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Review

Defibrotide: Properties and clinical use of an old/new drug ☆

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ABSTRACT

The drug named defibrotide (DFT) has been studied for many years. It has been shown to possess many activities: profibrinolytic, antithrombotic–thrombolytic, antiischemic (heart, liver, kidney, skin, brain), antishock, antiatherosclerotic, antirejection and anti-angiogenic. The previously displayed activities, as antithrombotic, profibrinolytic and anti-inflammatory, suggested its use in vascular disorders, as in the treatment of peripheral obliterative arterial disease and in thrombophlebitis. Some years after, the use of DFT in hepatic veno-occlusive disease has been also proposed. Even if DFT was considered for long time a multi-target drug, now it could be considered on the whole as a drug able to protect endothelium against activation. The present work reviews the more important experimental and clinical studies performed to detect DFT effects.

Contents

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1. Introduction

Once upon a time there was, in the field of pharmacology, a dominant concept: "one drug–one activity". But the accumulating evidences caused a change in the dominant ideas. The concept of "multi-target compounds" is now accepted by the scientific community.

In the present review we report the main experimental and clinical studies that are conducted for the discovery of a drug, named defibrotide (DFT), which can be considered a multi-target compound as well. It has a host of activities; however, if you consider DFT as a drug able to limit endothelial cell activation, each piece of the puzzle will neatly fit into its right place, and the host of activities becomes an array of activities. In fact the polyhedric inflammatory process is the common background of seemingly different illnesses, both acute and chronic. We come full circle: one drug, DFT–one activity, anti-endothelial cell activation activity (Fig. 1).

2. The history of defibrotide

The history of DFT began in 1968 when in Crinos' raw material department a fraction, containing phosphorus, was isolated from animal tissues. This fraction was called "Fraction P", the first name of DFT.

DFT's profibrinolytic activity was the first activity to be unraveled, thanks to the efforts of Mantovani (Pescador et al., 1983) and other authors (Porta et al., 1991; Mussoni et al., 1979; Klocking, 1992).

Niada discovered the second set of DFT's activities that is its antithrombotic and thrombolytic activities (Niada et al., 1981, 1982). Later this subject was explored in more detail by Fumagalli (Fumagalli et al., 1987, 1989), Breddin (Giedrojc and Breddin, 1991), Pescador (Tettamanti et al., 1992), Page (Paul et al., 1993), and Grodzinska (Grodzinska et al., 1987).

In 1982 Niada started a series of seminal experiments of myocardial infarction in a cat (Niada et al., 1985, 1986) which attracted investigators in this field both in Italy and abroad. All of them generated an astonishing amount of work outlining and clarifying the cardioprotective activity of DFT in both "in vitro" and "in vivo" animal models and in different animal species (Berti et al., 1986, 1987a, 1987b, 1988, 1990a, 1992, 1993; Hohlfeld et al., 1991, 1993; Lefer et al., 1990; Lobel and Schror, 1985; Radice et al., 1997; Rossoni et al., 1996, 1999b, 2000 Schror et al., 1989; Shin et al., 1998; Thiemermann et al., 1985, 1989).

DFT anti-ischemic activity was demonstrated in the kidney and in the liver too, by Bianchi (Berti et al., 1991), Rigotti (Ferraresso et al., 1993) and Ferrero (Ferrero et al., 1990) respectively.

Lefer was the first to discover and describe DFT anti-shock activity in different shock models (Aoki et al., 1988; Bitterman et al., 1988; Ma et al., 1991), followed by Schrör and Nowak (Hohlfeld et al., 1992), who were the first to claim DFT activity in multi organ failure (MOF).

The anti-atherosclerotic activity of DFT was mainly described by Pescador (Pescador et al., 1989; Pescador et al., 1995), Schrör (Schror et al., 1989) and Porta (Porta et al., 1994). Kahan (Ferraresso et al., 1993) and Ferrero (Ferrero et al., 1994) discovered and described DFT anti-rejection activity.

While the stream of the main DFT activities (at whole body or organ level), summarized above, was running, lots of activities both at cellular and molecular level were discovered and described as well.

Schrör (Bracht and Schror, 1994; Lobel and Schror, 1985, 1989), and Pescador (Pescador et al., 1995) found an evidence of antiplatelet activity of DFT, while Schrör (Hohlfeld et al., 1993; Schror et al., 1989), Lefer (Scalia et al., 1996a) and Rossoni (Rossoni et al., 1996) described its anti polymorphonucleate leukocyte (PMN) activity. Fumagalli, Pescador and Kahan discovered DFT anti leukocyte activity (Ferraresso et al., 1993; Fumagalli et al., 1987; Fumagalli et al., 1989; Pescador et al., 1995). As far as DFT activities at molecular level are concerned, we have to quote the seminal work of Niada, who first suggested that DFT increases the generation of prostacyclin I 2 (PGI 2) (Niada et al., 1981). This suggestion paved the way for the work of Schrör (Hohlfeld et al., 1992; Lobel and Schror, 1985, 1989; Schror et al., 1989), Berti (Berti et al., 1990b, 1993), Lefer (Bitterman et al., 1988), Rossoni (Rossoni et al., 2006) and Gryglewski (Gryglewski et al., 1989) on the ability of DFT to increase the generation of PGI2. In particular Berti discovered that DFT, besides increasing PGI2 generation, increases prostaglandin E 2 (PGE2) generation as well (Berti et al., 1988) and Schrör discovered that it decreases thromboxane A 2 (TXA2) generation (Bracht and Schror, 1994; Hohlfeld et al., 1992). Later Coccheri (who performed many clinical studies) discovered that DFT decreases the generation of leukotriene B 4 (LTB4) and Berti and Maclouf discovered that DFT decreases that of cysteinyl leukotrienes (CYSLT) (Rossoni et al., 1996). The list of DFT activities at molecular level goes on, showing the following properties of the drug: cyclic adenosine monophosphate (cAMP) increase in platelets, decrease of platelet activating factor (PAF) generation (Berti et al., 1990a), antagonism towards endothelin-1 contractile activity (Berti et al., 1990b), effect on nitric oxide (NO) increase (Berti

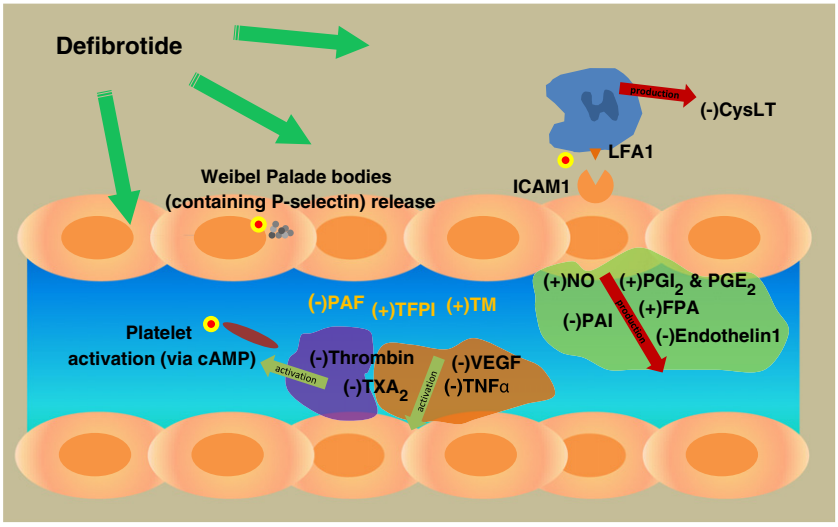

Fig. 1. Activities of defibrotide. Factors involved in coagulation are depicted un yellow; in green area factors released from EC; in orange area factors involved in EC activation; in purple area factors involved in platelet activation. Inhibition; (+) increase or (−) decrease. Green arrow = activation; red arrow = production.

et al., 1993; Masini et al., 1995), and anti tumor necrosis factor alpha (TNFα) activity (Schroder, 1995). Now let us look at some proteins in the blood and vascular wall and DFT effects on them. DFT increases tissue plasminogen activator (t-PA) and decreases plasminogen activator inhibitor (PAI) (Klocking, 1992). Fareed's work's main finding was to give evidence that DFT increases tissue factor pathway inhibitor (TFPI) (Cella et al., 2001). We have to quote the work of Ruan on the increase of thrombomodulin (TM) (Zhou et al., 1994). Ferrero and Lefer described DFT interference on leukocyte–endothelial cell interaction mediated by some adhesion molecules, as intercellular adhesion molecule/lymphocyte function associated antigen (ICAM-1/LFA-1) (Pellegatta et al., 1996) and P-selectin expression (Scalia et al., 1996a). DFT inhibits cytosolic calcium increase (Bracht and Schror, 1994). At last a look at some DFT molecular structures involved in some of its activities was achieved; DFT, through aptamers (GGTTGG ATT GGTTGG AND GGTTGG ATC GGTTGG) has been shown able to inhibit thrombin (Bracht and Schror, 1994), as well as cathepsin G (CATG) (Evangelista et al., 1992a), through a recurrence of alternating TG repeats (Gatto et al., 2008).

3. Defibrotide chemistry

Defibrotide is a mixture of 90% single-stranded phosphodiester oligonucleotides (length, 9-80mer; average, 50mer; average molecular mass, 16.5 ± 2.5 KDa) and 10% double stranded phosphodiester oligonucleotides derived from the controlled depolymerization of porcine intestinal mucosal DNA (Corbacioglu et al., 2012; Espinosa et al., 2011; Francischetti et al., 2012). That is, generally speaking, a heparin-like situation. Inside this mixture of oligonucleotides, aptamers (GGTTGGATTGGTTGG and GGTTGGATCGGTTGG) able to inhibit thrombin were discovered (Bracht and Schror, 1994). Later in a study carried out to discover inside defibrotide cathepsin G inhibiting aptamers the following consensus sequences were found: **GG**N_{1-7}**GG**N_{8-14}**GG**N_{1-6}**GG**N_{1-7}**GG**N_{1-6}**GG,GG**N_{10-13}**GG**N_{1-5}**GG**N_{1-5}**GG**N_{3-6} **GG**N_{2-7}**GG** and the sequence **GG**GTTGA**GG**GT**GG**ATTACGCCACGT-**GG**A GCTC**GG**ATCCACACATCCA**GG,** wherein N represents nucleotides and the figures represent the number of possible nucleotides at that site. More recently a second study, aimed at finding effective inhibitors of Cathepsin G, showed a recurrence of alternating TG repeats in the selected Cat G binders, adopting an extended conformation that grants maximal interaction with the highly charged protein surface (Gatto et al., 2008). Synthetic TG oligonucleotides of different length were tested to assess the dependence of Cat G binding on oligonucleotide length. $(dT\text{-}dG)_{60}$ and $(dT\text{-}dG)_{50}$ appeared to be more potent binders than $(dT\text{-}dG)_{40}$. Cat G recognition is poorer with 20–30 mer oligonucleotides (Gatto et al., 2008). All of the above is, generally speaking a heparin-like situation. Heparin is a mixture of chains of different length in which there are binding sites for the thrombin–antithrombin complex.

4. Pharmacokinetics

A study, carried out, by single oral or intravenous administration of [125I]-DFT, to man gave results, for radioactivity associated with DFT or related components, very close to those, previously obtained, in rats, by an administration of [125I]-DFT and [32P]-DFT, respectively, that is: half life of the distribution phase (T1/2α) in the range of minutes, half life of the elimination phase (T1/2β) in the range of hours, bioavailability in the range of 50–70% (Fisher et al., 1993, 1996).

5. The consistent picture of defibrotide activities

What do all of the above activities have to do with a single drug? In 1998 Hunt and Jurd (Hunt and Jurd, 1998) wrote: (Endothelial) "Activation entails a stereotyped series of processes although their effects are diverse and are seen differently by specialists in different disciplines. Immunologists study up-regulation of surface antigens and adhesion molecules while those in thrombosis research assess pro-thrombotic endothelial cell changes, and vascular biologists study changes in tone. All these effects however are components of endothelial cell activation and mutually interact in causing local inflammation". Endothelial cell activation seems to be a common pathogenic mechanism because it is induced by a wide range of agents such as certain bacteria and viruses, interleukin 1 (IL-1) and TNF, physical and oxidative stress, oxidized low density lipoproteins, anti-endothelial cell antibodies (Hunt and Jurd, 1998) (found in systemic autoimmune diseases such as vasculitis, systemic lupus erythematosus (Tsokos, 2011) and antiphospholipid syndrome (Hunt and Jurd, 1998)), ischemia–reperfusion (Tsao et al., 1990), hemorrhagic shock (Barroso-Aranda et al., 1995), traumatic shock (Scalia et al., 1996b), malignancy and complications of pregnancy (Periti, 2000). Endothelial cell activation is a graded rather than an all-or-nothing response; for example, changes in endothelial cell integrity range from simple increases in local permeability to major endothelial cell contraction exposing large areas of sub-endothelium (Hunt and Jurd, 1998). Activation may occur locally (Hunt and Jurd, 1998) or systemically (Hunt and Jurd, 1998; Kleinschmidt et al., 1998; Shayevitz et al., 1995) and this last systemic widespread inflammatory host response to variable insults is the patho-physiological basis of MOD and/or multi organ failure (MOF) (van Griensven et al., 2006). MOD/MOF has now been documented as a complication in a variety of infectious or non-infectious conditions such as acute pancreatitis, major surgery, hemorrhage, severe trauma, ischemia, burns and hypovolemic shock (Fortin et al., 2010; Parrillo, 1993). Mortality almost uniformly approaches 90%–100% when three or more organ systems fail. In a large-scale multicenter study 99 patients had three or more failing organ systems after 72 h of intensive therapy, of whom only two survived (Knaus et al., 1985). In the USA, the condition develops during 15% of all intensive care unit (ICU) admissions; it is responsible for up to 80% of all ICU deaths and results in ICU costs of more than $ 100,000 per patient or about $ 500,000 per survivor. Endothelial cell activation is also a key event in an unnatural situation as xeno-transplantation (Bach et al., 1995). It has been shown "in vitro" that human xenoreactive natural antibodies and complement as well as other mediators activate porcine endothelial cells with resultant coagulation and platelet aggregation, which are manifestations of thrombosis. Endothelial cell activation is also seen with rejection "in vivo" accompanied by inflammation and thrombosis, the very complications associated with xenograft rejection (Bach et al., 1995) (Table 1).

Table 1
The most important activities of defibrotide are reported.

DEFIBROTIDE ACTIVITIES	
Inflammation and vascular permeability	• Reduces cysteinyl leukotriene release • Reduces NFkB activation (bone marrow) displaying antiinflammatory function. • Reduces platelet activation
Leukocyte adhesion molecules	• Downregulates P-selectin expression. • Interferes with ICAM1/LFA1 system
Antithrombotic and thrombolytic effects	• Increases tPA, TFPI, TM • Reduces: PAI, PAF, thrombin
Mediator/cytokine production	• Increases PGE2 and PGI2 • Reduces IL-6 • Reduces VEGF • Reduces TXA2 • Reduces LTB4 • Reduces TNF
Regulation of HLA molecules	• Downregulates MHCI molecule expression • Reduces MHCII expression
Vascular tone	• Antagonizes endothelin I • Increases NO and NOS

According to Hunt and Jurd (1998), the changes of endothelial cell activation are:

- loss of vascular integrity;
- expression of leukocyte adhesion molecules;
- change in phenotype from anti-thrombotic to pro-thrombotic;
- cytokine production;
- up-regulation of human leukocyte antigen (HLA) molecules;
- changes in vascular tone.

Loss of vascular integrity can expose sub-endothelium and cause the efflux of fluids from the intravascular space (Hunt and Jurd, 1998).

Up-regulation of leukocyte adhesion molecules such as E-selectin, ICAM-1, and vascular cell adhesion molecule-1 (VCAM-1) allows leukocytes to adhere to endothelium and then move into the tissues (Hunt and Jurd, 1998), through a concerted action of other mediators such as TXA2, LTB4 (a potent chemotactic agent that initiates, coordinates, sustains and amplifies the inflammatory response (Devchand et al., 1996)), CYSLT (Rossoni et al., 1996), PAF and activated complement products and histamine (Kubes and Ward, 2000).

The pro-thrombotic effects of endothelial cell activation include loss of the surface anticoagulant molecules TM and heparan sulphate, and down-regulation of the activation of protein C pathway. In this regard, new-born infants totally deficient in protein C suffer from extensive thrombosis with inflammation in the microvasculature (Griffin, 1995), reduced fibrinolytic potential due to enhanced PAI type 1 release, loss of the platelet anti-aggregating effects of ecto-adenosine diphosphate phosphatases (ecto-ADPases) and PGI2, production of PAF and expression of tissue factor (TF) (Cicala and Cirino, 1998; Hunt and Jurd, 1998).

Cytokine synthesis occurred, including TNF, IL-1 and interleukin-6 (IL-6), as well as synthesis of chemokines, such as interleukin-8 (IL-8), monocyte chemoattractant protein 1 (MCP-1) (Hunt and Jurd, 1998; Ross, 1999) and platelet derived growth factor-BB (PDGF-BB) (Ross, 1993).

Expression of class II HLA molecules allows endothelial cells to act as antigen presenting cells (Hunt and Jurd, 1998; Ross, 1993, 1999). High levels of MHC class II cell surface receptor (HLA-DR) are present on the endothelium of the vessels from transplanted hearts (Ross, 1999). Inflammation is a defensive host response to numerous different forms of insult, and ideally it should last for a short time and be a very localized response as it operates through deadly cells and substances (Smith, 1994; Weiss, 1989). When this defensive host response lasts for decades and becomes excessive, the intended protection becomes a disease entity in itself, as happens in chronic inflammatory diseases such as cirrhosis, rheumatoid arthritis (McInnes and Schett, 2011; Sattar et al., 2003; Sattar and McInnes, 2005), glomerulosclerosis, pulmonary fibrosis, chronic pancreatitis (Ross, 1999) and, particularly, in atherosclerosis (Devchand et al., 1996; Pasceri and Yeh, 1999; Ross, 1993, 1999); in this last instance some talk about endothelial dysfunction (Busse and Fleming, 1996; Celermajer, 1997; Valdivielso et al., 2008), some of endothelial activation (Carluccio et al., 1999), and some of both of them (Chiba et al., 2001). So seemingly different disorders, for instance cardiovascular disease, rheumatoid arthritis, psoriasis and pre-eclampsia share a common background: inflammation (Barnes and Karin, 1997; Devchand et al., 1996; Pasceri and Yeh, 1999; Redman and Sargent, 2005; Ross, 1999).

6. Pharmacological properties of DFT: experimental studies

6.1. Loss of vascular integrity

DFT is active in local Shwartzman reaction (Niada et al., 1982), a model that involves close interaction between the inflammatory and hemostatic pathways (Subramaniam et al., 1996) and in carrageenin-induced pleurisy. Short oligodeoxynucleotides containing nuclear factor kB (NFkB) binding sites are effective in models of acute and chronic inflammation (Makarov, 2000), which is related to increase in vascular permeability. DFT abrogates NFkB activation in multiple myeloma and bone marrow stromal cell (Mitsiades et al., 2009). DFT decreases the generation of CYSLT (Rossoni et al., 1996) which may be involved as causative agents of pathologic vascular permeability (Cook and Halushka, 1989; Pfeifer et al., 1991; Rossoni et al., 1996). DFT deactivates platelets (Francischetti et al., 2012; Scalia et al., 1996a) which contribute to increased vascular permeability (Cicala and Cirino, 1998). DFT is active through aptamers, against thrombin (Scalia et al., 1996a) which enhances vascular permeability (Goldsack et al., 1998). DFT decreases the generation of TNF, which induces vascular permeability (Ferrero et al., 2001).

6.2. Expression of leukocyte adhesion molecules

DFT decreases leukocyte extravasation (Hohlfeld et al., 1993; Shin et al., 1998). DFT down-regulates P-selectin expression (Scalia et al., 1996a) (platelets, like leukocytes, roll on activated endothelium, and this rolling is mediated by P-selectin expressed on activated endothelium (Subramaniam et al., 1996)) and interferes with ICAM-1/LFA-1 adhesion system (Pellegatta et al., 1996). DFT decreases the generation of LTB4, one of the most powerful chemoattractants known (Devchand et al., 1996), provoked by histamine. P-selectin is induced in minutes in response to histamine (Kubes and Ward, 2000), CYSLT (Rossoni et al., 1996) (which enhance PMN adhesiveness (Damtew and Spagnuolo, 1997)), TXA2 (Hohlfeld et al., 1992; Scalia et al., 1996a) and PAF (Berti et al., 1990a), which directly primes superoxide generation and elastase release (Smith, 1994) from PMNs. DFT inhibits, probably through aptamers and/or a recurrence of alternating TG repeats (Gatto et al., 2008), CATG (Evangelista et al., 1992b), the main biochemical mediator of PMN–platelet cooperation (Evangelista et al., 1992b). DFT has, through aptamers, antithrombin activity (Bracht and Schror, 1994). In response to thrombin, P-selectin is rapidly redistributed from the membranes of Weibel–Palade bodies to the endothelial surface (McEver, 2001). DFT increases the generation of PGE2 (Berti et al., 1988) and PGI2 (Berti et al., 1988, 1990a; Bitterman et al., 1988; Gryglewski et al., 1989; Hohlfeld et al., 1992; Lobel and Schror, 1985), which suppress neutrophil activation (Smith, 1994), and favors the generation of NO (Masini et al., 1995). Accordingly, the NO synthase inhibitor L-arginine reduces endothelial expression of ICAM-1 and VCAM-1 (Adams et al., 1997).

DFT suppresses the increased gene expression of ICAM-1 in human microvascular endothelial cells (Mitsiades et al., 2009). DFT antagonizes, as well, the effect of metabolite of fludarabine (F-Ara), a strongly immunosuppressive purine analog with considerable anti-neoplastic and immunosuppressive activity. It also prevents the activation of macrovascular and microvascular endothelia caused by soluble factors released to blood by autologous hematopoietic stem cell transplantation (HSCT) (Eissner et al., 2002; Palomo et al., 2011).

TNF can promote the expression of adhesion molecules in endothelial cells which mediate the adherence of leukocytes to the endothelium also in the instance of reperfusion injury in the heart (Kupatt et al., 1999). DFT decreases the generation of TNF. Interaction of TNF with neutrophils triggers an increased expression of surface adherence glycoproteins, increase in of superoxide production, phagocytosis, enzyme release and aggregation of these cells. DFT increases the generation of protein C as well as inhibits neutrophil activation (Murakami et al., 1996). DFT inhibits $O2^-$ and β-glucuronidase release from human stimulated neutrophils (Schror et al., 1989). Since the activation of A2 receptors on neutrophils reduces their adherence, inhibiting the release of reactive oxygen species, DFT acts as an adenosine A2 receptor agonist (Bianchi et al., 1993). The above mechanism is confirmed by DFT inhibition of toll-like receptor ligand-dependent dendritic cell activation, by a mechanism that is blocked by the adenosine receptor antagonist 8-p-sulfo-phenyltheophylline, but not reproduced by synthetic poly-A,-C,-T and G. These results imply that aptameric sequences and

adenosine receptor are involved (Francischetti et al., 2012) in cell response to the drug.

Thrombin is formed following tissue injury. It promotes, besides the conversion of fibrinogen to fibrin, many cellular effects, including chemotaxis (monocytes and neutrophils), adhesion molecule expression, release of cytokines (Goldsack et al., 1998) and platelet aggregation (Goldsack et al., 1998). DFT has anti-thrombin activity (Bracht and Schror, 1994). DFT is active in leukocyte-dependent reperfusion injury models (Rossoni et al., 1996; Shin et al., 1998) in which endothelial activation is a strategic event during post-ischemic myocardial inflammation (Kupatt et al., 2000).

6.3. Change in phenotype from anti-thrombotic to pro-thrombotic

Venous thrombosis is associated with inflammation (Blann et al., 2000; Butenas et al., 2009; Downing et al., 1997; Henke et al., 2000; Wakefield et al., 1995, 2000) and new-born infants totally deficient in protein C suffer from extensive thrombosis with inflammation (Griffin, 1995). DFT has anti-thrombotic–thrombolytic activity in several different experimental models (Fumagalli et al., 1987; Fumagalli et al., 1989; Giedrojc and Breddin, 1991; Grodzinska et al., 1987; Niada et al., 1981, 1982; Paul et al., 1993; Tettamanti et al., 1992). DFT increases TM (Zhou et al., 1994), protein C plasmin activity, t-PA (Echart et al., 2012; Klocking, 1992), TFPI (Cella et al., 2001), which is also endowed with anti-inflammatory properties effects (Bajaj et al., 2001), PGI2 (Berti et al., 1988, 1990a; Hohlfeld et al., 1992; Lobel and Schror, 1985; Rossoni et al., 2006), PGI2 receptors on the surface of platelets and their affinity for PGI2 (Lobel and Schror, 1989), production of NO (Masini et al., 1995) and nitric oxide synthase (NOS) activity. DFT decreases PAF (Berti et al., 1990a), PAI (Klocking, 1992), TF (Falanga et al., 2003; Francischetti et al., 2012), which represents the link between inflammation and thrombosis (Penn and Topol, 2001) and TXA2 (Bracht and Schror, 1994; Hohlfeld et al., 1992). Coagulation is activated after the administration of TNF (Penn and Topol, 2001). DFT decreases the generation of TNF which causes the release of pro-coagulant TF from endothelial cells (Szotowski et al., 2005) as well as of thrombin (Scalia et al., 1996a) which induces endothelial TF expression (Viswambharan et al., 2004). DFT deactivates platelets (Bracht and Schror, 1994), monocyte/macrophages (Cirillo et al., 1991; Tettamanti et al., 1992) and PMNs (Hohlfeld et al., 1991; Scalia et al., 1996a; Schror et al., 1989; Shin et al., 1998). Leukocytosis and thrombocytosis are decreased by DFT (Pescador et al., 1995). DFT decreases leukocyte and platelet aggregates in thrombi (Fumagalli et al., 1987; Fumagalli et al., 1989). DFT attenuates complement activation (Francischetti et al., 2012) which is responsible to contribute to thrombosis (Kourtzelis et al., 2010). The ability of DFT to exert protective effects in different models of tissue and organ ischemia suggested also that it functions as a possible anti-ischemic drug (Coccheri and Nazzari, 1996), as confirmed by its anti-rejection activity.

6.4. Mediator/cytokine production

TNF is decreased by DFT. Endothelial cells are protected from TNFα noxius effects by DFT (Schroder, 1995). DFT counteracts the mitogenic activity of PDGF-BB. DFT increases the generation of PGE2 and PGI2 (Berti et al., 1988, 1990a; Gryglewski et al., 1989; Hohlfeld et al., 1992; Lobel and Schror, 1985) which inhibit IL-1 and interleukin-2 (IL-2) production (Ferraresso et al., 1993) and decreases the generation of TXA2 (Bracht and Schror, 1994; Hohlfeld et al., 1992) and LTB4 which enhance T cell proliferation (Ferraresso et al., 1993). LTB4 stimulates IL-1 and IL-2 production (Ferraresso et al., 1993). DFT normalizes elevated levels of IL-2. DFT suppresses the increase in secretion of IL-6 or vascular endothelial growth factor (VEGF) (Mitsiades et al., 2009). The vertebrate DNA is able to inhibit cytokine release from plasmid-triggered spleen cells (Wloch et al., 1998). Indeed, it has immune inhibitor activity (Hacker, 2000; Krieg et al., 1998). In fact DFT inhibits dendritic cell activation (Francischetti et al., 2012).

6.5. Regulation of human leucocyte antigen (HLA) molecules

DFT increases PGE2 and PGI2 generation (Berti et al., 1988, 1990a; Gryglewski et al., 1989; Hohlfeld et al., 1992; Lobel and Schror, 1985). PGE2 and PGI2 are known to decrease major histocompatibility complex (MHC) class II antigen expression (Ferraresso et al., 1993).

F-Ara, up-regulates MHC class I molecules. DFT down-regulates MHC class I molecule expression (Eissner et al., 2002). DFT either does not affect (Thiemermann et al., 1985) or actually reduces the synthesis of pro-rejection arachidonic acid metabolites thromboxane B2 (TXB2) and TXA2, respectively (Bracht and Schror, 1994; Hohlfeld et al., 1992). The above situation should promote graft acceptance (Ferraresso et al., 1993). Actually DFT prolonged the survival of transplant "in vivo" (Corsi et al., 1993; Ferraresso et al., 1993; Ferrero et al., 1994).

6.6. Changes in vascular tone

DFT antagonizes the vasoconstrictor activity of endothelin-1 (Rossoni et al., 1999a, 2000). Endothelin-1 is increased in inflammatory diseases as atherosclerosis and rheumatoid arthritis (Pasceri and Yeh, 1999). Atherosclerosis worsens relaxation (endothelium- and NO-dependent) to acetylcholine (Freiman et al., 1986; Hathaway et al., 2002). DFT counteracts atherosclerosis and noxious effects on vessel relaxation (Rossoni et al., 1999a, 2000). DFT increases the generation of NO (Masini et al., 1995) and NOS activity. Endothelial cell activation is inhibited by NO donors (Zampolli et al., 2000). The reduction in reactive oxygen species formation by DFT (Cirillo et al., 1991) would act to enhance the bioavailability of NO and thus potentiate the effectiveness of endothelium-generated NO (Lefer et al., 2001). DFT remarkably increases PGI2 production and reduces TXB2 levels (Rossoni et al., 2006).

7. Pharmacological properties of DFT: clinical studies

After the above detailed overview of DFT activities and action mechanism, now let us address a more practical issue: is DFT useful to humans?

7.1. Anti ischemic and antithrombotic activities

Some clinical results, especially in ischemic and microthrombotic conditions, have been previously revealed positive or promising (Coccheri and Biagi, 1991). In the past DFT has been used in the therapy of vascular disorders (Palmer and Goa, 1993). However, more recently also, in the PROVEDIS study, treatment with DFT in addition to elastic compression in patients with objectively assessed chronic deep vein insufficiency, mostly due to post-thrombotic syndrome, resulted in clinical benefits and prevented thrombotic complications harmful to the limb conditions (Coccheri et al., 2004). Moreover, DFT activity in peripheral arterial disease fits this scenario as well. In the DICLIS study (a double blind placebo controlled study), long-term administration of oral DFT improved the walking distance in patients with intermittent claudication (Violi et al., 2000).

7.2. Treatment of VOD

Now, DFT is proposed for treatment of patients with MOD/MOF or severe VOD.

Indeed, as reported above, endothelial cell activation may occur locally (Hunt and Jurd, 1998) or systemically (Hunt and Jurd, 1998; Kleinschmidt et al., 1998; Shayevitz et al., 1995) (this last condition is responsible for an estimated 100,000 deaths a year in the USA

(Evans and Smithies, 1999)) and this last systemic widespread inflammatory host response is the patho-physiological basis of MOD. Hence one could hypothesize that DFT is active in MOD/MOF and, actually, that was the case: in animals, 88% of pigs with MOF, treated with DFT, survived the otherwise lethal situation (Hohlfeld et al., 1992). In humans, the studies were performed in patients with venous occlusive disease (VOD). VOD is a life-threatening complication of high dose chemo/radiotherapy and human stem cell transplantation (HSCT) characterized by injury and inflammation to sinusoidal endothelial cells of the liver. It is also known as sinusoidal obstruction syndrome (SOS) (DeLeve et al., 2002; Shulman et al., 1994). The occurrence of clinical VOD presents disparity in incidence, depending on the type of transplant, conditioning therapies, patient-related risk factors and the criteria for diagnosis. Complications after bone marrow transplantation (BMT) are manifestations of systemic inflammatory response syndrome (Evans and Smithies, 1999; Takatsuka et al., 2000), that is endothelial cell activation and inflammation were evidenced in MOF (Richardson et al., 2002); 35% of DFT treated patients displayed complete resolution and survival past day +100 (Richardson et al., 2002). The above results were confirmed by the European compassionate-use study: 22 out of 40 evaluable patients with VOD achieved a complete response to DFT and resolution of signs/symptoms of VOD and end-organ dysfunction (EOD) (Chopra et al., 2000). In the subgroup of patients with either evidence of MOF or who met risk criteria predicting fatality, 10 out of 28 patients showed a complete response (Chopra et al., 2000). In this study 43% of patients were alive beyond day +100 (Chopra et al., 2000). Four of 8 patients with VOD, treated with DFT, were alive past 3 months and one woman with VOD, treated with DFT, was in excellent clinical condition at 6 months after the onset of liver VOD.

In a randomized phase II dose finding study, in severe (VOD + MOF) VOD patients, following HSCT, DFT was given at either 25 mg/kg day (n = 75) or 40 mg/kg/day (n = 74) by intravenous route. Survival rates at day +100, post HSCT, were 46% and 42% respectively with no significant difference between treatment arms (Richardson et al., 2010). Four patients with SOS and evidence of MOF were treated with iv. DFT and 75% of patients responded to therapy (Yakushijin et al., 2005).

Following HSCT, 71 children did not receive any specific VOD prophylaxis or therapy (controls). Other 91 children were given anti-thrombin III (AT III) replacement, in the case of decreased AT III activity (first group). If VOD was diagnosed clinically, high dose DFT and AT III therapy were combined (second group). The severity of VOD was determined according to the degree of MOD. The incidence of VOD was similar in both groups (13/71, 18% vs. 14/91, 15%). All 14 patients with VOD, receiving combined therapy, achieved complete remission and 93% (13/14) survived until day +100, compared to six survivors (46%) of first group (Haussmann et al., 2006).

DFT was active even when given by oral route to a patient with late-onset VOD, post autologous peripheral stem cell transplantation, with complete resolution of VOD by day +75 (Shah et al., 2009).

After the diagnosis of SOS, 14 patients, following HSCT, received DFT. The overall response rate was 78.56%, while it was 50% in severe SOS cases (Sucak et al., 2007).

Similarly, 14 children with VOD, following HSCT, received DFT at a mean dose of 33 mg/kg for a mean period of 16 days. The survival rate to day +100 was 79% (Bulley et al., 2007).

Of 180 pediatric patients, the 12%, with one or more risk factors for VOD, randomly allocated to the prophylactic DFT treatment, had VOD by 30 days after HSCT, compared with 20% of 176 controls (Corbacioglu et al., 2012).

Besides VOD, another vascular endothelial complication following stem cell transplantation is thrombotic microangiopathy (TMA) (Stavrou and Lazarus, 2010) which includes: hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Murrin and Murray, 2006). Mortality rate in TMA, associated with transplantation, ranges between 60% and 90% (Stavrou and Lazarus, 2010).

Five of 6 patients treated with DFT shoved a complete response and resolution of signs/symptoms of VOD and end-organ dysfunction and they were alive at day +100. Of 10 patient displaying episodes of HUS/TTP, treated with DFT, 6 showed complete resolution of HUS/TTP.

Twelve patients underwent BMT. TTP was mild in two patients and severe in 10 with central nervous system (CNS) dysfunction, renal impairment, gut or bladder bleeding and MOF. DFT was beneficial in about 50% of the patients who showed a good response to drug (Corti et al., 2002).

In some patients affected by acute renal failure, coagulation abnormalities (low platelet count, high levels of fibrin degradation products) and neurological manifestations, DFT administration caused disappearance of neurological manifestations and normalization of coagulation parameters. All patients are alive at 7–22 months of follow-up (Bonomini et al., 1985).

In anti-phospholipid antibody syndrome (APS) endothelial cells are perturbed (Rand, 2002) and one mechanism that proposed to account for this unusual pro-thrombotic state is the activation of endothelial cells by anti-phospholipid antibodies (Williams et al., 2000) as well as injury to endothelium and platelet activation (Rand, 2002): activation of endothelial cells enhance the expression of ICAM-1, VCAM-1 and P-selectin (Pierangeli et al., 2001). DFT was active in 2 out of 2 patients with APS, normalizing increased levels of endothelin-1, IL-2, TNF and PAI. Hypertension, impaired platelet aggregation and abnormal bleeding time were normalized by DFT as well.

More recently DFT prophylaxis has been shown to reduce incidence of VOD in pediatric HSCT (Corbacioglu et al., 2012).

In conclusion, DFT may be effective in the prophylaxis and treatment of VOV (Guglielmelli et al., 2012).

7.3. Effects of DFT on tumor angiogenesis

Twenty four patients with relapsed/refractory multiple myeloma were enrolled and given melphalan, prednisone and thalidomide. DFT was administered orally. In all patients, the complete response plus very good partial response rate was 9% and the partial response rate was 43%. The 1 year progression-free survival and 1-year overall survival rates were 34% and 90% respectively (Palumbo et al., 2010). These data were confirmed by an “in vitro” study in which DFT was able to protect endothelial cells by thalidomide-mediated cell death without interfering with its anti-tumor effect (Echart et al., 2012).

In another set of experiments DFT “in vitro” reduced vessel formation. Similarly DFT blocked sprouting from cultured rat aortic rings. Angiogenesis was also blocked “in vivo” in a model of nude mice (Koehl et al., 2007). It was proposed that DFT directly interferes with migration and tube formation of endothelial cells which is dependent on mTOR-p70S6k activity (Koehl et al., 2007).

VOD occurred in 5 of 35 patients with Wilms tumor, treated with chemotherapy. Two patients developed MOF. Three patients were treated with DFT, while two patients received supportive measures only. Four patients recovered, three of them had received DFT. They are all alive and well after 35 months (Cesaro et al., 2011). One of two patients not treated with DFT died of MOF (Cesaro et al., 2011).

The obtained results suggest that DFT may belong to the new generation of anti-cancer drugs that can prevent tumor angiogenesis. In multiple myeloma, DFT may overcome the pro-thrombotic effect of thalidomide on endothelial cells (Guglielmelli et al., 2012).

8. Discussion and concluding remarks

Taken globally the seemingly different DFT activities [pro-fibrinolytic, anti-thrombotic-thrombolytic, anti-ischemic (heart, liver, kidney), anti-shock and anti-atherosclerotic] fit a more global anti-endothelial cell activation activity.

Taking into account that DFT contains aptamers (Bracht and Schror, 1994) DFT could represent a natural library of oligonucleotides but with some boundaries. The boundaries are that, being DFT derived from nuclear DNA, it must not have any stimulatory activity on the immune system (Hacker, 2000; Krieg et al., 1998; Wloch et al., 1998).

The boundaries are derived from the constraints imposed by evolutionary pressures which had to assure both a working genetic information and lack of immune stimulatory activity in the instance of nuclear DNA breakdown when a body cell dies. In this contest vertebrate DNA is not an immuno-stimulator, in fact it is able to inhibit cytokine release from plasmid-triggered spleen cells (Wloch et al., 1998). Hence to expect that DFT could contain aptamers good for any pharmacological task, could be out of any evolutionary standpoint. Actually we do have to expect that DFT has activities addressed to dampen and eliminate any inflammatory activity.

From the data, so far collected in animals and humans, it appears that DFT is useful in those diseases, the underlying pathology of which is systemic endothelial activation and inflammation.

The concept of multi-target compound has emerged in the field of inflammation (Celotti and Laufer, 2001). For many years the beneficial effects of the statins were attributed to their cholesterol lowering effect. But statins were found to exert other activities. They are antioxidant, anti-thrombotic and vasculo-protective, for instance (Lefer et al., 2001). Last but not least DFT directly interferes with migration and tube formation of endothelial cells which is dependent on mTOR-p70S6k activity (Koehl et al., 2007). mTOR is involved in nutrient signaling pathways that regulate longevity in various organisms and mammals (Fontana et al., 2010). Rapamycin inhibition of mTOR pathway increases mouse life span (Fontana et al., 2010). Rapamycin is anti-angiogenetic as well (Guba et al., 2002).

DFT prophylaxis has been shown to reduce the incidence of VOD in pediatric HSCT (Corbacioglu et al., 2012). Furthermore, DFT has been demonstrated to exhibit therapeutic potential to treat severe malaria (Francischetti et al., 2012). Finally, further preclinical and clinical investigations are needed to assess the role of DFT in the treatment of patients with multiple myeloma (Guglielmelli et al., 2012).

Interestingly, drug safety evaluation of DFT has been recently performed, displaying a safety profile of such product (Richardson et al., 2013).

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