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Antioxidant activity and protective effect of suramin against oxidative stress in collagen induced arthritis

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ABSTRACT

It is imperative to interrupt the link between arthritis and regulation of oxidative stress with the administration of antioxidants. Suramin is known for its anti-inflammatory, antineoplastic and antiangiogenic activities implying its possible antioxidant property. In this study, the antioxidant activity of suramin in cell free system was found to be higher than L-ascorbic acid (L-AA) with respect to its scavenging effect on nitric oxide (NO), hypochlorous acid and hydrogen peroxide radicals. Besides, suramin was found to be nontoxic to cultured RAW cells even at high concentrations along with marked inhibition of NO production. Suramin was found to curb the inflammation associated with the collagen induced arthritis (CIA) model. Administration of suramin significantly reduced the malondialdehyde and protein carbonyl content in joints, liver, kidney and spleen of rats as studied *ex vivo*. Furthermore, the increased antioxidant enzymes such as SOD, catalase, GST, GPx and GR activities in the tissues were restored significantly after suramin treatment. *In silico* experiments using Vlife MDS4.4-GRIP docking method showed strong affinity of suramin towards erythrocyte catalase followed by glutathione peroxidase thus corroborating with the findings of antioxidant enzyme assays. Our studies clearly indicate that suramin has remarkable antioxidant potential and can ameliorate arthritis *via* modulation of oxidative stress.

1. Introduction

Oxidative stress (OS) is one of the major underlying causes of many health disorders with varied severity and durations. The diseases manifested as a result of increased OS range from cancer, cardiovascular diseases, inflammatory and autoimmune diseases like rheumatoid arthritis (RA), to ageing and associated ailments like Alzheimer's disease, *etc.* resulting in widespread morbidity and mortality in the global population (Pham-Huy et al., 2008). In the development and progression of RA, there is a major contribution of the reactive oxygen species (ROS) and reactive nitrogen species (RNS) to bring about the OS. So, these ROS and RNS are considered as risk and enhancer factors for such autoimmune diseases, thus entailing a significant relationship between OS and RA (Filippin et al., 2008). There is a definite role of free radicals in the pathophysiology of inflammatory synovitis as these are produced by the local cells like macrophages, lymphocytes, neutrophils and endothelial cells at the site (Hadjigogos, 2003). Other ROS such as hypochlorous acid and singlet oxygen are also significantly involved in the degenerative joint pathogenesis (Campo et al., 2003). So, it is imperative to state that effective strategy for alleviation of RA should include neutralization of ROS through nutritional, pharmacological and environmental means (van Vugt et al., 2008).

Collagen induced arthritis (CIA) is an animal model mimics RA by sharing clinical and pathological features, thereby portray the fact that inflammation is strongly associated with oxidative stress (Schurgers et al., 2011). Organ or tissue specific oxidative damage is a parameter to ascertain the antioxidant potential of experimental compounds being administered in model animals (Tkachenko et al., 2014). Antioxidants have been reported to be effective in the reduction of inflammation and many standard antioxidants are indeed anti-inflammatory in nature and *vice-versa*. Compounds such as curcumin, resveratrol, rutin, quercetin and many more show both antioxidant and anti-inflammatory activities (Sarkar et al., 2013). Suramin also known as Bayer 205 and Germanin is a symmetric polyanionic naphthylurea commonly used as antihelminthic drug for the treatment of trypanosomiasis and onchocerciasis (McGeary et al., 2008). It exhibits an extremely broad range of biological actions such as anticancer, anti-inflammatory, anti-proliferative and antiviral activity. Apart from these, suramin also antagonizes venom toxicity and has an ability to bind various growth factors

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and cytokine such as TNF-α inhibiting their interaction with respective receptors. Suramin also impairs translation by inhibiting initiation and elongation processes (Trapp et al., 2007). Plasma concentration of acute phase proteins (APPs) reflects the progressive status of RA (Cylwik et al., 2012). These APPs are also found to possess the properties of disease biomarkers and it was shown that the administration of suramin has been able to normalize the levels of APPs, hence exhibited anti-inflammatory activity (Sahu et al., 2012). Both anti-inflammatory and antioxidant properties of suramin have not been studied in detail. Here we have emphasized on the fact that the antioxidant property of suramin which indeed renders its anti-inflammatory nature and may be an underlying basis of its multifaceted therapeutic spectrum.

There is very little information about anti-oxidant potential of suramin in *in vitro* (cell free system), cell culture, *in silico* and *in vivo* (CIA animal model).The objective of this study is to evaluate the antioxidant property of suramin. Firstly, we evaluated the oxidative, reductive and free radical scavenging power of suramin using various chemical and biological model systems. Secondly, suramin was checked in macrophage cell culture for its nitric oxide inhibitory potential and associated cytotoxic activity. Thirdly, suramin was administered intraperitoneally in CIA rat model for the suppression of arthritic symptoms by maintaining the homeostasis of endogenous antioxidant system (both enzymatic and non enzymatic). Lastly, the affinity of suramin to antioxidant enzymes (Cu-Zn SOD, catalase and peroxidase) was demonstrated through docking studies. Indomethacin was used as a positive control in this study. The objective of the present work is to define the therapeutic effectiveness of suramin as a potent antioxidant agent that can abrogate arthritis-mediated cartilage/bone degradation, oxidative stress and associated inflammation *in vivo*.

2. Materials and methods

The key chemical compounds studied in this article are suramin sodium salt (PubChem CID: 8514), curcumin (PubChem CID: 969516) and L-ascorbic acid (PubChem CID: 54670067). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), L-nitro-arginine methyl ester (L-NAME), (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), antibiotic-antimycotic solution, fetal bovine serum, 2,4-dinitrophenylhydrazine (DNPH), Griess reagent, trichloroacetic acid, sodium nitroprusside, ferric chloride, hypochlorous acid, reduced glutathione, oxidized glutathione, tetramethoxypropane, dimethyl sulfoxide (DMSO), glutathione reductase, hydrogen peroxide, catalase, and indomethacin were purchased from Sigma Aldrich, India. All other chemicals and solvents used were of analytical grade.

2.1. In vitro antioxidant assays of suramin

Suramin was tested for its antioxidant capability using different assays in a cell free system. Both L-ascorbic acid and curcumin were taken as reference or standard antioxidants. All the three compounds *viz*., suramin, curcumin and L-ascorbic acid were dissolved in 5% DMSO in PBS with increasing concentrations of 5, 10, 25, 50, 100, 250 and 500 μg/ml (5 to 500 μg/ml) and used in all the assays.

2.1.1. DPPH radical scavenging activity of suramin was estimated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and done according to the procedure of Sharma et al. (2011)

Methanolic DPPH solution (1.3 mg/ml (3.3 mM), 150 μl) was added to 3 ml of methanol and this was recorded as control. Concentrations ranging from (5 to 500 μg/ml) of both suramin and standards were tested as already mentioned in Section 2.1. The mixtures were vortex-mixed and kept for incubation in dark for 15 min after which the decrease in the DPPH absorption was measured at 517 nm. A non-linear regression analysis method was employed to calculate the IC_{50} values for the estimation of the scavenging activity of suramin on DPPH reduction.

2.1.2. Nitric oxide radical inhibition activity of suramin was evaluated using the method of Temraz and El-Tantawy (2008)

Briefly, the reaction mixture with a total volume of 3 ml containing sodium nitroprusside or SNP (10 mM, 2 ml), and suramin (5 to 500 μg/ml) in phosphate buffer saline pH 7.4 was incubated at 25 °C for 2 h 30 min. Half milliliter of the reaction mixture was added to 1 ml of sulfanilic acid (SA) reagent (SA 0.33% in 20% glacial acetic acid) and kept undisturbed for 5 min for diazotization reaction. Then, 1 ml of NED (0.1% in water) was mixed and incubated for 20 min at 25 °C. A pink color was developed in diffused light. The optical densities of the solutions were recorded at 540 nm against a corresponding blank sample.

2.1.3. Total antioxidant capacity of suramin was evaluated by the method illustrated by Sharma et al. (2011)

Suramin (0.3 ml) and standards at all the concentrations were combined with 3 ml of reagent solution (0.6 M H_2SO_4, 4 mM ammonium molybdate and 28 mM sodium phosphate) followed by incubation at 95 °C for 1.5 h. The absorbance of the solutions was recorded at 695 nm against blank after cooling to room temperature. Methanol (0.3 ml) was used in the blank sample.

2.1.4. Hypochlorous acid scavenging assay was done as per the method of Aruoma et al. (1989), where the scavenging activity was evaluated by measuring the decrease in absorbance of catalase at 404 nm

Hypochlorous acid (HOCl) was prepared immediately before the experiment by adjusting the pH of a 10% (v/v) solution of sodium hypochlorite to 6.2 with 0.6 M H_2SO_4, and the concentration of HOCl was determined by measuring the absorbance at 235 nm using the molar extinction coefficient of 100 M^{-1} cm^{-1}. The scavenging activity was evaluated by measuring the decrease in absorbance of catalase at 404 nm. The final volume of 1 ml of reaction mixture contained 50 mM phosphate buffer (pH 6.8), catalase (7.2 mM), HOCl (8.4 mM) and increasing concentrations of suramin and other standards. The assay mixture was incubated at 25 °C for 20 min and the absorbance was measured against an appropriate blank (Hazra et al., 2008).

2.1.5. Hydrogen peroxide (H_2O_2) radical scavenging activity of suramin was determined using some modifications in the method of Ruch et al. (1989)

Hydrogen peroxide concentration was estimated by recording the absorbance at 230 nm using molar absorbance coefficient of 81 M^{-1} cm^{-1}. Solution of H_2O_2 (4 mM) was prepared in PBS (pH 7.4) and the concentration of H_2O_2 was determined spectrophotometrically. Concentration gradient of suramin and standards (4 ml each) prepared in distilled water were added to 0.6 ml H_2O_2-PBS solution. Absorbance of H_2O_2 at 230 nm was taken after 10 min against a blank solution containing suramin in PBS without H_2O_2 (Venkatachalam et al., 2012).

2.1.6. In vitro lipid peroxidation inhibition assay

The antioxidant property of suramin was done by lipid peroxidation inhibition assay based on the method of Silva and co-workers (Silva et al., 2005). Young Wistar rat brain homogenate (1:10 w/v) was prepared in 50 mM Tris-HCl buffer (pH 7.4) followed by centrifugation at 3000 rpm for 10 min. An aliquot (100 μl) of supernatant was mixed with suramin and standards of all the concentrations followed by addition of 100 μl each of 1 mM $FeCl_3$ and 1 mM L-ascorbic acid to induce lipid peroxidation and incubated for 1 h at 37 °C. TCA (500 μl of 28%) was used to stop the reaction and then 380 μl of 2% TBA was added with heating at 95 °C for 30 min, to generate the color. Then, the