmaceuticals (Jadcherla, Mahaboobnagar). Carrageenan was obtained from (Sigma Aldrich,Bangolore ). RSV was procured from Navachetan (New Delhi, India).

Solvents used for quantitative analysis (Merck, India) and all other chemicals and reagents which were used in the study are of analytical grade.

## Animals

Albino male Wistar rats weighing 200–250 g were used for the study. The animals were procured from the central animal facility at Kakatiya University, Warangal. The rats were group housed in polypropylene cages (38 x 23 x 10 cm) with not more than 5 animals per cage. They were maintained under standard laboratory conditions with a natural light-dark cycle (14± 1 h light; 10 + 1 h dark) and were allowed free access to standard dry rat diet and tap water ad libitum. All procedures described were reviewed and approved by the Institutional Animal Ethics Committee (IAEC/35/UCPSc/KU/2016). The rats were divided into different groups of 6 animals each.

## Method

### Carrageenan induced Paw edema model

1% w/v suspension of carrageenan is prepared freshly in normal saline & injected into sub plantar region of the left hind paw. Test drug is administered orally/i.p. Paw volume upto the ankle joint measured in drug treated & untreated groups & 3hrs after carrageenan challenge using a plethysmograph filled with mercury. Edema is found out & % reduction in edema is calculated using the formula.

% reduction in edema= mean edema in control group / mean edema in treated control group - mean edema in drug treated groupx100.

All the animals were divided into V groups containing 6 animals each ( n=6).Group(III)

resveratrol 30mg/kg p.o, group(IV) naproxen 25mg/kg p.o, and group(V) combination received drug treatment for a period of three weeks.

On the 21st day of the study, after an hour blood samples were obtained from retro orbital sinus at time intervals of 0.5,1,2,3,4,5,6 and 7 hours and samples were stored at -80ºC till further analysis. Next day all the groups except Group (I) received 1% w/v suspension of carrageenan, injected in to the subplantar region for 1 day. Group I received only vehicle i.e., Normal saline. Each animal was observed in separate cage for atleast 30 minutes after carrageenan induced inflammation.

## Determination of Naproxen in Plasma samples

### Stock solutions and standards

Stock solutions of naproxen were prepared by dissolving naproxen in methanol resulting in a solution containing 1mg/ml. This solution was diluted 20-fold to give a working solution of 50 µg/ml. Stock solution of internal standard was prepared by dissolving ibuprofen in methanol to give 1mg/ml concentration.

### Treatment of Plasma samples

The working solutions (100µl) were separately transferred to clean dry centrifugation tubes. Plasma (500 µl) was added to each tube. These were vortex mixed for 2 min before adding 400 µl of methanol. These were vortex mixed before centrifugation at 5000 rpm for 5minsSupernatant was separated and loaded into HPLC vials before injecting 50 µl into the HPLC system.

### Calibration curves

Calibration curves were constructed in rat plasma. These involved replicate analysis of plasma samples spiked with varying concentrations of Naproxen ( 0.5,1,2,5,10,20 and 40µg/ml) and a fixed concentration of the internal standard (10 µg/ml). The calibration curve of naproxen is a plot of ( PAR) peak area ratio of the drug to the internal standard as a function of the drug concentration (C).

### Pharmacokinetic analysis

The pharmacokinetic parameters, Peak serum concentrations [ Cmax] and time to reach peak concentration [ tmax] were directly obtained from concentration-time data. In the present study AUC0-t refers to AUC from 0 to 7 hours, which was determined by linear trapezoidal rule, and AUC0-∞ refers to AUC from 0 to infinity.

The AUC0-∞ was calculated using the formula AUC0-t +[Clast/ Kcl] where Clast is the concentration in mg/ml at the last time point and Kcl is the elimination rate constant.

## Results

During the study period, no serious adverse events related to drug were reported. The pharmacokinetic parameters and mean plasma concentration–time profiles of NAP after pretreatment with RSV are shown in (Table 1) and (Table 2), respectively.

| TIME (h) | NAP | NAP+RSV |
|---|---|---|
| 0 | 0±0 | 0±0 |
| 1 | 2.1±0.24 | 2.8±0.29 |
| 2 | 5.6±0.32 | 6.1±0.35 |
| 4 | 8.6± 0.72 | 8.3± 0.68 |
| 6 | 4.3±0.28 | 8.4±0.31 |
| 8 | 3.2±0.18 | 4.8±0.20 |
| 10 | 2.1±0.9 | 3.5±0.12 |
| 12 | 1.3±0.8 | 2.3±0.2 |

**Table1:** Mean serum concentration (ug/ml) of Naproxen and naproxen in presence of resveratrol (SDT & MDT) in inflammatory rats.

| PK PARAMETER | Naproxen | NAP+RSV |
|---|---|---|
| $C_{max}$ (µg/ml) | 1.8±0.72 | 2.95±0.7** |
| $t_{max}$ (h) | 1.4±0 | 2.82±0 |
| AUC $_{0-t}$ (µg/ml/h) | 4.81±1.8 | 7.61±2.7*** |
| $t_{½}$ (h) | 10.7±0.05 | 14.3±0.06** |
| Clearance (l/hr) | 14.83±5.45 | 6.01±2.85*** |
| $V_d$ (ml) | 22.92±8.68 | 12.96±7.32*** |

**Table2:** Mean Pharmacokinetic parameters of Naproxen in presence of resveratrol in Inflammatory rats

**Mean ± SD**: ***significant at p<0.001; ** significant at p<0.01; *significant at p<0.05 compared to Naproxen control

The plasma NAP concentrations were increased after RSV pretreatment when compared to control phase. The mean Cmax (1.8±0.72 versus 2.95±0.7µg/mL, p<0.05), mean AUC (4.81±1.8 versus 7.61±2.7µgh/mL, p<0.05) and mean t1/2 (10.7±0.05 versus 14.3±0.06 h, p<0.05) values were increased respectively, after RSV pretreatment as compared to that of control phase.On the other hand, mean CL/F (14.83±5.45 versus 6.01±2.85 L/h, p<0.05) and mean Vd/F (22.92±8.68 versus 12.96±57.32 L, p<0.05) values were decreased respectively, after RSV pretreatment as compared to the control phase. However, there was no significant change observed in tmax of NAP between RSV treatment and control phases.

The pharmacodynamic study states that the percentage inhibition of mean paw edema for resveratrol treated group was 51.1±0.1, naproxen treated group was 48.2±0.5, combination of naproxen and resveratrol treated group was 53.1±0.2.

## Pharmacodynamic data

| | TREATMENT | | | |
|---|---|---|---|---|
| Time (h) | Control | RSV | NAP | RSV+NAP |
| 0 | 31.2±0.4 | 34.3±0.1 | 35.1±0.4 | 36.2±0.5 |
| 1 | 42.3±0.1 | 51.1±0.1 | 48.2±0.5 | 53.1±0.2 |
| 2 | 60.6±0.1 | 63.2±0.4 | 59.1±0.3 | 64.3±0.9 |
| 3 | 58.2±0.3 | 54.1±0.2 | 55.1±0.1 | 59.3±0.1 |
| 4 | 55.3±0.4 | 52.1±0.3 | 48.5±0.3 | 43.2±0.2 |

**Table 3:** % Inhibition of Mean Paw edema

## Discussions

Herbal medicines have been widely used as a complementary or alternative treatment for a variety of diseases, rehabilitation and health care. Herbal medicines contain more than one pharmacologically active ingredient and are commonly used with many prescribed drugs.

From the literature it is evident that, RSV was proposed to block the transcription of various CYPs through antagonism of the nuclear aryl hydrocarbonreceptor (AHR).On the other hand, inhibition of CYP activity by RSV could lead to safety problems by altering the pharmacokinetics of co-administered drugs

Thus, the present study evaluated the effect of RSV treatment on the pharmacokinetics of naproxen in rats by using naproxen as a CYP1A2 substrate.

On account of its good tolerance when administerd, naproxen represents a promising CYP1A2 probe substrate to assess the CYP1A2 enzyme activity. Alterations in the catalytic activity of CYP1A2 enzyme can change the pharmacokinetics of naproxen. Hence, naproxen is used as a probe drug for assessing the CYP1A2enzyme activity in the study.

Our results suggest that oral administration of RSV significantly altered the pharmacokinetics and enhanced the bioavailability of naproxen through the inhibition of CYP1A2 enzyme in rats. In this study, we found that treatment with RSV resulted in significant increase in mean Cmax, AUC, T1/2 and a significant decrease in mean CL/F, Vd/F of naproxen as compared to control. Although mean Tmax values of naproxen were increased after RSV treatment phase but they were statistically insignificant. The increasing Cmax and AUC values indicate that enhanced exposure of naproxen after RSV treatment. On the other hand, the decreasing CL/F and increasing T1/2 values indicate the inhibition of elimination of Naproxen upon RSV treatment.

Based on these findings, reservetrol acts as an inhibitor of CYP1A2 mediated metabolism of naproxen in rats. Consequently, the bioavailability of naproxen was increased via the inhibition of CYP1A2enzyme activity.

Therefore, combination of naproxen along with RSV may represent a novel approach to reduce the dosage and results in reduced gastrointestinal side effects of naproxen.

The mean percent paw volume in control and drug treated animals was compared. These findings suggest that the inhibition of sustained phase of paw edema following subplantar injection of carrageenan in naproxen and reservetrol treated animals is enhanced compared to only naproxen treated group.

These changes in the pharmcokinetics and pharmacodynamics of naproxen when co-administered with resveratrol may be due to the inhibition of CYP1A2 enzyme by resveratrol.

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