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(54)【発明の名称】移植器官の拒絶反応の治療および予防における（Ｒ）－イブプロフェンメタンスルホンアミドおよびその塩の使用

(57)【要約】
移植器官の拒絶反応から生ずる機能障害の治療用および予防用薬剤を製造するための、（Ｒ）－イブプロフェンメタンスルホンアミドの使用について記載する。特に、移植腎の拒絶反応の予防および治療のための、（Ｌ）－リジン塩のような（Ｒ）－イブプロフェンメタンスルホンアミドの無毒性塩の使用について記載する。

【特許請求の範囲】
【請求項１】
虚血／再灌流障害の予防用または治療用薬剤を製造するための、（Ｒ）－イブプロフェンメタンスルホンアミドまたはその無毒性塩の使用。
【請求項２】
移植器官の拒絶反応から生ずる機能障害の予防用または治療用薬剤を製造するための、（Ｒ）－イブプロフェンメタンスルホンアミドまたはその無毒性塩の使用。
【請求項３】
該無毒性塩がＬ－リジン塩またはＤＬ－リジン塩である、請求項１または２に記載の使用。
【請求項４】
該無毒性塩がＬ－リジン塩である、請求項３に記載の使用。
【請求項５】
該移植器官が移植腎である、請求項２に記載の使用。
【請求項６】
（Ｒ）－イブプロフェンメタンスルホンアミドまたはその無毒性塩を、許容される担体に混合した形で含有する、移植器官の拒絶反応から生ずる機能障害を予防または治療するための医薬組成物。
【請求項７】
（Ｒ）－イブプロフェンメタンスルホンアミドまたはその無毒性塩を、許容される担体に混合した形で含有する、虚血／再灌流障害を予防または治療するための医薬組成物。
【請求項８】
該無毒性塩がＬ－リジン塩である、請求項６または７に記載の医薬組成物。
【発明の詳細な説明】
【０００１】
本発明は、移植器官の拒絶反応から生ずる機能障害の治療用および予防用薬剤を製造するための、（Ｒ）－イブプロフェンメタンスルホンアミドおよびその無毒性塩の使用に関する。
発明の背景
器官移植、特に腎臓タイプの移植は、改良された免疫抑制剤摂取、器官保存および手術前後ケアの導入により、この数十年で相当な進歩を遂げた。それにも関わらず、特に長期間の予後を改善する点については改善の余地がある。器官の回復、保存および移植に二次的に生ずる初期の虚血／再灌流障害は、続いて起こる悪化および移植障害に関係している。腎移植において、同種移植片の即時機能の欠如は、移植後第１週の間における透析の必要性として一般に広く定義されている遅延移植機能（ＤＧＦ）として知られている。遅延移植機能は移植直後期間における最も一般的な同種移植合併症で、初期は死体腎移植の５０％までが罹っている（Ｏｊｏ ＡＯ ｅｔ ａｌ．、Ｄｅｌａｙｅｄ ｇｒａｆｔ ｆｕｎｃｔｉｏｎ：ｒｉｓｋ ｆａｃｔｏｒｓ ａｎｄ ｉｍｐｌｉｃａｔｉｏｎｓ ｆｏｒ ｒｅｎａｌ ａｌｌｏｇｒａｆｔ ｓｕｒｖｉｖａｌ．Ｔｒａｎｓｐｌａｎｔａｔｉｏｎ ６３、１１：１６２０－１６２８、１９９７）。異なる病因が移植した同種移植片のＤＧＦを生じさせ得るが、蓄積された実験および臨床上の証拠は、虚血後再灌流の同種移植片への障害がＤＧＦ発生の原因となる主な事象であることを示唆している。ＤＧＦと早期拒絶の組合せが乏しい移植片生存率の厳しい指標であり、ＤＧＦの発生が急性拒絶増加の危険性をもたらすというのが一致した合意である（Ｃａｒｍｅｌｌｉｎｉ Ｍ ｅｔ ａｌ．、Ｄｅｌａｙｅｄ ｇｒａｆｔ ｆｕｎｃｔｉｏｎ ａｄｖｅｒｓｅｌｙ ａｆｆｅｃｔｓ ｏｎｅ－ｙｅａｒ ｇｒａｆｔ ｓｕｒｖｉｖａｌ ｏｆ ｃａｄａｖｅｒｉｃ ｒｅｎａｌ ｔｒａｎｓｐｌａｎｔｓ．Ｔｒａｎｓｐｌａｎｔ Ｐｒｏｃ ２８、１：３５９－３６０、１９９６）。虚血／再灌流障害の病因は、現在はサイトカインおよび特に表面接着分子が関与することが知られており、その発現が炎症細胞の付着を開始させる。急性腎虚血を生じた実験動物からの証拠では、細胞間接着分子－１（ＩＣＡＭ－１）

が障害後急速に上方制御され、好中球、Ｔ細胞およびマクロファージの浸潤が続いて生ずることを示している。多形核細胞（ＰＭＮ）に対し強力な走化性効果を有するサイトカインであるインターロイキン－８（ＩＬ－８）は、再灌流に続く上皮細胞の活性化により生じ、最終的に虚血／再灌流障害による遅延移植機能に導く複雑な事象の一因となり得る。近年、ＩＬ－８の生物的活性を選択的に阻害する新規化合物が発見されている。その中でも、Ｒ（－）－Ｎ－［２－（４－イソブチルフェニル）プロピオニル］－メタンスルホンアミド（以後、（Ｒ）－イブプロフェンメタンスルホンアミドと称する）およびそのリジン塩（以後、ＤＦ１６８１Ｂと称する）が、国際特許出願ＷＯ００／２４７１０号に、ＩＬ－８により誘導される好中球の走化性の生体外での選択的阻害剤として記載されており、したがって好中球依存性病理の治療に望ましく好適である。

【０００２】

現在では、ＤＦ１６８１Ｂはマウスモデルの生体中で走化性を阻害し、マウスおよびラットの虚血／再灌流障害の異なるモデルにおいてＰＭＮ浸潤を阻害することが示されている。

【０００３】

実際は、最近の技術水準によれば、ＩＬ－８誘導走化性を選択的に阻害することは、移植した器官を機能障害から保護するには充分な条件ではない。事実、科学文献には移植腎の機能回復遅延の病因に関与する多くの因子が同定されており、その因子の中でＩＬ－８は確かに最も重要な因子ではないようである：例えば、ＩＬ－８はＩＬ－３および可溶性ＣＤ２３（Ｋｕｔｕｋｃｕｌｅｒ Ｎ．ｅｔ ａｌ．、Ｔｒａｎｓｐｌａｎｔａｔｉｏｎ、１９９５、５９（３）、３３３－４０）と共に、とにかく拒絶現象が全くない移植患者においてもこれらのマーカーが高レベルで存在している場合は、器官拒絶の診断には使用できないと報告されている。その上、科学文献には、ＩＬ－３、ＩＬ－８およびＣＤ２３に加えて、例えばＩＬ－１β、ＩＬ－２、ＩＬ－１０、ＩＬ－１７、ＭＩＰ－１β、ＭＣＰ－１などのような、移植器官の機能回復遅延の可能性ある病因因子として、種々の他の可能性ある後炎症性分子が同定されている。文献データから、炎症反応または少なくとも白血球の漸増加の特異的阻害剤が、器官移植おける再灌流障害、特に腎臓における障害の阻害には必要であると思われる。

発明の説明

上で検討した先行技術からの予想に反して、驚くべきことに、（Ｒ）－イブプロフェンメタンスルホンアミドおよびそのリジン塩（Ｌ－リジンまたはｄｌ－リジン）は器官移植、特に腎臓移植における器官障害からの保護において効果的であることを発見した。更に、同じ化合物が虚血／再灌流障害の予防において活性であることが示された。

【０００４】

その活性は、以下に詳細に開示されるラットにおける腎移植実験モデルで示され、ＤＦ１６８１Ｂが同系腎移植に続く虚血／再灌流障害直後の腎機能保存において活性であり、また、虚血後再灌流に続いて起こる移植における白血球浸潤を予防することが証明された。

【０００５】

成熟雄性Ｌｅｗｉｓラット（ＲＴ１１）（Ｃｈａｒｌｅｓ Ｒｉｖｅｒ、Ｃａｌｃｏ Ｉｔａｌｉａ Ｓ．ｐ．Ａ、イタリア）を用いた。全ての動物は飼料および水を自由摂食できるようにした。このようなラットをドナーおよび移植片レシピエントとして使用した同系腎移植モデルにおいて研究を行った。ドナー動物をレプトフェンで麻酔した。左腎は尿管を付着物から外して調製した。腎動脈を腎静脈から切開して分離した。ドナー腎臓および尿管を「一括して」取除き、ヘパリン１０００Ｕ／ｍｌを含有するＢｅｌｚｅｒ（ＵＷ）をざっとかけた。それから腎臓を氷冷したＢｅｌｚｅｒ（ＵＷ）溶液中に移植まで４～６時間（冷虚血）放置した。レシピエントは左腎を除去して調製した。ＤＦ１６８１Ｂによる動物処理を以下にまとめた。腎移植片を移植前に食塩溶液で洗浄した。レシピエントおよびドナー腎動脈間並びに腎静脈間を末端－末端吻合により吻合した。血管のクランプは３０分後に解放した（温虚血）。ドナーおよびレシピエントの尿管を末端－末端付着した。その後もとのままの右腎を除去した。腎機能回復の指標として毎日の尿排出を測定す

るために、動物を個々の代謝ケージに入れた。１６および２４時間後に腎機能を血漿クレアチニン濃度で評価した。腎移植２４時間後、動物を屠殺した。腎移植片を除去し、スライスして、光学顕微鏡による従来の組織学的分析用にＤｕｂｏｓｑ－Ｂｒａｚｉｌ溶液中に置いた。その上、さらなる腎臓片を液体窒素中で冷凍し、炎症細胞浸潤の免疫組織化学的分析用に用いた（多形核細胞、ＭＨＣクラスＩＩ陽性細胞）。

【０００６】

以下の動物実験群について考察した：

第１群（ｎ＝３）　４時間冷虚血に曝し、ＩＬ－８阻害剤ＤＦ１６８１Ｂで処理した腎移植片のラットレシピエント。

第２群（ｎ＝３）　４時間冷虚血に曝し、賦形剤で処理した腎移植片のラットレシピエント。

第３群（ｎ＝３）　６時間冷虚血に曝し、ＩＬ－８阻害剤ＤＦ１６８１Ｂで処理した腎移植片のラットレシピエント。

第４群（ｎ＝３）　６時間冷虚血に曝し、賦形剤で処理した腎移植片のラットレシピエント。

【０００７】

レシピエントは実験前日に前処理を行った（１５ｍｇ／ｋｇ　皮下注射）。当該動物は、移植腎の再灌流直前にＤＦ１６８１Ｂ（１５ｍｇ／ｋｇ）の静脈内注射を受けた。当該化合物（１５ｍｇ／ｋｇ　皮下注射）の追加投与を移植の２時間後に実施した。対照動物には、ＤＦ１６８１Ｂで処理した動物と同じ投与方法を用いて同じ時間に賦形剤を与えた。

【０００８】

加えて、腎同種異系移植に続く虚血／再灌流障害の直後の腎機能保存においてＤＦ１６８１Ｂが活性であることを証明した。以下の動物実験群について考察した：

第１群（ｎ＝９）　６時間の冷虚血に曝し、賦形剤で処理したＬｅｗｉｓラットの腎移植片のＢｒｏｗｎ　Ｎｏｒｗａｙラットレシピエント。

第２群（ｎ＝５）　６時間の冷虚血に曝し、ＩＬ－８阻害剤ＤＦ１６８１Ｂで処理したＬｅｗｉｓラットの腎移植片のＢｒｏｗｎ　Ｎｏｒｗａｙラットレシピエント。

【０００９】

レシピエントは実験前日に前処理を行った（２０ｍｇ／ｋｇ　皮下注射）。当該動物は、移植腎の再灌流直前にＤＦ１６８１Ｂ（２０ｍｇ／ｋｇ）の静脈内注射を受けた。当該化合物（２０ｍｇ／ｋｇ　皮下投与）の追加投与を移植の２時間後に実施した。対照動物には、ＤＦ１６８１Ｂで処理した動物と同じ投与方法を用いて同じ時間に賦形剤を与えた。

【００１０】

データは、多重比較ではノンパラメトリックなＫｒｕｓｋａｌ－Ｗａｌｌｉｓ検定、またはＴｕｋｅｙ－Ｃｉｃｃｈｅｔｔｉ検定を用いて解析した。

結果

図１および表１は、４および６時間冷虚血に曝し、同系腎移植を受けた後１６および２４時間におけるＬｅｗｉｓラットの血漿クレアチニン濃度を示す。４時間の虚血腎を受けた動物では血漿クレアチニンが術後増加し、１６および２４時間で非虚血同系腎移植を受けた動物の対照群に見られる値より顕著に高い値に達した。ＤＦ１６８１Ｂ処理により動物は腎機能低下から保護され、２４時間での血漿クレアチニン値が非虚血同系腎移植を受けた動物の値とかなり近似していた（表２）。４時間虚血後の同系腎移植を受けた動物より顕著に高い血漿クレアチニンレベルにより立証されるように、予想通り、６時間虚血はより重症な腎機能障害を誘発した（図１および表１）。ＤＦ１６８１Ｂは顕著に血漿クレアチニン濃度を減少させたが、依然として非虚血同系腎移植を受けた動物で測定したより相当高いレベルであった。

【００１１】

移植後２４時間において検討した移植腎における白血球浸潤の詳細な免疫組織学的評価を表２にまとめる。４時間冷虚血を受けた腎臓においては、多数の顆粒球が間質で、それより少ない範囲で糸球体内部および周囲領域において見られた。当該化合物はＰＭＮの浸潤

を低減させ、虚血／再灌流で引き起こされる尿細管の変形を減ずる。糸球体内部領域での顆粒球の計測は数値的に行ったが、ＩＬ－８阻害剤で顕著には低下しなかった。細胞浸潤は、４時間虚血後の値と比較して、６時間虚血後では必ずしも増加しなかった。一方で、間質性炎症細胞はＤＦ１６８１Ｂにより有意に低下した（ｐ＜０．０１）。好中球に関しては、ＭＨＣクラスＩＩ細胞の数は４および６時間虚血後の移植腎でより少なかった。細胞は主に間質で検出されたが（表３）、それらの数はＩＬ－８阻害剤では影響されなかった。

【００１２】

４および６時間冷虚血後に移植した腎臓から得た切片で観察し、移植２４時間後に検討した糸球体、間質および尿細管障害の組織化学的スコアを表４に示す。光学顕微鏡により、移植腎には尿細管上皮細胞の退行性変化、特に近位尿細管の細胞腫脹、空胞化および壊死により現される特徴がみられた（表４）。ＤＦ１６８１Ｂは、虚血４時間後の尿細管変化を低減するが正常化はしなかった。加えて、尿細管円柱が全ての腎臓で見られた。局所的虚血変化が６時間虚血に曝された腎臓にのみ糸球体で検出され、それはＤＦ１６８１Ｂで妨げられた。

【００１３】

したがってＤＦ１６８１Ｂは冷虚血に次ぐ腎機能障害を予防することができる。当該化合物は細胞浸潤の数を減少させ、虚血により誘発される尿細管変化を減少させる。データは他の動物で確認された。６時間虚血は非常に重度の腎機能障害を誘発する。

【００１４】

Ｌｅｗｉｓラットから同種異系腎移植を受けた後１６および２４時間のＢｒｏｗｎ　Ｎｏｒｗａｙラットにおける血清クレアチニン濃度に対するＤＦ１６８１Ｂの効果を表５に示す。血清クレアチニンレベル増加の顕著な防止は２０ｍｇ／ｋｇの処置を受けた全てのラットで一貫して観察された。

【００１５】

上記のデータは（Ｒ）－イブプロフェンメタンスルホンアミドまたはそのリジン塩（Ｌ－リジンまたはｄｌ－リジン）が、どのように医療の実践において有利に使用できるかを明確に示している。

【００１６】

この目的のために、（Ｒ）－イブプロフェンメタンスルホンアミドまたはそのリジン塩は、移植手術の前および後に経口、非経口、直腸または局所経路で投与される医薬組成物に好適に製剤化されるであろう。好適な製剤には、カプセル、錠剤、座薬、シロップ、ドロップ、懸濁液、乳濁液、注射用滅菌溶液、注射用の滅菌凍結乾燥粉末のバイアル、放出制御製剤、経皮性製剤、軟膏などが含まれる。このような製剤の製造に使用される手法および担体は全体的には慣用されるものであり、例えばＲｅｍｉｎｇｔｏｎ’ｓ　Ｐｈａｒｍａｃｅｕｔｉｃａｌ　Ｓｃｉｅｎｃｅｓ　Ｈａｎｄｂｏｏｋ、Ｍａｃｋ　Ｐｕｂ．　Ｃｏ．、Ｎｅｗ　Ｙｏｒｋ、ＵＳＡ、ＸＶＩＩ　Ｅｄ．に記載されるようなものである。

【００１７】

医薬製剤は好ましくは１～５００ｍｇ、より好ましくは１０～１００ｍｇの（Ｒ）－イブプロフェンメタンスルホンアミドまたは等量のそのリジン塩を含有する単位剤形で投与される。より多用量のものも状況に応じて考えられる。投与は単回投与または好適な時間間隔、通常は手術の数日前から数週間後までに亘り複数回投与することができる。（Ｒ）－イブプロフェンメタンスルホンアミド、またはリジン塩のようなその許容できる塩は、必要であれば他の補助的な薬剤または、ともかく有用な作用の例えば抗炎症剤、免疫抑制剤、鎮痛剤、抗血栓剤と組合せて投与できる。

【実施例１】

【００１８】

Ｒ－イブプロフェン－メタンスルホンアミドの製造

塩化チオニル（７．４ｍＬ）中のＲ（－）－２－（４－イソブチルフェニル）－プロピオン酸（Ｒ－イブプロフェン、４ｇ、０．０１９ｍｏｌ）の懸濁液を４時間還流した；それ

から自然に冷却するまで室温で放置した。過剰の塩化チオニルは減圧下で蒸発させた。

【００１９】

塩化チオニルの最後の痕跡を数滴の乾燥ジオキサンで残留物を２回洗浄して除去し、減圧下で溶媒を蒸発させた。Ｒ（－）－２－（４－イソブチルフェニル）－プロピオニルクロリド４．６６ｇ（０．０１９ｍｏｌ）が黄色油状物として得られ、それを数ｍｌの無水テトラヒドロフラン（ＴＨＦ）に溶解した。

【００２０】

別に、メタンスルホンアミド（２．３ｇ、０．０２４３ｍｏｌ）を、無水ＴＨＦ（２８ｍＬ）中のカリウムｔｅｒｔ－ブトキシド（２．７３ｇ、０．０２４４ｍｏｌ）の懸濁液に加え、混合物を室温で３０分間攪拌した。その後、上で得たＲ（－）－２－（４－イソブチルフェニル）－プロピオニルクロリド（４．６６ｇ、０．０１９）溶液を攪拌しながら加え、反応混合物を室温で一晩攪拌した。

【００２１】

分離した無機塩をろ過して除去し、溶媒は減圧下で蒸発させ、油状残留物をCH_2Cl_2（３０ｍＬ）およびリン酸１ナトリウム飽和溶液の間で分配した。有機相は水（２×１０ｍＬ）で洗浄し、水相はCH_2Cl_2（２×１０ｍＬ）で抽出した。合わせた有機抽出物はNa_2SO_4で乾燥し、溶媒は蒸発させ、それから無水ＭｅＯＨ（１０ｍＬ）中の油状残留物溶液に濃硫酸２小滴を加えて未反応Ｒ（－）－２－（４－イソブチルフェニル）－プロピオン酸の痕跡をメチルエステル化した。混合物を一晩室温に保ち、溶媒は減圧下で注意深く蒸発させ、残留物は水（１０ｍＬ）および塩化メチレン（２５ｍＬ）間で分配した。水相は廃棄し、有機相は$NaHCO_3$飽和溶液（２×２０ｍＬ）で抽出した。塩基性相を合わせ、濃塩酸で酸性にしてCH_2Cl_2（３×１５ｍＬ）で抽出した。通常の洗浄で中性とした後、合わせた有機相をNa_2SO_4で乾燥し、溶媒を減圧下で蒸発させると、１．８６ｇ（０．００６６ｍｏｌ）のＲ（－）－Ｎ－［２－（４－イソブチルフェニル）プロピオニル］－メタンスルホンアミドが得られた：融点１０３～１０５℃（分解）；$[\alpha]_D$＝－６８（ｃ＝１；CH_3OH）；

【００２２】

【化１】

^{1}H-NMR

(DMSO-d_6) δ 7.3 (d, 2H J=8 Hz); 7.09 (d, 2H J=7 Hz); 3.42 (q, 1H, J=8 Hz); 2.8 (s, 3H); 2.45 (d, 2H, J=7 Hz); 1.55 (m, 1H); 1.3 (d, 3H, J=8 Hz), 0.95 (d, 6H, J=7 Hz).

【実施例２】

【００２３】

Ｒ－イブプロフェン－メタンスルホンアミド　Ｌ（＋）－リジン塩（ＤＦ１６８１Ｂ）の製造

水（１．３ｍＬ）中のＬ（＋）－リジン（１２９ｍｇ；０．８８ｍｍｏｌ）の溶液を、メタノール（１ｍｌ）中のＲ（－）－Ｎ－［２－（４－イソブチルフェニル）プロピオニル］－メタンスルホンアミド（２５０ｍｇ；０．８８ｍｍｏｌ）の溶液に加えた。溶媒を蒸発させ、残留物をエチルエーテル（５ｍＬ）と合わせ、一晩室温で攪拌した。分離した結晶性で、強吸湿性の物質を窒素雰囲気下で素早くろ過し、フィルター上にて無水エチルエーテルで洗浄し、減圧下５０℃で２時間乾燥すると、Ｒ（－）－Ｎ－［２－（４－イソブチルフェニル）プロピオニル］－メタンスルホンアミドのＬ（＋）－リジン塩が淡黄色粉末として３６０ｍｇ得られた。$[\alpha]_D$＝－１７．３°（ｃ＝１．１５；CH_3OH）；

【００２４】

【化２】

^{1}H-NMR (D_2O): δ7.30 (dd, 4H, J=8Hz), 3.77 (t, 1H, J=7Hz), 3.65 (q, 1H, J=7Hz), 3.05 (m, 5H), 2.52 (d, 2H, J=7Hz), 1.92 (m, 2H), 1.75 (m, 2H), 1.50 (m, 3H), 1.40 (d, 3H, J=7Hz), 0.90 (6H, d, J=7Hz).

【００２５】
【表１】

同系腎移植を受けたラットにおける血清クレアチニンに対するＤＦ１６８１Ｂの効果

ラット	群	血漿クレアチニン (mg/dl) 16h	24h
10 gr l	賦形剤 (4h)	2.27	2.58
11		1.82	1.14
12		2.24	2.49
13		2.30	2.09
		2.16±0.23*	**2.08±0.66***
1A	DF 1681B (4h)	1.09	0.99
2A		0.74	0.64
3A		0.89	0.71
		0.91±0.18°	**0.78±0.19°**
2D	賦形剤 (6h)	2.76	2.91
3D		2.39	3.64
4D		3.58	3.27
		2.91±0.61**	**3.27±0.37****
1E	DF 1681B (6h)	2.32	1.89
2E		2.54	1.87
3E		1.84	1.72
		2.23±0.36*Δ	**1.83±0.09#**
	対照範囲	0.5-	0.6
	（非虚血同系移植腎）		

データは平均±ＳＤで表す

*p<0.05,**p<0.01 対 対照
°p<0.05 対 賦形剤 4h
#p<0.05 対 賦形剤 6h
Δp<0.05 対 DF1681 4h

【００２６】
【表２】

各動物につき無作為に選択した少なくとも１０カ所の高倍率（×４００）顕微鏡視野において計測した顆粒球数に対するＤＦ１６８１Ｂの効果

ラット		顆粒球				
		糸球体内	糸球体周囲	血管内	血管周囲	間質
10gr1	賦形剤 (4h)	6.2±6	4±4	0.3±1	10±6	21.6±11
11		4.7±4.4	5.3±3.4	1.7±2.9	9.7±9.9	25.8±11.6
12		18.1±20.2	7.6±8.1	15±14.8	12±7	39.8±29.9
13		33±11.3	74.3±43.9	9.4±7.2	48±29	135.7±31.5
		15.5±13.1	22.8±34.4	6.6±6.9	19.9±18.7	55.7±53.9
1A	DF1681B (4h)	7.4±4.7	5.3±4.5	1.5±2.4	3±0.8	9.1±6.4
2A		14±15.4	4	0	8±2.6	11±4.8
3A		7±2.9	7±8	8±5.3	5.3±4.6	16.2±8.3
		9.5±3.9	5.4±1.5	3.2±4.3	5.4±2.5*	12.1±3.7*
2D	賦形剤 (6)	10.4±5.8	7.3±4.3	3.3±2.0	5.8±3.3	25.4±22
3D		12.3±9.5	2.9±1.1	1.4±2.1	5.6±2.7	23.6±18
4D		10.5±7.6	2.8±2.1	0	1.3±2.3	23.1±6.0
		11.1±1.1	4.3±2.6	1.6±1.7	4.2±2.5	24±1.2
1E	DF1681B (6h)	5.0±6.0	1.0±1.8	0.2±0.4	1.0±1.3	2.5±4.6
2E		3.7±3.0	3.2±1.9	0	6.8±8.9	4.8±5.6
3E		7.7±6.5	2.2±1.2	3.0±4.0	4.5±4.4	1.6±1.2
		5.5±2.0°	2.1±1.1	1.1±1.7	4.1±2.9	3±1.7°

データは平均±ＳＤで表す

*p<0.05 対 賦形剤 4h

°p<0.05 対 賦形剤 6h

【００２７】

【表３】

各動物につき無作為に選択した少なくとも１０カ所の高倍率（×４００）顕微鏡視野において計測したＭＨＣＩＩ陽性間質細胞数に対するＤＦ１６８１Ｂの効果

ラット			MHC II +
10gr1	賦形剤 (4h)		11.5±7
11			16.6±5
12			7.7±2.8
13			12.8±7.2
			12.2±3.7
1A	DF1681B (4h)		5.1±6.2
2A			9.1±2
3A			11.9±6.3
			8.7±3.4
2D	賦形剤 (6h)		6.9±2
3D			17.3±8.9
4D			21.3±3.6
			15.2±7.4
1E	DF1681B (6h)		10.5±4.7
2E			13.1±7
3E			19.7±3.3
			14.4±4.7

データは平均±ＳＤで表す

【００２８】
【表４】

腎臓障害についての半定性的スコア

ラット		組織障害		
		糸球体 (スコア)	間質 (スコア)	尿細管 (スコア)
10	賦形剤 (4h)	0	2	1.3
11		0	2	1
12		-	-	-
13		0	3	0.7
		0	**2.3±0.6**	**1±0.3**
1A	DF1681B (4h)	0	2.5	1.5
2A		0	2	0.7
3A		0	2	0.5
		0	**2±0.3**	**0.7±0.6**
2D	賦形剤 (6h)	1	2	1.33
3D		1	2.5	1.33
4D		1	2.5	1.2
		1	**2.3±0.3**	**1.3±0.1**
1E	DF1681B (6h)	0	3	1.7
2E		0	3	1.7
3E		0	3	1.2
		0	**3**	**1.5±0.3**

データは平均±ＳＤで表す

【００２９】
【表５】

同種異系腎移植を受けたラットにおける血清クレアチニンに対する
ＤＦ１６８１Ｂの効果

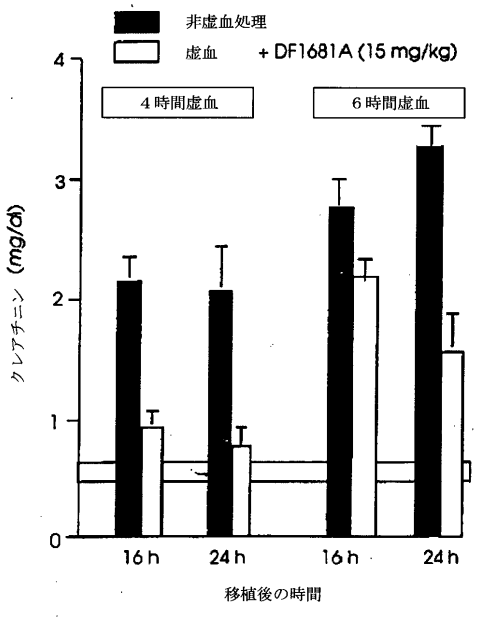
Brown Norwayラット	群	血漿クレアチニン (mg/dl)	
		16h	24h
1T	賦形剤	1.4	1.74
2T		1.27	1.20
3T		0.82	0.96
4T		2.23	2.28
5T		2.67	2.67
6T		2.23	2.29
7T		1.66	2.37
8T		1.67	1.6
9T		1.86	1.9
平均 ±		1.76	1.86
sd		0.56	0.59
1Z	DF1681B	1.05	1.3
2Z		1.2	1.39
3Z		1	0.87
4Z		0.84	0.69
5Z		1.01	0.85
平均 ±		1.02	1.02
sd		0.12	0.3

【図面の簡単な説明】

【００３０】

【図１】データは平均±ＳＤで示す。□は虚血に曝していない（対照非虚血）同系移植片を受けた対照動物における血清クレアチニンの対照範囲を示す。

【図１】


非虚血処理
虚血
+ DF1681A (15 mg/kg)
4時間虚血
6時間虚血
クレアチニン (mg/dl)
4
3
2
1
0
16h
24h
16h
24h
移植後の時間

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as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designation US

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designation US*

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(54) **Title:** USE OF (R)-IBUPROFEN METHANESULFONAMIDE AND SALTS THEREOF IN THE TREATMENT AND PREVENTION OF REJECTION REACTIONS OF TRANSPLANTED ORGANS

(57) **Abstract:** The use of (R)-ibuprofen methanesulfonamide is described for the preparation of medicaments for the treatment and prevention of functional injury resulting from rejection reactions of transplanted organs. In particular, the use of non-toxic salts of (R)-ibuprofen methanesulfonamide, such as the (L)-lysine salt, is described for the prevention and the treatment of rejection reactions of transplanted kidneys.

"USE OF (R)-IBUPROFEN METHANESULFONAMIDE AND SALTS THEREOF IN THE TREATMENT AND PREVENTION OF REJECTION REACTIONS OF TRANSPLANTED ORGANS".

The present invention relates to the use of (R)-ibuprofen methanesulfonamide and non-toxic salts thereof for the preparation of medicaments for the treatment and prevention of functional injury resulting from rejection reactions of transplanted organs.

Background of the invention

Organ transplantation, especially of renal type, has made substantial strides in the past few decades, with the introduction of improved immunosuppressive regimens, organ preservation, and pre- and postoperative care. Nevertheless, there is considerable room for improvement, particularly in terms of improving long-term outcome. Initial ischemia/reperfusion injury occurring secondary to organ retrieval, storage, and transplantation has been associated with subsequent deterioration and transplant failure. In renal transplantation the absence of immediate allograft function is known as delayed graft function (DGF) which is commonly and broadly defined as the need of dialysis during the first week after transplantation. Delayed graft function is the most common allograft complication in the immediate posttransplant period, affecting up to 50% of primary cadaveric renal transplants (*Ojo AO et al. Delayed graft function: risk factors and implications for renal allograft survival. Transplantation 63, 7:968-974, 1997; Koning OHJ et al. Risk factors for delayed graft function in cadaveric kidney transplantation. Transplantation 63, 11:1620-1628, 1997*). Although different etiologies may cause DGF of implanted allograft, there is accumulating experimental and clinical evidence suggesting that post-ischemic reperfusion injury to allograft may represent the major key event responsible for the occurrence of DGF. There is unanimous agreement that the combination of DGF and early rejection is a severe indicator of poor graft survival and that the occurrence of DGF leads to an increased risk of acute rejection (*Carmellini M et al. Delayed graft function adversely affects one-year graft survival of cadaveric renal transplants. Transplant Proc 28, 1:359-360, 1996*). The pathogenesis of ischemia/reperfusion injury is now known to involve

cytokines and particularly surface adhesion molecules, the expression of which initiates the attachment of inflammatory cells. Evidence from experimental animals with acute renal ischemia has shown that the intercellular adhesion molecule-1 (ICAM-1) is promptly up-regulated after injury and that neutrophil, T cell, and macrophage infiltrations subsequently occur. Interleukin-8 (IL-8), a cytokine with a potent chemotactic effect for polymorphonuclear cells (PMN), can be generated by the activation of endothelial cells that follows reperfusion and may contribute to the complex events ultimately leading to delayed graft function due to ischemia/reperfusion injury. Recently, new compounds that selectively inhibit the biological activity of IL-8 have been discovered. Among these, R (-)-N-[2-(4-Isobutylphenyl)propionyl]-methanesulfonamide, hereinafter referred to as (R)-ibuprofen methanesulfonamide, and its L-lysine salt (hereinafter referred to as DF 1681B), have been described in international patent application WO 00/24710 as selective *in vitro* inhibitors of the chemotaxis of neutrophils induced by IL-8 and therefore desirably suitable for the treatment of neutrophil-dependent pathologies.

DF 1681B has now been shown to inhibit chemotaxis *in vivo* in a mouse model and to inhibit PMN infiltration in different models of ischemia/reperfusion injury in mice and rats.

Indeed, according to the current state of the art, the selective inhibition of IL-8-induced chemotaxis is not a sufficient condition for the protection of a transplanted organ from functional injury. In fact, the scientific literature identifies numerous factors involved in the etiology of the delay in functional recovery of the transplanted kidney, among which factors, IL-8 does not certainly appear as one of the most important: for example, IL-8, together with IL-3 and soluble CD 23, (*Kutukculer N. et al., Transplantation, 1995, 59(3), 333-40*) is reported to be of no diagnostic use for organ rejection given that, in any case, high levels of these markers were also present in transplant patients who were wholly free from rejection phenomena. Moreover, in addition to IL-3, IL-8 and CD 23, scientific literature identifies various other possible pro-inflammatory molecules as possible pathogenetic factors of the delay in the functional recovery of the transplanted

organ, such as, for example, IL-1beta, IL-2, IL-10, IL-17, MIP-1beta, MCP-1, etc. It follows that, from the literature data, an aspecific inhibitor of the inflammatory response or, at the least, of leukocyte recruitment would appear necessary for the inhibition of reperfusion injury in organ transplantation, especially that of kidneys.

Description of the invention

It has now surprisingly been found out that, contrary to any expectations from the prior art discussed above, (R)-ibuprofen methanesulfonamide and its lysine salt (L-lysine or DL-lysine) are effective in the protection from functional injury in organ transplantation, particularly that of kidneys. Moreover, the same compounds were shown to be active in the prevention of ischemia/reperfusion injury.

Such an activity has been demonstrated in an experimental model of kidney transplantation in rats, disclosed in detail herein after, in which DF 1681B proved active in the preservation of renal function immediately after ischemia/reperfusion injury which follows syngeneic kidney transplantation, also preventing leukocyte infiltration in the transplant which occurs following post-ischemic reperfusion.

Adult male Lewis rats (RT11) (Charles River, Calco Italia S.p.A, Italy) were used. All animals were allowed free access to food and water. The study was performed in a syngeneic kidney transplant model using such rats as donor and graft recipients. Donor animals were anaesthetized with leptofen. The left kidney was prepared by freeing the ureter from the attachments. The renal artery was separated from the renal vein by dissection. The donor kidney and ureter were removed "en bloc" and flushed with Belzer (UW) containing 1000 U/ml of heparin. Then the kidney was placed in an iced Belzer (UW) solution for 4 - 6 hours (cold ischemia) until transplant. Recipients were prepared by removal of the left kidney. Animal treatment with DF 1681B is summarized below. Kidney grafts were washed with saline solution before transplant. An anastomosis was created between the recipient and the donor renal artery as well as renal vein with end-to-end anastomosis. Vascular clamps were released after 30 minutes (warm ischemia). Donor and recipient ureters were attached end-to-end. The native right kidney was then removed. Animals were placed in individual metabolic cages for

measurements of daily urine output as an index of renal function recovery. After 16 and 24 hours, renal function was assessed by measuring plasma creatinine concentration. Twenty-four hours after kidney transplantation, the animals were sacrificed. The kidney graft was removed, cut in slices and put in Dubosq-Brazil solution for the analysis of conventional histology by light microscopy. Moreover, additional kidney fragments were frozen in liquid nitrogen and used for immunohistochemical analysis of inflammatory cell infiltrate (polymorphonuclear cells, MHC class II positive cells).

The following experimental groups of animals were considered:

Group 1 (n.=3) rats recipients of a kidney graft exposed to 4 hours cold ischemia and treated with the IL-8 inhibitor DF 1681B.

Group 2 (n.=3) rats recipients of a kidney graft exposed to 4 hours cold ischemia and treated with the vehicle.

Group 3 (n.=3) rats recipients of a kidney graft exposed to 6 hours cold ischemia and treated with the IL-8 inhibitor DF 1681B.

Group 4 (n.=3) rats recipients of a kidney graft exposed to 6 hours cold ischemia and treated with the vehicle.

The recipients were pretreated the day before the experiment (15mg/kg s.c.). The animals received an intravenous injection of DF 1681B (15mg/kg) immediately before reperfusion of the transplanted kidney. Additional administration of the compound (15mg/kg s.c.) was performed 2 hours after transplantation. Control animals were given vehicle at the same time points and using the same administration method as for animals treated with DF 1681B.

In addition, DF 1681B proved active in the preservation of renal function immediately after ischemia/reperfusion injury which follows kidney allotransplantation. The following experimental group of animals were considered:

Group 1 (n=9) Brown Norway rats recipients of a kidney graft of Lewis rats exposed to 6 hours cold ischemia and treated with vehicle.

Group 2 (n=5) Brown Norway rats recipients of a kidney graft of Lewis rats exposed to 6 hours cold ischemia and treated with the IL-8 inhibitor DF 1681B.

The recipients were pretreated the day before the experiment (20 mg/kg s.c.). The animal received an intravenous injection of DF 1681B (20 mg/kg) immediately before reperfusion of the transplanted kidney. Additional administration of the compound (20 mg/kg s.c.) was performed 2 hours after transplantation. Control animals were given vehicle at the same time points and using the same administration method as for animals treated with DF 1681B.

Data were analyzed using the non-parametric Kruskal-Wallis test for multiple comparisons or the Tukey-Cicchetti test.

Results

Figure 1 and table 1 show plasma creatinine concentrations in Lewis rats at 16 and 24 hours after receiving a syngeneic kidney transplant, pre-exposed to 4 and 6 hour cold ischemia. In animals receiving 4 hour ischemic kidneys, plasma creatinine increased after surgery reaching values that at 16 and 24 hours were significantly higher than values observed in a control group of animals receiving a non-ischemic syngeneic kidney transplant. Treatment with DF 1681B protected animals from renal function deterioration, the values of plasma creatinine at 24 hours being fairly comparable to those of animals receiving a non-ischemic syngeneic kidney transplant (table 2). As expected, 6 hours ischemia induced a more severe renal function impairment, as documented by significantly higher plasma creatinine levels than in animals receiving a syngeneic kidney transplant after 4 hour ischemia (figure and table 1). DF1681B significantly reduced plasma creatinine concentrations to levels that, however, were still significantly higher than those measured in animals receiving a non-ischemic syngeneic kidney transplant.

Detailed immunohistologic evaluation of leukocyte infiltration in transplanted kidney, studied at 24 hours post-transplant, is summarized in table 2. In kidneys undergoing 4 hour cold ischemia a large number of granulocytes was found in the interstitium and, to a lesser extent, in intra and peri-glomerular areas. The compound decreases the PMN infiltrate and attenuates the tubular variations induced by ischemia/reperfusion. Granulocyte count in the intraglomerular area was numerically but not significantly

lowered by the IL-8 inhibitor. The cellular infiltrates did not consistently increase after 6 hour ischemia compared to values after 4 hour ischemia. Again, interstitial inflammatory cells were significantly lowered ($p<0.01$) by DF 1681B. With respect to neutrophils, the number of MHC class II cells was lower in kidneys transplanted following 4 and 6 hour ischemia. Cells were detected mainly in the interstitium (table 3) and their number was not affected by the IL-8 inhibitor.

Histologic scores of glomerular, interstitial and tubular injury observed in sections from kidneys transplanted after 4 and 6 hour cold ischemia and studied 24 hours after transplantation are shown in table 4. By light microscopy transplanted kidneys were characterized by degenerative changes of tubular epithelial cells predominantly in proximal tubuli, manifested by cell swelling, vacuolization and necrosis (table 4). DF 1681B attenuated but did not normalize tubular changes after 4 hour ischemia. In addition, tubular casts were found in all kidneys. Focal ischemic changes were detected in the glomeruli only in kidneys exposed to 6 hour ischemia and they were prevented by DF 1681B.

DF 1681B is thus able of preventing renal function impairment secondary to cold ischemia. The compound reduces the number of cellular infiltrates and attenuates tubular changes induced by ischemia. Data have been confirmed in other animals. 6 hour ischemia induces a very severe renal function impairment.

The effect of DF 1681B on serum creatinine concentrations in Brown Norway rats at 16 and 24 hours after receiving an allogeneic kidney transplant from Lewis rats is shown in Table 5. A significant prevention of increased serum creatinine levels was observed consistently in all rats receiving 20 mg/kg of the treatment.

The above data clearly show how (R)-ibuprofen methanesulfonamide or its lysine salt (L-lysine or DL-lysine) can be advantageously used in medical practice.

For this purpose, (R)-ibuprofen methanesulfonamide or its lysine salt will be suitably formulated in pharmaceutical compositions which may be administered in oral, parenteral, rectal or topic route, before and after transplantation surgery. Examples of suitable formulations include capsules, tablets, suppositories, syrups, drops,

suspensions, emulsions, injectable sterile solutions, vials of sterile lyophilized powders for injection, controlled release formulations, transdermal formulations, ointments and the like. The techniques and carriers used for the preparation of such formulations are wholly conventional, as described for example in Remington's Pharmaceutical Sciences Handbook, Mack Pub. Co., New York, USA, XVII Ed.

The pharmaceutical formulations are to be preferably administered in unit dosage forms containing from 1 to 500 mg, more preferably from 10 to 100 mg, of (R)-ibuprofen methanesulfonamide or the equivalent of its lysine salt. Higher doses can also be considered, depending on the circumstances. The administration can be a single one or divided into more administrations spread over a suitable time period, normally from some day before surgery until some weeks afterwards. (R)-Ibuprofene methanesulfonamide or an acceptable salt thereof, such as lysine salt can, if necessary, be administered in combination with others drugs of complementary or, in any case, useful action, for instance anti-inflammatory agents, immunosuppressants, analgesics, antithrombotic agents.

Example 1

Preparation of R-ibuprofen-methanesulfonamide.

A suspension of R(-)-2-(4-Isobutylphenyl)-propionic acid (R-ibuprofen, 4g, 0.019 mol) in thionyl chloride (7.4 mL) was refluxed for 4 h; then left to cool spontaneously at r.t. The thionyl chloride in excess was evaporated off under vacuum.

The last traces of thionyl chloride were removed washing twice the residual mass with a few drops of dry dioxane and evaporating the solvent under vacuum. 4.66g (0.019 mol) of R(-)-2-(4-Isobutylphenyl)-propionyl chloride were obtained as yellow oil, which was dissolved in a few ml of anhydrous tetrahydrofuran (THF).

Separately, methanesulfonamide (2.3g, 0.0243 mol) was added to a suspension of potassium tert-butoxide (2.73g, 0.0244 mol) in anhydrous THF (28 mL) and the mixture was stirred for 30 min at r.t. After that, the solution of R(-)-2-(4-Isobutylphenyl)-propionyl chloride (4.66g, 0.019) as obtained above was added under stirring, keeping the reaction mixture stirred overnight at r.t.

The separated inorganic salts were filtered off, the solvent was evaporated off under vacuum and the oily residue was partitioned between CH_2Cl_2 (30 mL) and a monosodium phosphate saturated solution. The organic phase was washed with water (2x10 mL) and the aqueous phases were extracted with CH_2Cl_2 (2x10 mL). The combined organic extracts were dried over Na_2SO_4 and the solvent was evaporated off, then the solution of the oily residue in anhydrous MeOH (10 mL) was added with two micro-drops of concentrated sulfuric acid, to esterify to methyl ester any traces of untransformed R(-)-2-(4-Isobutylphenyl)-propionic acid. The mixture was kept overnight at r.t., the solvent was cautiously evaporated under vacuum, the residue was partitioned between water (10 mL) and methylene chloride (25 mL). The aqueous phases were discarded and the organic phase was extracted with a $NaHCO_3$ saturated solution (2x20 mL). The basic phases were combined, acidified with conc. HCl and extracted with CH_2Cl_2 (3x15 mL). After the usual washings to neutrality, the combined organic extracts were dried over Na_2SO_4 and the solvent was evaporated off under vacuum to obtain 1.86g (0.0066 mol) of R(-)-N-[2-(4-Isobutylphenyl)propionyl]-methanesulfonamide: m.p.103-105°C (dec.); $[\alpha]_D$=-68 (c=1; CH_3OH); ^{1}H-NMR (DMSO-d_6) δ 7.3 (d, 2H J=8 Hz); 7.09 (d, 2H J=7 Hz); 3.42 (q, 1H, J=8 Hz); 2.8 (s, 3H); 2.45 (d, 2H, J=7 Hz); 1.55 (m, 1H); 1.3 (d, 3H, J=8 Hz), 0.95 (d, 6H, J=7 Hz).

Example 2

Preparation of R- ibuprofen-methanesulfonamide L (+)-lysine salt (DF 1681B).

A solution of L(+)-lysine (129mg; 0.88 mmol) in water (1.3 mL) was added to a solution of R(-)-N.[2-(4-Isobutylphenyl)propionyl]-methanesulfonamide (250 mg; 0.88 mmol) in 1 ml of methanol. The solvent was evaporated off and the residual mass was taken up with ethyl ether (5mL) and stirred overnight at room temperature. The crystalline, highly hygroscopic material which separated was filtered quickly under nitrogen atmosphere, washed on the filter with anhydrous ethyl ether and dried under vacuum at 50°C for 2 h to give 360mg of R(-)-N-[2-(4-Isobutylphenyl)propionyl]-methanesulfonamide salt of L(+)-lysine as pale yellow powder. $[\alpha]_D$=-17.3° (c=1.15; CH_3OH); ^{1}H-NMR (D_2O): δ7.30 (dd, 4H, J=8Hz), 3.77 (t, 1H, J=7Hz), 3.65 (q, 1H,

J=7Hz), 3.05 (m, 5H), 2.52 (d, 2H, J=7Hz), 1.92 (m, 2H), 1.75 (m, 2H), 1.50 (m, 3H), 1.40 (d, 3H, J=7Hz), 0.90 (6H, d, J=7Hz).

Table 1

"Effect of DF1681B on serum creatinine in rats receiving syngeneic kidney transplant"

Rat	Group	Plasma Creatinine (mg/dl)	
		16h	**24h**
10 gr 1	Vehicle (4h)	2.27	2.58
11		1.82	1.14
12		2.24	2.49
13		2.30	2.09
		2.16±0.23*	**2.08±0.66***
1A	DF 1681B (4h)	1.09	0.99
2A		0.74	0.64
3A		0.89	0.71
		0.91±0.18°	**0.78±0.19°**
2D	Vehicle (6h)	2.76	2.91
3D		2.39	3.64
4D		3.58	3.27
		2.91±0.61**	**3.27±0.37****
1E	DF 1681B (6h)	2.32	1.89
2E		2.54	1.87
3E		1.84	1.72
		2.23±0.36*Δ	**1.83±0.09#**
	Range Control	0.5-	0.6
	(non ischemic syngeneic kidney)		

Data are expressed as mean±SD

*p<0.05,**p<0.01 vs Control
°p<0.05 vs Vehicle 4h
#p<0.05 vs Vehicle 6h
Δp<0.05 vs DF1681 4h

Table 2

"Effect of DF1681B on the number of granulocytes counted in at least 10 randomly selected high-power microscopic fields (X400) for each animal".

Rat		Granulocytes				
		Intraglom	*Periglom.*	*Intravasc.*	*Perivasc.*	*Inrterstitial*
10gr1	Vehicle (4h)	6.2±6	4±4	0.3±1	10±6	21.6±11
11		4.7±4.4	5.3±3.4	1.7±2.9	9.7±9.9	25.8±11.6
12		18.1±20.2	7.6±8.1	15±14.8	12±7	39.8±29.9
13		33±11.3	74.3±43.9	9.4±7.2	48±29	135.7±31.5
		15.5±13.1	**22.8±34.4**	**6.6±6.9**	**19.9±18.7**	**55.7±53.9**
1A	DF1681B (4h)	7.4±4.7	5.3±4.5	1.5±2.4	3±0.8	9.1±6.4
2A		14±15.4	4	0	8±2.6	11±4.8
3A		7±2.9	7±8	8±5.3	5.3±4.6	16.2±8.3
		9.5±3.9	**5.4±1.5**	**3.2±4.3**	**5.4±2.5***	**12.1±3.7***
2D	Vehicle (6)	10.4±5.8	7.3±4.3	3.3±2.0	5.8±3.3	25.4±22
3D		12.3±9.5	2.9±1.1	1.4±2.1	5.6±2.7	23.6±18
4D		10.5±7.6	2.8±2.1	0	1.3±2.3	23.1±6.0
		11.1±1.1	**4.3±2.6**	**1.6±1.7**	**4.2±2.5**	**24±1.2**
1E	DF1681B (6h)	5.0±6.0	1.0±1.8	0.2±0.4	1.0±1.3	2.5±4.6
2E		3.7±3.0	3.2±1.9	0	6.8±8.9	4.8±5.6
3E		7.7±6.5	2.2±1.2	3.0±4.0	4.5±4.4	1.6±1.2
		5.5±2.0°	**2.1±1.1**	**1.1±1.7**	**4.1±2.9**	**3±1.7°**

Data are expressed as mean±SD.
*p<0.05 vs. Vehicle 4h
°p<0.05 vs. Vehicle 6h

Table 3

"Effect of DF1681B on the number of MHC II positive interstitial cells counted in at least 10 randomly selected high-power microscopic fields (X400) for each animal".

Rat			MHC II +
10gr1	Vehicle (4h)		11.5±7
11			16.6±5
12			7.7±2.8
13			12.8±7.2
			12.2±3.7
1A	DF1681B (4h)		5.1±6.2
2A			9.1±2
3A			11.9±6.3
			8.7±3.4
2D	Vehicle (6h)		6.9±2
3D			17.3±8.9
4D			21.3±3.6
			15.2±7.4
1E	DF1681B (6h)		10.5±4.7
2E			13.1±7
3E			19.7±3.3
			14.4±4.7

Data are expressed as mean±SD.

Table 4

"Semi-quantitative score for renal damage"

Rat		Histological damage		
		Glomerular (score)	*Interstitial* (score)	*Tubular* (score)
10	Vehicle (4h)	0	2	1.3
11		0	2	1
12		-	-	-
13		0	3	0.7
		0	**2.3±0.6**	**1±0.3**
1A	DF1681B (4h)	0	2.5	1.5
2A		0	2	0.7
3A		0	2	0.5
		0	**2±0.3**	**0.7±0.6**
2D	Vehicle (6h)	1	2	1.33
3D		1	2.5	1.33
4D		1	2.5	1.2
		1	**2.3±0.3**	**1.3±0.1**
1E	DF1681B (6h)	0	3	1.7
2E		0	3	1.7
3E		0	3	1.2
		0	**3**	**1.5±0.3**

Data are expressed as mean±SD.

Table 5

"Effect of DF 1681B on serum creatinine in rats receiving allogeneic kidney transplant".

Brown Norway Rat	Group	Plasma Creatinine (mg/dl)	
		16h	24h
1T	Vehicle	1.4	1.74
2T		1.27	1.20
3T		0.82	0.96
4T		2.23	2.28
5T		2.67	2.67
6T		2.23	2.29
7T		1.66	2.37
8T		1.67	1.6
9T		1.86	1.9
Mean±		**1.76**	**1.86**
sd		**0.56**	**0.59**
1Z	DF1681B	1.05	1.3
2Z		1.2	1.39
3Z		1	0.87
4Z		0.84	0.69
5Z		1.01	0.85
Mean±		**1.02**	**1.02**
sd		**0.12**	**0.3**

CLAIMS

1. Use of (R)-ibuprofen methanesulfonamide, or a non-toxic salt thereof, for the preparation of medicaments for the prevention or the treatment of ischemia/reperfusion injury.
2. Use of (R)-ibuprofen methanesulfonamide, or a non-toxic salt thereof, for the preparation of medicaments for the prevention or the treatment of functional injury resulting from rejection reactions of transplanted organs.
3. Use according to claim 1 or 2, wherein the non-toxic salt is the L-lysine or DL-lysine salt.
4. Use according to claim 3, wherein the non-toxic salt is the L-lysine salt.
5. Use according to claim 2, wherein said transplanted organs are transplanted kidneys.
6. Pharmaceutical compositions for the prevention or the treatment of functional injury resulting from rejection reactions of transplanted organs containing (R)-ibuprofen methanesulfonamide, or a non-toxic salt thereof, in admixture with an acceptable carrier.
7. Pharmaceutical compositions for the prevention or the treatment of ischemia/reperfusion injury, containing (R)-ibuprofen methanesulfonamide, or a non-toxic salt thereof, in admixture with an acceptable carrier.
8. Pharmaceutical compositions according to claims 6 or 7, wherein the non-toxic salt is the L-lysine salt.

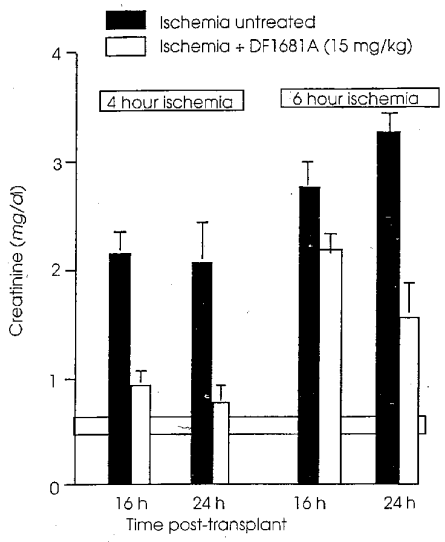

Figure 1. Data are mean ± SD.□range values of serum creatinine in control animals receiving a syngeneic graft not exposed to cold ischemia (control non ischemic).

Figure 1

【国際公開パンフレット（コレクトバージョン）】

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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designation US*
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WO 02/062330 A3

(54) Title: USE OF (R)-IBUPROFEN METHANESULFONAMIDE AND SALTS THEREOF IN THE TREATMENT AND PREVENTION OF REJECTION REACTIONS OF TRANSPLANTED ORGANS

(57) Abstract: The use of (R)-ibuprofen methanesulfonamide is described for the preparation of medicaments for the treatment and prevention of functional injury resulting from rejection reactions of transplanted organs. In particular, the use of non-toxic salts of (R)-ibuprofen methanesulfonamide, such as the (L)-lysine salt, is described for the prevention and the treatment of rejection reactions of transplanted kidneys.

【国際調査報告】

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/00946

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/18 A61P37/06 A61P9/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
MEDLINE, CHEM ABS Data, EPO-Internal, WPI Data, PAJ, EMBASE, SCISEARCH, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 24710 A (ALLEGRETTI MARCELLO ;CESTA MARIA CANDIDA (IT); COLOTTA FRANCESCO () 4 May 2000 (2000-05-04)	1,3,4, 6-8
Y	page 2, line 24 -page 3, line 10 page 5, line 6 - line 32 page 11, line 12 - line 13 example 7	2,5
Y	LI L ET AL: "The relationship between cytokines in MLC supernatants and acute rejection after renal transplantation." TRANSPLANTATION PROCEEDINGS. UNITED STATES NOV 2000, vol. 32, no. 7, November 2000 (2000-11), pages 2531-2534, XP002223824 ISSN: 0041-1345 Conclusion page 2533 -page 2534	2,5

☐ Further documents are listed in the continuation of box C. ☒ Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 December 2002	29/01/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Strack, E

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INTERNATIONAL SEARCH REPORT
Information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0024710 A	04-05-2000	IT 1303249 B1	06-11-2000
		AU 1037500 A	15-05-2000
		BR 9914741 A	03-07-2001
		CN 1324344 T	28-11-2001
		CZ 20011441 A3	17-10-2001
		EE 200100233 A	15-08-2002
		WO 0024710 A1	04-05-2000
		EP 1123276 A1	16-08-2001
		HU 0103793 A2	28-03-2002
		JP 2002528434 T	03-09-2002
		NO 20012000 A	20-06-2001
		PL 347947 A1	22-04-2002
		SK 5382001 A3	03-12-2001
		TR 200101124 T2	22-10-2001

Form PCT/ISA/210 (patent family annex) (July 1992)

フロントページの続き

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