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• REVIEW •

Pharmacology of tetrandrine and its therapeutic use in digestive diseases

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INTRODUCTION

Tetrandrine (Tet) is a dibenzylisoquinoline alkaloid isolated from Stephania tetrandra S. Moore, a Chinese herbal medicine. In the past decade, lots of studies demonstrated that Tet has multiple bioactivities. It is promising to use Tet as an antifibrogenetic in liver or lung fibrosis with or without portal or pulmonary hypertension, as well as an immunomodulating and anticarcinoma drug.

PHARMACOLOGY

Ca^{2+} channel blocking activity

Abnormal Ca^{2+} signaling and elevated concentration of intracellular free Ca^{2+} are the basic pathophysiological events involved in various diseases. As a Ca^{2+} antagonist, Tet can inhibit extracellular Ca^{2+} entry, int ervene in the distribution of intracellular Ca^{2+}, maintain intracellular Ca^{2+} homeostasis, and then disrupt the pathological processes. As shown in whole cell patch-clamp recordings, Tet blocked bovine chromaffin cells voltage-operated Ca^{2+} channel current in a time- and concentration-dependently manner. In rat phaeochromocytoma PC 12 cells, 100 μmol·L^{-1} Tet abolished high K^+ (30 mmol·L^{-1}) - induced sustained increase in cytoplasmic Ca^{2+} concentration, inhibit bombesin-induced inositol triphosphate accumulation in NIH/3T3 fibroblast and abolish Ca^{2+} entry[1]. In rat glioma C6 cells, studied with fluorometric ratio method, Tet did not affect the resting cytoplasmic Ca^{2+} concentration, but it inhibited IP3 accumulation and the sustained and peak elevation of cytoplasmic Ca^{2+} concentration induced by bombesin and thapsigargin, a microsomal Ca^{2+}-ATPase inhibitor, in a dose-dependent manner. The dose of Tet needed to abolish the sustained and peak elevation of cytoplasmic Ca^{2+} concentration induced by bombesin and thapsigargin was 30 μmol·L^{-1}[2]. Bickmeyer *et al*[3] demonstrated that NG108-15 cells treated with 100 μM Tet for seven minutes could block voltage-dependent Ca2+ entry induced by depolarization with 50 mM KCl. Tet could block non-voltage-operated Ca^{2+} entry activated by intracellular Ca^{2+} store depletion induced by thapsigargin and could release intracellular Ca^{2+} in HL-60 cells, and could therefore increase concentration of intracellular free Ca^{2+}, elicit therapeutic effects. We have previ ously demonstrated that Tet could concentration-dependently block extracellular Ca^{2+} entry into hepatocytes, promote mitochondria Ca^{2+}-uptake, and inhibit Ca^{2+}-mobilizing from mitochondria. However, the blockade of Ca^{2+} channel played the most important role in maintaining Ca^{2+} homeostasis, but not intracellular Ca^{2+} distribution.

In the presence of extracellular Ca^{2+} (1.3 μmol·L^{-1}), glutamate, serotonin and histamine significantly increased the intracellular Ca^{2+} concentration in a dose-dependent manner. 30ìmol·L^{-1} Tet significantly inhibited the increase in intra cellular Ca^{2+} concentration induced by glutamate, serotonin and histamine by 28.0%, 46.8% and 29.0%. In Ca^{2+} free Hanks' solution, Tet did not produce a significant inhibitory effect on the increase in intracellular Ca^{2+} concentration caused by serotonin and histamine. These results indica ted that Tet conducted blocking of Ca^{2+} influx from the extracellular site via NMDA, 5-HT_2 and histamine type I receptor-operated Ca^{2+} channels and has no obvious effect on the Ca^{2+} release from intracellular Ca^{2+} stores[4]. In addition, Tet also inhibited extracellular Ca^{2+} entry and intracellular Ca^{2+} mobilization induced by norepinhprine and angiotonin II via corresponding receptor respectively[3,5]. Taking together, in different tissues and different kinds of cells, Tet may block the Ca^{2+} channel through different mechanisms. It can block the voltage- and/or receptor-operated Ca^{2+} channel. Nevertheless, in some kind of carcinoma cells, Tet does not affect Ca^{2+} channel, but promote Ca^{2+} release from intracellular stores and elevate the cytosolic free Ca^{2+} concentration.

Immunomodulating activity

Clinically, Stephania tetrandra S. Moore has been thought to be effective in treating autoimmune diseases such as rheumatoid arthritis and systemic lupus erythe matosus. Tet, the active ingredient isolated from *Stephania tetrandra* S. Moore, has potential immunomodul ating and anti-inflammatory effects. T-lymphocytesplay a critical role as autoactive and pathogenic population in autoimmune and inflammatory diseases. Some experimental data showed that, through down-regulating the protein kinase C (PKC) signaling, interleukin-2 secretion and the expression of the T cell activation antigen (CD71), Tet inhibited phobol 12-myristate 13-acetate (PMA)+ionomycin-induced T cell proliferation dependent on interleukin-2 receptor alpha chain and CD69, such an action was unrelated to Ca^{2+} channel blockade[6]. Tet (0.1-10 μmol·L^{-1}) significantly inhibited neutrophil-monocyte chemotactic factor-1 upregulation and adhesion to fibrinogen induced by N-formyl-methionyl-leucyl-phenylalanimne and PMA. Tet at 0.1-100 μmol·L^{-1} caused dose- and time-dependent loss of cell viability of mouse peritoneal macrophages, guinea-pig alveolar macrophages and mouse macrophage-like J774 cells, reduced production of oxygen free radical oxygen, down-regulated synthesis and

release of some pro-inflammatory cytokines[7,8].

Nuclear transcription factor kappa B (NF-kappa B) is a multiprotein complex which regulates a variety of genes concerned with immunity and inflammation. For the alveolar macrophages, Tet could inhibit the activation of their NF-kappa B and NF-kappa B-dependent reporter gene expression induced by endotoxin , PMA, and silica in a dose-dependent manner. Western blot analysis suggested that the inhibitory effects of tetrandrine on NF-kappa B activation could be attributed to its ability to suppress signal-induced degradation of I kappa B alpha, a cytoplasmic inhibitor of the NF-kappa B transcription factor[7,9].

Conducting tumor cell apoptosis

To induce tumor cell apoptosis is one of the important chemotherapeutic strategies for malignent tumors. Tet inhibited both proliferation and clonogenicity of human leukemic U937 cells at an optimal concentration of 2.5 $mg \cdot L^{-1}$. The characteristic morphological changes of apoptosis were observed under light microscopy and DNA fragmentation was noted by gel electrophoresis in these cells. Moreover, flow-cytometric detection of surface phosphatidyl serine expression of cells after treatment with Tet confirmed the induction of apoptosis in these cells[10]. Tet concentration-dependently inhibited the proli feration of human leukemic HL-60 cells. Morphological observation and DNA analysis revealed that Tet caused cell shrinkage with the formation of apoptotic bodies, and showed clear evidence of DNA fragmentation[11]. Tet was found to induce pronounced morphological changes characteristic of apoptosis and extensive DNA fragmentation in the human BM13674 cell line 8 h after treatment[12]. The induction of apoptosis by Tet was much more rapid in CEM-C7 cells (4 h) than in the same cells treated with glucocort icoids (40 h), and did not require de novo protein synthesis[13]. These results indicate that Tet may have value as an anti-neoplastic agent.

Reversing multidrug resistance (MDR)

The occurrence of MDR to chemotherapeutic drugs is a major problem for successful cancer treatment. The overexpression of cell membrane P-glycoprotein (P-gp) is one of the major mechanisms of MDR. P-gp pumps antitumor drugs out of tumor cells, causing drug resistance.

Tet (3 $\mu mol \cdot L^{-1}$) reduced the paclitaxel concentration required to achieve 50% inhibition of cell growth (EC50) of HCT15 (P-gp-positive) cells about 3100-fold, and also reduced the EC50 value of actinomycin D about 36.0-fold in the cells. Meanwhile, Tet had no effect on the cytotoxicity of the drugs to SK-OV-3 (P-gp-negative) cells[14]. The non-cytotoxic concentrations of Tet potentiated the growth-inhibitory actions of doxorubicin (Dox) in the Har-resistant HL60 cells. The colony formation efficencies were reduced from 60% by Dox to 0.2% by Tet + Dox. Retardation of the G2M phase cells was increased. But Tet did not potentiate Dox cytotoxity in the sensitive HL60 cells. Dox accumulation in the harringtonine-resistant HL60 cells treated by with was increased. These results indicated that Tet enhanced the cytotoxicity of MDR-related drugs via modulation of P-gp[15]. In addition, Tet could also inhibit platelet- activating factor-induced human platelet aggregation and decrease thromboxane B2 production and thrombus formation[16].

THERAPEUTIC USE IN DIGESTIVE SYSTEM DISEASES

Protective effects on hepatocyte injury

Hepatocyte lesions are common and very important clinically[A17-A21]. Chen *et al*[22] observed the effects of Tet on hepatocytic injury ind uced by CCl_4. The result showed that, compared with control group, Tet (1-1000 $nmol \cdot L^{-1}$) increased viability of liver cell (from 71% to 72%-89%), reduced lactate dehydrogenase release, and malondialdehyde (MDA) formation. Tet prevented the increase of the intracellular Ca^{2+} concentration and the attenuation of the membrane microflow of liver cells. Tet (30 $mg \cdot kg^{-1} \cdot d^{-1}$ via gavage for two wk) could markedly reduce the elevation of serum alanine aminotransferase, alkaline phosphatatase and MDA induced by azathioprine. The level of reductive glutathione and SOD were not different from the normal control group. Histological changes in the Tet-treated group were slight[23]. The protective effect on CCl_4- or azathioprine-injured hepatocytes may be elicited by inhibiting the lipid peroxidation, improving the membrane microflow, and lessening the Ca^{2+} concentration. With flow-cytometric technique, we demonstrated that 10-60 $mg \cdot L^{-1}$ Tet could concentration-dependently accelerate the G1 phase cells transforming to S phase cells, and increase the level of DNA in the S phase and protein in the G1, G2 phase cells significantly. Further stu dies indicated that the effect of Tet in promoting hepatocytes proliferation was not related to blockade of Ca^{2+} influx[24].

Anti-hepatofibrogenetic activity

Tet could significantly reduce the degree of experimental hepatic fibrogenesis induced by CCl_4 in rats; the levels of serum hyaluronic acid and procollagen peptide were decreased, and the liver dysfunction was ameliorated, Tet could also obviously inhibit extracellular matrix formation and collagen deposition. In the liver tissue of rats treated with Tet, hepatic stellate cell (HSC) activation, proliferation, and transformation were down-regulated; the number of desmin-positive cells were reduced significantly. The anti-fibrotic effect of Tet had no significant difference from that of colchicine[25]. HSC activation, proliferation, and transforming into fibroblast are the putative events in hepatic fibrogenesis. Tet could significantly inhibit conventional cultured HSC activation and type I and type III collagen mRNA expressions and protein synthesis were down-regulated. Tet could block HSC proliferation colla gen synthesis induced by platelet-derived growth factor (PDGF), reduce the level of PDGF, PDGF receptor (PDGF-R beta1), transforming growth factor beta1 (TGF beta1) and alpha-smooth muscle actin mRNA, and also down-regulate the au tocrine of PDGF, PDGF-R beta1, TGF beta1. These data suggest that Tet may block hepatic fibrogenesis directly and/or through inhibiting cytokine express ions[26,27]. After taking Tet orally for three months, liver functions of the patients with cirrhosis were obviously improved. Administrat ion Tet for six to eighteen months, serum levels of PIIIP and HA of the patients were markedly reduced. Histological examination showed that, compared with pretreatment or placebo, inflammatory cell infiltration was reduced, and even abolished, and that the deposition of ECM, type I and type III collagen were decreased significantly[28].

Anti-portal hypertension

Portal hypertension is one of important manifestations of the patients with cirrhosis. Upper gastrointestinal hemorrhage caused by portal hypertension commonly led to the patient's death. After injecting Tet intravenously (2.0, 6.0 and 20.0 $mg \cdot kg^{-1}$), portal venous pressure and mean arterial pressure were assessed in cirrhotic rats induced by CCl_4. The results

demonstrated that Tet induced dose-dependent decreases in portal venous pressure and mean arterial pressure. The maximum percentage reductions of portal venous pressure after Tet in the three different dosages were 5.4%±1.0%, 9.2%±0.8%, and 23.7%±1.2% of baseline, respectively. Total peripheral resistance was also reduced by Tet[29,30]. In portal hypertensive rats induced by partial portal vein ligation, Tet (4, 8, 16 and 24 mg·kg^{-1}) induced dose-dependent decreases of portal venous pressure and mean arterial pressure after intravenous infusion. Tet (16 mg·kg^{-1}) caused the portal venous pressure decreasing from a baseline of 12.5 mmHg to 10.0 mmHg, and the mean arterial pressure from a baseline of 90 mmHg to 80 mmHg. At 24 mg·kg^{-1}, Tet reduced portal venous pressure and mean arterial pressure to 20.3%±2.4% and 28.4%±1.4% of baseline, respectively[31]. The effects of Tet on portal hypotension may be attributed to its actions of blocking voltage- and receptor-operated Ca^{2+} channels in vascular smooth muscle cells, inhibiting intracellular Ca^{2+} mobilization and dilating peripheral blood vessels. We had previously observed its clinical therapeutic effects on portal hypertension. Taking Tet orally for 2 consecutive years, the esophageal variceal pressure and the portal blood flow in cirrhotic patients with portal hypertension were significantly reduced. The proportion of patients with no recurrent gastrointestinal bleeding during 2 years' medication of tetrandrine was 87.9%. It is suggested that Tet would be effective for cirrhotic patients with portal hypertension in preventing recur rent variceal bleeding[32].

Therapeutic effect on portal hypertensive gastropathy

Portal hypertensive gastropathy is caused by dysfunction of submucosal circulation and gastric mucosal barrier damage. Recent studies found that Tet increased prostgalandin E2, GMBE and GAM secretion, reduced the degree of gastric mucosa injury, and lowered the portal pressure. This result indicates that Tet may be useful in portal hypertensive gastropathy.

Preventing pancreatic islet beta cells from toxic injury

Pancreatic islet beta cells could be damaged by alloxan (50 mg·k g^{-1} i.v.)[aa] in rats, and diabetic animal models were thus prepared. Pancreatic islet beta cells density in experimental groups pretreated with Tet (100 mg·kg^{-1} via gavage) at 1.5 hours and 5 hours prior to alloxan injection increased from the control value of 13±4 to 62±9 and 65±7 (P<0.001). When the doses of Tet decreased from 100 mg·kg^{-1} to 50 mg·kg^{-1} and 25 mg·kg^{-1}, the pancreatic islet beta cell density were 45±5 and 38±4 (P<0.01 and P<0.001)[33].

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