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Changes of materials that scavenge 1,1-diphenyl-2-picrylhydrazyl radicals in plasma by per-oral administration of Kampo medicine, Ninjin-yoei-to in rats

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Abstract

The Kampo medicine, Ninjin-yoei-to, scavenged 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals in a dose-dependent fashion as did ascorbic acid and α-tocopherol. Ninjin-yoei-to, which is composed of 12 herbs, had a potent DPPH radical scavenging ability. We investigated the transition of the materials that scavenge DPPH radicals in plasma after oral administration of Ninjin-yoei-to to rats. When 1.0 g kg^{-1} Ninjin-yoei-to was administered, the DPPH radical scavenging ability increased at 30 min and biphasic peaks were observed at 2 h and at 10 h. From the response–time profile, kinetic parameters including values for K_a (absorption rate constant), t_{max} (peak concentration time), t½ (half-life) and MRT (mean residence time) of the radical scavenging ability in plasma could be calculated for DPPH radicals. K_a values were 0.53 ± 0.03 and 0.36 ± 0.07 h, t_{max} values were 2.1 ± 1.04 and 8.56 ± 2.69 h, t½ values were 1.60 ± 0.12 and 3.39 ± 1.72 h, and MRT values were 4.14 ± 1.59 and 8.18 ± 2.55 h, respectively. These parameters calculated from the antioxidation dynamics were considered to offer a very meaningful procedure for examining the effects of Ninjin-yoei-to.

Introduction

Although the active moiety of Kampo medicines (Japanese herbal medicine) producing their pharmacological effects in man has not been clarified, Kampo medicines are widely used for the treatment of many diseases, for example, pneumonia, bronchitis, chronic hepatitis and other inflammatory disorders. Some Kampo medicines are known to possess a free-radical scavenging activity (Ueda et al 1995; Egashira et al 1999a), since they consist of many herbs containing many low- and high-molecular weight compounds such as flavonoids, saponins, tannins, polyphenols and others (Bors & Saran 1987; Hatano et al 1989; Okuda et al 1990). Previously, we reported the free radical scavenging ability of Ninjin-yoei-to (Egashira et al 1999b) and it was considered that this radical scavenging ability was one of the effects of the Kampo medicine. Although many unidentified substances exist in Kampo medicines, it seems relevant to know the dynamics of the radical scavenging ability of Kampo medicines for evaluating their efficacy.

In this study, we have investigated the transition of the materials that scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals in plasma after oral administration of Ninjin-yoei-to, a type of Kampo medicine, in rats.

Materials and Methods

Animals

Animal care and handling were performed according to the Oita Medical University guidelines for the care and use of laboratory animals.

Male Wistar rats (Seac Laboratory Animals, Inc., Fukuoka, Japan; 250–300 g) were housed in an environmentally controlled room (20–23 °C, 50–60% humidity, illuminated 07:00–19:00 h). Food and water were freely available.

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Drugs

Ninjin-yoei-to (Tsumura, Tokyo, Japan) is a dried decoction of a mixture of 12 herbs. A total of 3 g Ginseng radix (*Panax ginseng* C.A. Mey.), 4 g Angelicae radix (*Angelica acutiloba* Kitagawa.), 2 g Paeoniae radix (*Paeonia lactiflora* Pall.), 4 g Rehmanniae radix (*Rehmannia glutinosa* Liboschitz var. *purpurea* Makino.), 4 g Atractylodis rhizoma (*Atractylodes japonica* Koide, ex Kitam.), 4 g Hoelen (*Poria cocos* Wolf.), 2.5 g Cinnamomi cortex (*Cinnamomum cassia* Bl.), 1.5 g Astragali radix (*Astragalus membranaceus* Bge.), 2 g Aurantii nobilis pericarpium (*Citrus unshiu* Markov), 2 g Polygalae radix (*Polygala tenuifolia* Willd.), 1 g Schisandrae fructus (*Schisandra chinensis* Baill.) and 1 g Glycyrrhizae radix (*Glycyrrhiza uralenis* Fisher *Glycyrrhiza glabra* L.) were boiled in 10-times their weight of water for 1 h. The resultant extract was spray dried. This decoction was spray dried to give a powdered extract. Ascorbic acid and α-tocopherol (Wako, Osaka, Japan) were used as control drugs.

Administration of drugs

Ninjin-yoei-to ($1.0\,g\,kg^{-1}$) was administered orally to a group of rats. Another group of rats received α-tocopherol ($200\,mg\,kg^{-1}$) and another group received ascorbic acid ($200\,mg\,kg^{-1}$), both known antioxidants. Saline (0.1 mL/rat) was administered orally to another group as a control. Blood ($100\,\mu L$) was sampled into a heparinized syringe before drug administration (0 h), and at 1, 2, 4, 6, 10, 12, 24 and 48 h after administration ($n = 4$). Immediately after being sampled, the blood was centrifuged ($3000\,rev\,min^{-1}$, 10 min) to obtain plasma for analysis. The plasma samples thus obtained were stored at $-30\,^{\circ}C$ until analysis. We re-examined the radical scavenging ability of this plasma on DPPH radicals. This study was performed according to the Oita Medical University guidelines for the care and use of laboratory animals.

Assay of DPPH radicals

For the analysis of DPPH radicals by an electron spin resonance (ESR) spectrometer (JES-RE1X, Jeol Ltd, Tokyo, Japan) (Takayama et al 1994), $100\,\mu L$ 30 mM DPPH (Sigma, St Louis, MO) ethanol solution and $100\,\mu L$ of a suspension of Ninjin-yoei-to, the 12 herbs that comprise Ninjin-yoei-to, ascorbic acid or α-tocopherol were placed in a test tube and mixed for 10 s. The mixture was transferred to a special flat cell. The ESR parameters were: magnetic field 339.6 ± 10 mT, Mn 553, field modulation 0.79×0.1, time constant 0.3 s, sweep time 3 min, power 10, gain $\times 1000$. After 60 s, the signal intensity was evaluated from the peak height of the third signal of DPPH radicals.

Data analysis

Results are shown as the means $\pm$ s.d. for four experiments. Data were statistically analysed by one-way analysis of variance techniques with nonparametric Kruskal-Wallis multiple comparison procedure. The responses, radical scavenging activity vs time data, were analysed by compartmental and noncompartmental kinetic approaches using a nonlinear least-squares computer program based on differential equations (MULTI) and developed by Yamaoka et al (1978b, 1981). Individual curves of data from the oral administration of the drugs were fitted to polyexponential equations. Based on the Akaike information criterion (Yamaoka et al 1978a), the most suitable equations were determined. The kinetic parameters were derived from the optimum equations. The values for the apparent absorption rate constant (K_a), the peak concentration time (t_{max}) and the half-life ($t\frac{1}{2}$) were shown as the results of a compartmental kinetic approach. The mean residence time (MRT) value, an index for duration of efficacy, was presented as the result of moment analysis, a noncompartmental kinetic approach.

Results

Scavenging effects of Ninjin-yoei-to, α-tocopherol, ascorbic acid and the 12 herbs that comprise Ninjin-yoei-to on DPPH radicals in-vitro

We examined the radical scavenging ability of Ninjin-yoei-to on DPPH radicals with an ESR spectrometer and compared the effects of Ninjin-yoei-to with those of α-tocopherol and ascorbic acid.

The typical ESR spectra of DPPH radicals as well as the radical scavenging effects of Ninjin-yoei-to, α-tocopherol and ascorbic acid on DPPH radicals are shown in Figure 1. Ninjin-yoei-to had a concentration-dependent radical-trapping ability for stable DPPH radicals

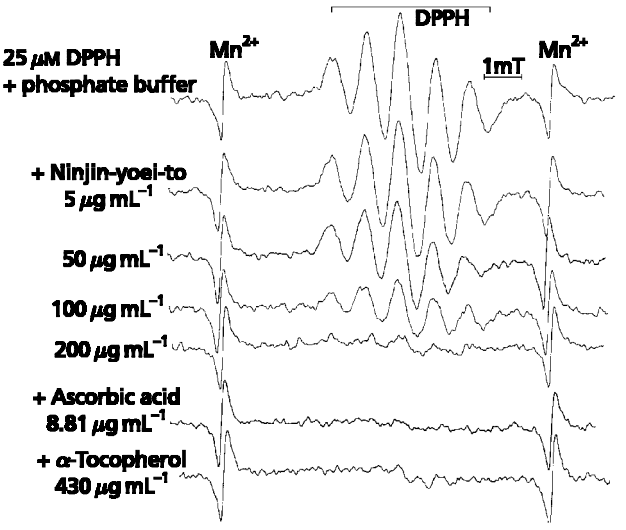

Figure 1 Scavenging effects of Ninjin-yoei-to, ascorbic acid or α-tocopherol on DPPH radicals, *in vitro*. The top ESR spectrum was recorded as the control without drug. The other spectra were recorded after adition of Ninjin-yoei-to, ascorbic acid or α-tocopherol.

Table 1 Scavenging effects of the 12 herbs of Ninjin-yoei-to on DPPH radicals.

Herb/drug	SC50 ($\mu g\,mL^{-1}$)
Ginseng radix	290 ± 11
Angelicae radix	110 ± 9
Paeoniae radix	10 ± 2
Rehmanniae radix	280 ± 18
Atractylodis rhizoma	70 ± 15
Hoelen	130 ± 21
Cinnamomi cortex	6 ± 1
Astragali radix	120 ± 31
Aurantii nobilis pericarpium	110 ± 23
Polygalae radix	50 ± 22
Schisandrae fructus	82 ± 8
Glycyrrhizae radix	31 ± 19
Ninjin-yoei-to	83 ± 33
α-Tocopherol	18 ± 8
Ascorbic acid	8 ± 1

Values are expressed as the concentrations that scavenged 50% of DPPH radicals (SC50).

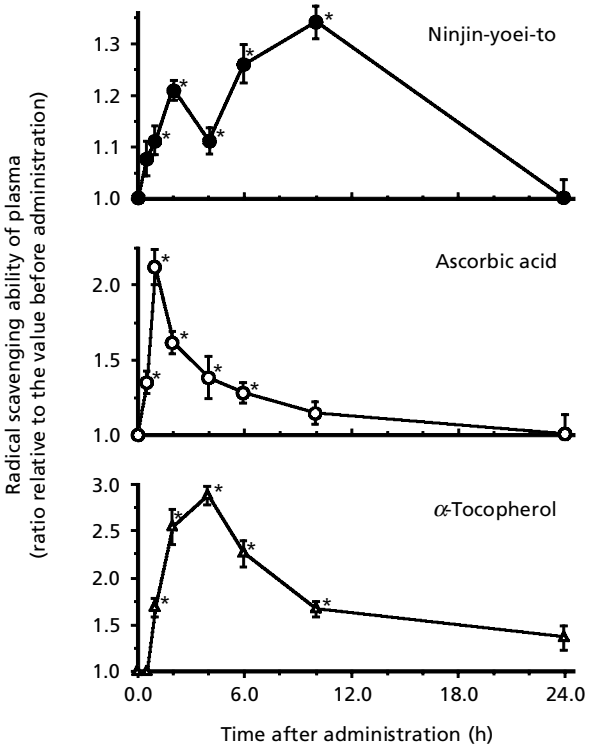

Figure 2 Transition of DPPH radical scavenging ability in plasma after oral administration of Ninjin-yoei-to, ascorbic acid or α-tocopherol. The ESR signal ratio was calculated by dividing the simultaneously recorded Mn^{2+} by the signal height of DPPH radicals. Each pont (ESR signal ratio) is expressed as the value after subtracting the radical scavenging ability in plasma after oral administration of saline for Ninjin-yoei-to, ascorbic acid or α-tocopherol. The ESR signal ratio was 0.5 ± 0.1 relative to DPPH radicals in plasma after oral administration of saline (control rats). ●, Ninjin-yoei-to $1.0\,g\,kg^{-1}$; ○, ascorbic acid $200\,mg\,kg^{-1}$; △, α-tocopherol $200\,mg\,kg^{-1}$. $P < 0.05$ vs control (ANOVA with Kruski–Wallis's multiple test).

Table 2 Kinetic parameters of the DPPH radical scavenging ability after a single administration of Ninjin-yoei-to, ascorbic acid or α-tocopherol.

Parameter	Ninjin-yoei-to	Ascorbic acid	α-Tocopherol
K_a (h)	0.53 ± 0.03 0.36 ± 0.07	1.54 ± 0.08	0.30 ± 0.04
t_{max} (h)	2.10 ± 1.04 8.56 ± 2.69	1.25 ± 0.98	4.10 ± 1.35
t½ (h)	1.60 ± 0.12 3.39 ± 1.72	2.21 ± 0.10	5.82 ± 0.35
MRT (h)	4.14 ± 1.59 8.18 ± 2.55	5.51 ± 1.31	15.7 ± 3.1

Each value is expressed as the mean ± s.e.m. for four rats.

(500–5 $\mu g\,mL^{-1}$). Next, we examined the DPPH radical scavenging ability of the 12 constituent herbs of Ninjin-yoei-to. These herbs had a concentration-dependent and potent DPPH radical trapping ability (data not shown). The respective concentrations that scavenged 50% of DPPH radicals (SC50 values) were calculated from the dose–response curves, and the results are shown in Table 1. The intensity of the DPPH radical scavenging ability with the 12 herbs was in the following order: Cinnamomi cortex > Paeoniae radix > Glycyrrhizae radix > Polygalae radix > Atractylodis rhizoma > Schisandrae fructus > Aurantii nobilis pericarpium = Angelicae radix > Astragali radix > Hoelen > Rehmanniae radix > Ginseng radix.

Changes of DPPH radical scavenging ability in plasma over time after administration of Ninjin-yoei-to, ascorbic acid or α-tocopherol

Figure 2 shows a representative profile of the transition of DPPH radical scavenging ability in plasma after oral administration of Ninjin-yoei-to, $1.0\,g\,kg^{-1}$ (Figure 2). The radical scavenging ability increased biphasically after the administration of Ninjin-yoei-to and showed peak values at 2 and 8 h. The peak levels gradually returned to the control level by 24 h. The administration of ascorbic acid or α-tocopherol rapidly increased the DPPH radical scavenging ability in plasma also and showed peak values at approximately 1 and 4 h, respectively. These increases diminished gradually and the levels returned to the control value (Figure 2).

Kinetic parameters of radical scavenging ability after administration of Ninjin-yoei-to, ascorbic acid or α-tocopherol

We attempted to monitor the dynamics of the radical scavenging ability using the pharmacokinetic technique as a marker instead of the drug concentration. The kinetic parameters of DPPH radical scavenging ability in plasma were calculated and are shown in Table 2. The K_a (absorption rate constant) values of substances that scavenged

DPPH were almost identical with that of α-tocopherol for DPPH radical scavenging. The velocity of t_{max} (peak concentration time) was in the following order: ascorbic acid, Ninjin-yoei-to in the first peak, α-tocopherol and Ninjin-yoei-to in the second peak. The t½ (half-life) of the first peak was the shortest compared with the values for ascorbic acid and α-tocopherol.

Discussion

Some members of the herbal medicinal system known as Kampo medicine are expected to be effective for oxidative-related damage due to the scavenging abilities for reactive oxygen radicals because they consist of many herbs containing compounds such as flavonoids, saponins, tannins, polyphenols and others (Bors & Saran 1987; Larson 1988; Hatano et al 1989; Okuda et al 1990). β-Carotene and flavonoids, which are widely used as therapeutic agents, are known to act as strong O_2^- and ·OH scavengers, as well as singlet oxygen ($^1O_2$) quenchers (Sorata et al 1984; Husain et al 1987). These scavenging abilities contribute in part to the effects of Kampo medicine including Ninjin-yoei-to. We reported previously on the free radical scavenging ability of Ninjin-yoei-to (Egashira et al 1999b). In this study, we have examined the radical scavenging ability of Ninjin-yoei-to on DPPH radicals in detail. Ninjin-yoei-to consists of 12 herbs containing many low- and high-molecular weight compounds such as terpenoids, flavonoids, tannins and others. Of the 12 herbs, the Cinnamomi cortex and Paeoniae radix particularly strongly scavenged DPPH radicals, since tannins and polyphenols are contained in these herbs. Due to these effects, DPPH radicals are strongly scavenged by Ninjin-yoei-to.

It is thought that the clinical effects of Kampo medicine are achieved only with a cocktail of complex plant extracts. Therefore, we investigated the dynamics of the radical scavenging activity in plasma as a marker for monitoring the absorption of Ninjin-yoei-to. A typical profile of the transition of DPPH radical scavenging activity in plasma after oral administration of Ninjin-yoei-to was biphasic and showed peak values at 2 and 8 h. It is very interesting that the DPPH radical scavenging ability was biphasic. This result suggested that the intestinal absorption velocity of the components that had radical scavenging ability differed, suggesting that some components were absorbed early, while the absorption of others was delayed.

Many attempts have been made to clarify the active components of Kampo medicines and their pharmacodynamic properties (Yanagisawa et al 1992; Uchida et al 1995). Tashiro (1997) proceeded to establish a method for the serum sampled after per-oral administration of Kampo medicine, which was considered to be a kind of "crude drug", and this method was used for pharmacological research. Moreover, these researchers have been seeking to establish a model system of serum pharmacology by monitoring oriental herbal drugs. In this study, we attempted to monitor the dynamics of their radical scavenging activity using the pharmacokinetics as a marker instead of the drug concentration according to Figure 2. These values were thought to be critical parameters for comparing the beneficial effects of Kampo medicine. The response–time profile differed from the results reported previously in rat after a single administration of Sho-saiko-to (Egashira et al 1999a). The kinetic parameters of DPPH radical scavenging ability after the administration of Ninjin-yoei-to were different compared with Sho-saiko-to. The kinetic parameters after the administration of Sho-saiko-to were: $K_a = 0.13 \pm 0.03$ h, $t_{max} = 6.2 \pm 2.3$ h, $t½ = 8.76 \pm 1.35$ h and $MRT = 20.1 \pm 4.7$ h. These differences were considered to be due to the differences in the species of herbs that comprised the Kampo medicine and of the species of radical scavenging materials contained in these herbs.

From these results, the parameters calculated from the dynamics of antioxidation were considered very meaningful for examining the effects of Ninjin-yoei-to, and from now on will serve for evaluating these effects. It is considered that examination of the radical scavenging ability of Kampo medicines is a good method for evaluating the efficacy of Kampo medicines that have multiple components and multiple properties.

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