# The Identification of Bio-available and Active Components in Oxihumate

by

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**Abstract**

**Key Words:** Humic acid; oxihumate; chemical analysis; fractionation; HPLC; TLC; fluorescence spectra; UV spectra; IR spectra; infrared spectra; absorption; baboon model; rat model; distribution; pharmacokinetics; isolated gut segments; contact hypersensitivity; DNFB; chemiluminescence; CR3; human neutrophil adhesion molecules; anti-oxidant activity; anti-inflammatory; immuno-modulating

Humic acids are ubiquitous organic compounds found in soils and waters of the world. These organic molecules, derived from plant materials, have been isolated from many natural sources like seawater, marshes, soils, peat and coal. Despite extensive studies, the chemical structure of humic acids has not yet been elucidated and several theories have been proposed as to its structure. They have been found to have growth-stimulating effects on plants and medicinal properties in animals and humans.

Oxihumate is a semi-synthetic humic acid derived from a bituminous coal by a mild oxidation process. This synthetic humic acid has similar physical and chemical characteristics to humic acids isolated from different natural resources. Extensive toxicological studies have shown that there are no toxic effects to orally administered oxihumate at concentrations as high as 300mg/kg body weight.

In this study it was found that oxihumate could be sub-fractionated into at least seven sub-fractions using differential solubility in increasing concentrations of organic solvent, mixtures in which humic acid was reputed not to dissolve. Several analytical techniques were applied to each isolated sub-fraction in an attempt to determine the complexity and main chemical structures and to identify which sub-fractions possess the greatest anti-inflammatory properties. Thin layer and high pressure liquid chromatography, infrared, UV and fluorescent spectrophotometry, ash content and EDS microanalyses were performed on each isolated sub-fraction, all of which proved to still be complex mixtures of compounds.

The biological activity of the sub-fractions measured using immuno-fluorescent and chemiluminescent bioassays for anti-inflammatory properties indicated that the activity appeared to be associated with the complete mixture of compounds in the humic acid complex and not in the isolated or extracted sub-fractions.

Absorption studies using isolated rat gut segments and radioactively labelled oxihumate indicated that several compounds are absorbed from the lumen of the gastro intestinal tract

(GIT) and that the absorption rates depended on the GIT segment and reached a plateau within two hours.

As the GIT absorption studies showed uptake, the effect that orally administered oxihumate had on a dinitro-fluoro-benzene (DNFB) induced contact hypersensitivity rat model was tested and found to have a limited effect on the typical reaction at 61mg/kg. Brown coal humic acid and prednisolone, a steroidal anti-inflammatory, both showed significant and greater inhibition effects on the hypersensitivity response.

A further part of the study used 13 baboons that were dosed orally or rectally with radioactive iodide-123 labelled oxihumate. All the experimental baboons presented a distribution that compared almost exactly with that of free iodide controls. Initially however, the rectally dosed baboon showed a different pattern of distribution indicating uptake in the liver and gall bladder and only later showed the same distribution as the other animals. Excreted radioactivity was almost exclusively via the urine and this radioactivity was found to be mostly free iodide. The conclusions drawn from the baboon experiments was that the oxihumate appeared to be absorbed from the gut at a slower rate than the rate of metabolism of these absorbed compounds resulting in the release of free iodide.

The results of this study indicate that oxihumate can be sub-fractionated but that the isolated sub-fractions are still complex mixtures of compounds. The isolated sub-fractions appear to have less anti-inflammatory activity than the complete oxihumate indicating that the activity could be due to a combination of effects by different compounds. Oxihumate appears to have a unique combination of immuno-modulating properties that makes it a promising candidate as an anti-inflammatory agent.

## OPSOMMING

**Sleutelwoorde:** Humiensuur; oksihumaat; chemiese analise; HDVC; dunlaag chromatografie; fluoressensie spektra; UV spektra; IR spektra; infrarooi spektra; absorpsie; bobbejaan model; rot model; verspreiding; farmakokinetika; geïsoleerde derm segmente; kontak hipersensitiwiteit; DNFB; chemiluminessensie; CR3; menslike neutrofiel adhesie molekules; anti-oksidant aktiwiteit; anti-inflammatoriese middel; immuno-moduleer middel.

Humiensure kom wyd verspreid voor en is in meeste grond en water aanwesig. Dié organiese molekules wat meestal van plant materiaal afkomstig is, is al uit verskeie bronne soos seewater, moerasse, grond, feen en steenkool geïsoleer. Ten spyte van uitgebreide studies en verskeie teorieë, is die chemiese struktuur van humiensure nog nie vasgestel nie. Dit is bekend dat hulle groei-stimulerende effekte op plante en terapeutiese eienskappe in diere en die mens het.

Oksihumaat is 'n semi-sintetiese humiensuur wat deur 'n matige oksidasie proses vanuit bitumineuse steenkool gemaak word. Hierdie semi-sintetiese humiensuur het soorgelyke fisiese en chemiese eienskappe aan humiensure wat vanaf verskillende natuurlike bronne geïsoleer is. Uitgebreide toksikologiese studies het gewys dat orale toediening van oksihumaat met konsentrasies van 300mg/kg liggaamsmassa, geen toksiese of newe effekte het nie.

In hierdie studie is bewys dat oksihumaat in minstens sewe sub-fraksies verdeel kon word, gebasseer op oplosbaarheids verskille in water met toenemende konsentrasies organise oplosmiddels, middels waarin humate na bewering onoplosbaar beskou te wees. Om die kompleksiteit, chemiese struktuure en anti-inflamatoriese eienskappe van elk van die geïsoleerde sub-fraksies te karakteriseer, is daar van verskillende analitiese tegnieke gebruik gemaak. Dun-laag en hoëdruk chromatografie, infrarooi-, UV-, en fluoressensie spektrofotometrie, as-inhoud en EDS mikroanaliese was op elke geïsoleerde sub-fraksie uitgevoer, wat bewys het dat al die sub-fraksies nog steeds komplekse mengsels van komponente is.

Die biologiese aktiwiteit van die sub-fraksies is gemeet deur immuno-fluoressensie en chemiluminessensie toetse wat aandui dat die hoogste aktiwiteit en anti-inflamatoriese eienskappe geassosieer word met die volledige humiensuur mengsel en nie by die geïsoleerde sub-fraksies nie.

Opname studies, waar geïsoleerde rot dermsegmente en radioaktief gemerkte oksihumaat gebruik is, het aangedui dat verskeie komponente wel opgeneem word uit die dermkanaal en dat die opname tempo afhankilik is van die spesifieke dermsegment. Afplatting van opname het binne twee ure plaasgevind.

Aangesien opname bewys is, is 'n vergelykende studie gedoen om die effek van 'n mondelinge toediening van 61mg/kg oksihumaat op dinitro-fluoro-benseen (DNFB) geinduseerde kontak hipersenstiewiteit in rot modele te toets. Daar is gevind dat slegs bruinsteenkool-humiensuur en prednisoloon, 'n bekende steroïedale anti-inflamatoriese middel, die hipersenstiewiteits reaksie betekenisvol geinhibeer het.

In 'n verdere studie is 13 bobbejane oraal of rektaal met radioaktief gemerkte oksihumaat gedoseer. Al die eksperimentele bobejane het 'n verspreiding soortgelyk aan die kontrole diere, waar net vrye jodied toegedien is, getoon. Die rektaal gedoseerde bobbejaan het wel in die begin 'n ander verspreiding getoon veral in die lewer en galblaas, maar dit het binne 24 uur dieselfde gelyk as al die ander. Radioaktiwiteit was slegs in die vorm van vrye jodied in die uriene uitgeskei. 'n Moontlike afleiding wat gemaak kan word is dat opname vanuit die spysverteeringskanaal stadiger is as wat metabolisme in die liggaam plaasvind en dus word net die vrygestelde jodied se verspeiding waargeneem.

Die resultate van hierdie studie toon dat oksihumaat wel gefraksioneer kan word, maar dat die geïsoleerde sub-fraksies nog steeds komplekse mengsels is. Die geïsoleerde sub-fraksies besit minder anti-inflamatoriese aktiewiteit as die volledige oksihumaat, moontlik omdat die anti-inflammatoriese effek 'n gekombineerde effek van verskillende komponente is. Dit blyk dat oksihumaat 'n unieke kombinasie van immuno-moduleerende eienskappe het, wat maak dat oksihumaat 'n belowende anti-inflamatoriese middel is wat verdere navorsing verg.

## ABBREVIATIONS

| | |
|---|---|
| AA | Arachidonic acid |
| APC | Antigen presenting cells |
| APCI | Atmospheric pressure chemical ionisation |
| AUC | Area under curve |
| CD | Clusters of differentiation (The number following the CD indicates which surface antigen is referred to). |
| CD4+ | T-lymphocyte presenting the CD4 surface antigen. A T-helper cell |
| CD8+ | T-lymphocyte presenting the CD8 surface antigen. A cytotoxic T-cell |
| CE | Capillary electrophoresis |
| CNS | Central nervous system |
| Con A | Concanavalin A |
| COX-1 | Cyclooxygenase 1, prostaglandin H synthase type 1 (constitutive) |
| COX-2 | Cyclooxygenase 2, prostaglandin H synthase type 2 (inducible) |
| CR3 | Complement 3 receptor adhesion molecules, a leukocyte surface marker |
| DMSO | Dimethyl sulphoxide |
| DNFB | 2,4-Dinitro-fluorobenzene |
| DTH | Delayed type hypersensitivity |
| $E_4/E_6$ | The ratio of absorbance measured at 465nm divided by the absorbance measured at 665 nm for a compound in solution |
| eV | Electron volt |
| FAB | Fast ion bombardment |
| GC | Gas chromatography |
| GM-CSF | Granulocyte macrophage colony stimulating factor |
| HPLC | High performance liquid chromatography |
| HUVEC | Human umbilical cord arterial endothelial cells |
| ICAM-1 | Intercellular adhesion molecule type 1 |
| ICAM-2 | Intercellular adhesion molecule type 2 |
| Ig | Immunoglobulin |
| IL- | Interleukin- (the number refers to the particular interleukin) |
| INF-γ | Interferon –γ |
| IV | Intra-venous |
| kD | Kilo-Dalton, molecular mass/1000 |
| LAK | Lymphokine activated killer cells |

| | |
|---|---|
| LPS | Lipopolysaccharide, a gram negative bacterial cell wall antigen |
| $LTB_4$ | Leukotriene $B_4$ |
| MALDI TOF | Matrix assisted laser desorption ionisation time-of -flight |
| MHC-II molecules | Major histocompatibility complex type 2 molecules |
| MO | Monocytes |
| MØ | Macrophages |
| mRNA | Messenger ribose nucleic acid |
| MS | Mass spectroscopy |
| NF-κB | Nuclear transforming factor - κB |
| NK | Natural killer cells, large granular lymphocytes |
| nm | nanometre |
| NMR | Nuclear magnetic resonance spectroscopy |
| PAF | Platelet activating factor, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphate |
| PAGE | poly-acrylamide gel electrophoresis |
| PAI-1 | Plasminogen activator inhibitor - 1 |
| $PGD_2$ | Prostaglandin $D_2$ |
| $PGE_2$ | Prostaglandin $E_2$ |
| $PGF_{2\alpha}$ | Prostaglandin $F_{2\alpha}$ |
| $PGI_2$ | Prostaglandin $I_2$ |
| pH | Negative logarithm of the concentration of hydronium ions, a measure of the acidity of an aqueous solution |
| PHA | phytohemagluttinin |
| PMNL | Polymorphonuclear leukocytes |
| PPAR-α | Peroxisome proliferator-activated receptor-α |
| ROS | Reactive oxidant species |
| SEC | Size exclusion chromatography |
| sIgD | Soluble immunoglobulin D |
| sIgM | Soluble immunoglobulin M |
| Th 1 | T-lymphocyte helper cells type 1 |
| Th 2 | T-lymphocyte helper cells type 2 |
| TNF-α | Tumour necrosis factor – α, a pro-inflammatory cytokine |
| TNF-β | Tumour necrotic factor-β |
| t-PA | Tissue plasminogen activator |
| $TxA_2$ | Thromboxane $A_2$ |
| UV | Ultraviolet light |
| VCAM-1 | Vascular adhesion molecule type 1 |