

LES NOUVEAUX PRODUITS À ACTIVITÉ HORMONALE SÉLECTIVE SELECTIVE ESTROGEN RECEPTOR MODULATORS AND SELECTIVE ACTION STEROIDS

EM-652 (SCH57068), a pure SERM in the mammary gland and endometrium

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ABSTRACT: In order to minimize the risks of endometrial cancer and the development of resistance to antiestrogen therapy, we have synthesized the orally active antiestrogen EM-652 which is the most potent of the known antiestrogens and exerts pure antiestrogenic activity in the mammary gland and endometrium. EM-652 inhibits the AF1 and AF2 functions of both ER α and ER β while the inhibitory action of OH-TAM is limited to AF2. EM-652 thus inhibits Ras-induced transcriptional activity and blocks SRC-1-stimulated activity of the two receptors. The absence of blockade of AF1 by OH-TAM could explain why resistance develops to tamoxifen

treatment. Not only the development, but also the growth of established DMBA-induced mammary carcinoma is inhibited by treatment with EM-800, the prodrug of EM-652. This compound is the most potent antiestrogen to inhibit the growth of human breast cancer ZR-75-1, MCF-7 and T-47D cells in vitro. When incubated with human Ishikawa endometrial carcinoma cells, EM-800 has no stimulatory effect on the estrogen-sensitive parameter alkaline phosphatase activity. When administered to ovariectomized animals, EM-800 prevents bone loss, and lowers serum cholesterol and triglyceride levels. EM-800 has shown benefits in women with breast

cancer who had failed tamoxifen. The above-summarized preclinical and clinical data clearly suggest the interest of studying EM-652 (SCH57068) in the neoadjuvant and adjuvant settings and, most importantly, for the prevention and treatment of breast and uterine cancer.

Key words: Antiestrogen – EM-652 – SCH57068 – Breast cancer – Uterine cancer – Chemoprevention – Osteoporosis – Estrogen receptor

L'EM-652 (SCH57068), un SERM principalement actif sur la glande mammaire et l'endomètre

RÉSUMÉ : Pour réduire au minimum les risques de cancer de l'endomètre et le développement de résistance au traitement par les anti-estrogènes, nous avons synthétisé un anti-estrogène oralement actif, l'EM-652, qui est le plus puissant des anti-estrogènes connus et exerce une activité anti-estrogénique pure dans le sein et l'endomètre. L'EM-652 inhibe les activités AF1 et AF2, des RE α et RE β alors que l'action inhibitrice du 4OH-TAM est limitée à AF2. L'EM-652 inhibe aussi l'activité transcriptionnelle induite par Ras et bloque l'activité stimulée par SRC-1 des deux récepteurs. L'absence de blocage d'AF1 par le 4OH-TAM pourrait expliquer pourquoi une résistance se développe sous traitement

par le tamoxifène. Non seulement le développement, mais aussi la croissance du carcinome mammaire induit par le DMBA, sont inhibés par le traitement avec l'EM-800, la prodrogue d'EM-652. Ce composé est l'anti-estrogène le plus puissant pour inhiber la prolifération des cellules mammaires cancéreuses humaines ZR-75-1, MCF-7 et T-47D *in vitro*. Quand il est incubé avec des cellules de carcinome endométrial humain Ishikawa, l'EM-800 n'a aucun effet activateur sur l'activité de phosphatase alcaline induite par les estrogènes. Quand il est administré à des animaux ovariectomisés, l'EM-800 prévient la perte osseuse et abaisse les taux sériques de cholestérol et de triglycérides. L'EM-800 a montré

des effets bénéfiques chez des femmes avec un cancer du sein résistant au tamoxifène. Les données précliniques et cliniques résumées ci-dessus suggèrent clairement l'intérêt d'étudier EM-652 (SCH57068) dans les situations néoadjuvantes et adjuvantes et, le plus important, pour la prévention et le traitement des cancers du sein et de l'utérus.

Mots clés : Anti-estrogène – EM-652 – SCH57068 – Cancer du sein – Cancer de l'utérus – Chimio-prévention – Ostéoporose – Récepteur des estrogènes

Introduction

Knowing that absolute tissue- or even gene-specific action of antiestrogens is now possible, the objective of pharmaceutical research is to design compounds which will act in a beneficial way in all the tissues of special interest for women's health. Breast and uterine cancer were estimated to represent 36.5% of all new cancer cases and 17.6% of all cancer deaths in women in the

United States in 2000 [1] while osteoporosis and cardiovascular disease are the main causes of morbidity and mortality at postmenopause. The ideal compound would thus be the one having preventive as well as curative effects on all these diseases which most frequently affect women's health. What could only be a dream a few years ago has become a reality: recent discoveries of pharmaceutical research offer women the hope to achieve a marked reduction in the

incidence of breast and uterine cancer while protecting against bone loss and fracture as well as reducing the risk of cardiovascular disease.

Among all risk factors, estrogens are well recognized to play the predominant role in breast cancer development and growth [2-5]. Considerable attention has thus focused on the development of blockers of estrogen biosynthesis and action [6-12].

Since the first step in the action of estrogens in target tissues is binding to the estrogen receptor [13, 14], a logical approach for the prevention and treatment of estrogen-sensitive breast cancer is the use of antiestrogens, or compounds which block the interaction of estrogens with their specific receptor. Until very recently, however, no agent with pure antiestrogenic activity under *in vivo* conditions has been available.

Stimulated by the need of an improved therapy for breast cancer, considerable efforts have thus been devoted to the synthesis of compounds which would exert pure antiestrogenic activity in the mammary gland and uterus. While tamoxifen has beneficial effects on breast cancer, it clearly acts as an estrogen agonist in the endometrium with an increased rate of endometrial carcinoma in women taking tamoxifen under chronic conditions [15, 16]. Moreover, it is most likely that a pure antiestrogen will have beneficial effects superior to those of tamoxifen on breast cancer prevention and treatment.

In order to meet the objective of a completely tissue-specific antiestrogen, a long series of benzopyran derivatives were synthesized in our laboratory with the objective of developing an orally active compound having pure antiestrogenic activity in the mammary gland and uterus. EM-652 was thus the compound selected for clinical development (Fig. 1).

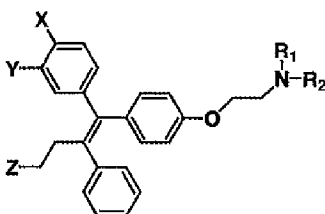
Characteristics of EM-652 (SCH57068)

EM-652 blocks both AF-1 and AF-2 sites of ERS

In addition to the classical hormone activation pathway, a number of steroid receptors including ER α and β have been shown to be activated by non steroidal agents (Fig. 2) including dopamine, growth factors and PKA activators [17, 18].

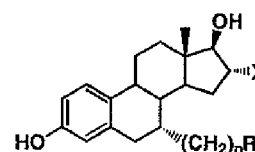
To investigate whether EM-652 could efficiently block this effect, we used the wild-type H-Ras and its dominant active form H-Ras^{V12} in our transfection experiments [19]. Thus, inductions by both Ras forms were completely abolished with the addition of EM-652 in the medium, as with ICI 182,780, thus suggesting that EM-652 is

A- TRIPHENYLETHYLENES



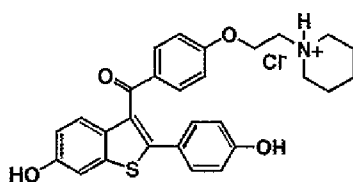
Tamoxifen X=Y=Z=H, R₁=R₂=CH₃
 Droloxifene X=H, Y=OH, Z=H, R₁=R₂=CH₃
 Toremifene X=Y=H, Z=Cl, R₁=R₂=CH₃
 Idoxifene X=I, Y=Z=H, R₁, R₂=C₄H₉

B- STEROIDALS



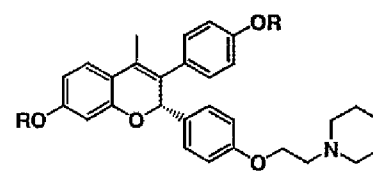
ICI 164,384 X=H, n=10, R=CON(CH₃)C₄H₉
 EM-139 X=Cl, n=10, R=CON(CH₃)C₄H₉
 ICI 182,780 X=H, n=9, R=SO(CH₂)₃C₂F₅

C- BENZOTHIOPHENES



Raloxifene

D- BENZOPYRANS



EM-652 R=H
 EM-800 R=COC(CH₃)₃

Fig. 1. Molecular structures of antiestrogens

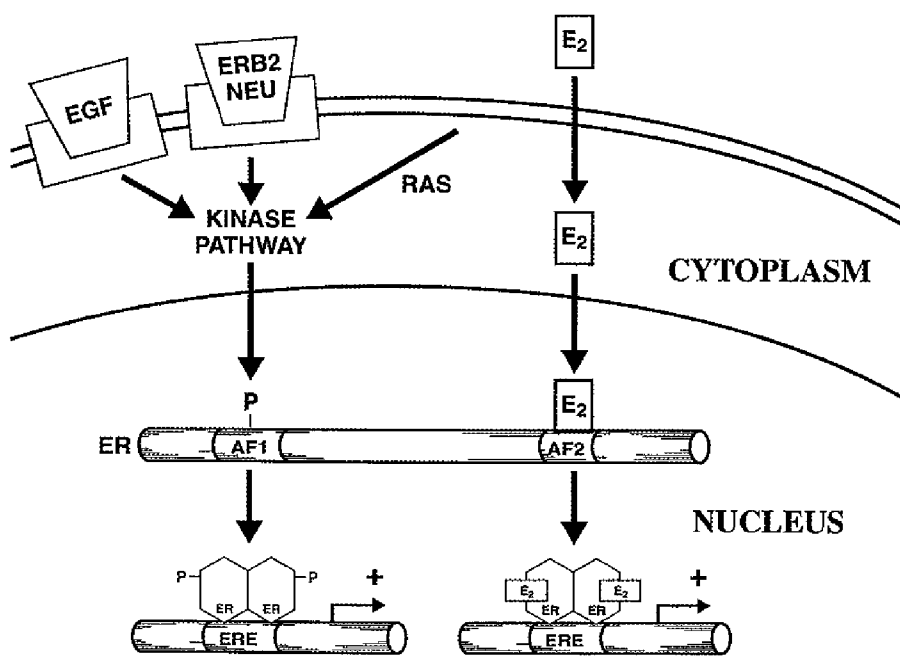


Fig. 2. Schematic representation of the dual activation mechanisms of ER by the AF-1 and AF-2 sites

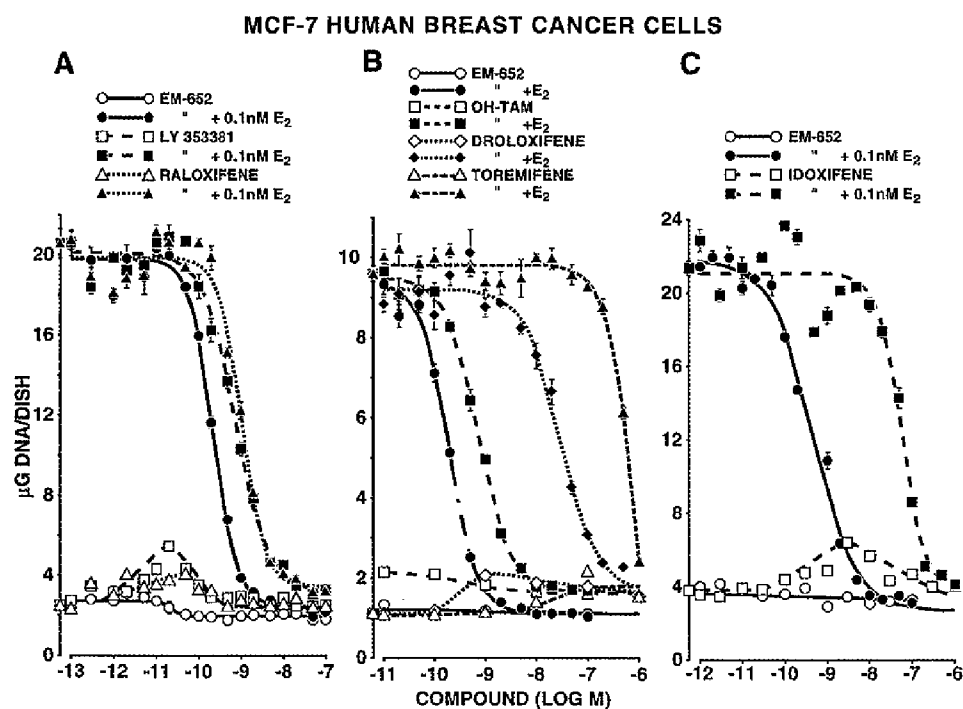


Fig. 3A-C. Effect of increasing concentrations of EM-652, LY353381, raloxifene, OH-tamoxifene, droloxifene, toremifene or idoxifene on basal and E₂-induced cell proliferation in MCF-7 human breast cancer cells. Three days after plating, cells were exposed for 7 (panel A and C) or 8 (panel B) days to the indicated concentrations of compounds in the presence or absence of 0.1 nM E₂. Media were changed at 2- or 3-day intervals

effective in blocking the AF-1 activity of ER α . The same experiment was also conducted on ER β where H-Ras and H-Ras^{V12} augmented the E₂ response in a similar manner. Again, EM-652 and ICI 182,780 abolished the Ras effect on ER β in the presence of E₂.

Inhibition of the growth of human breast cancer cells in vitro

After 8 days of treatment of MCF-7 cells in the absence of E₂, OH-tamoxifen, droloxifene, and toremifene all led to a 75% to 100% increase in cell proliferation which was dose-dependent: the stimulation by OH-tamoxifen was observed at concentrations as low as 0.01 nM, the lowest concentration used, while droloxifene gave the first significant effect at 1 nM and toremifene stimulated basal MCF-7 cell proliferation at 100 nM (Fig. 3B) [20]. In the same experiment, EM-652, OH-tamoxifen, droloxifene and toremifene inhibited the proliferative action of E₂ at respective IC₅₀ values of 0.19, 0.73, 30.58, and > 500 nM.

It can also be seen in Fig. 3A that the marked increase in MCF-7 cell proliferation induced by a 7-day incubation with 0.1 nM

E₂ was competitively blocked by a simultaneous exposure to EM-652, LY353381 or raloxifene at respective IC₅₀ values of 0.23 \pm 0.02, 0.77 \pm 0.09 and 1.07 \pm 0.12 whereas as illustrated in Fig. 3C, the antagonistic activity of EM-652 or idoxifene was exerted at respective IC₅₀ values and 0.49 \pm 0.08 and 58 \pm 12.8. It can also be seen in Fig. 3A and 3C that, here again, the basal proliferation of MCF-7 cells was not affected by EM-652, whereas incubation with 0.02 nM LY353381, 0.05 nM raloxifene or 2 nM idoxifene increased significantly this estrogen-sensitive parameter by 120, 63, and 70%, respectively (all p < 0.01).

Inhibition by EM-800 of the stimulatory effect of tamoxifen on the growth of human breast cancer xenografts in nude mice

As mentioned above and demonstrated in a series of studies with human breast cancer cell lines *in vitro* and *in vivo* [21-26] and supported by clinical observations [27-32], it seems reasonable to suggest that the loss of positive response to tamoxifen treatment in breast cancer patients could be, at least in part, due to the intrinsic estrogenic activity of the compound, as well as its lack of bloc-

kade at the AF-1 site of ERs. This explanation is supported by the finding that human breast cancer cell lines showing resistance to tamoxifen retain their sensitivity to specific antiestrogens *in vitro* [25, 33-35] as well as *in vivo* in nude mice [26, 36-37].

Since human breast carcinoma xenografts in nude mice are the closest available model of human breast cancer, we have compared the effect of EM-800 and tamoxifen alone and in combination on the growth of ZR-75-1 breast cancer xenografts in nude mice. An example of the direct stimulatory effect of tamoxifen on the growth of human breast cancer can be seen on Figure 4. In fact, at 161 days, the daily oral administration of 200 μ g of tamoxifen caused a 5-fold stimulation of size of the ZR-75-1 human breast cancer xenografts compared to ovariectomy while EM-652.HCl, in agreement with its pure antiestrogenic activity in the mammary gland had no stimulatory effect. That the stimulatory effect of tamoxifen on tumor growth is an estrogenic effect is demonstrated in the same experiment by the observation of the complete reversal of the stimulatory effect of tamoxifen by simultaneous administration of the pure antiestrogen EM-652.HCl.

Pure antiestrogenic activity of EM-652 and EM-800 in human endometrial adenocarcinoma Ishikawa cells

Direct comparison of the estrogen-like activity of these mixed agonist/antagonist compounds can best be seen in Fig. 5. Incubation with the indicated concentrations of LY353381, raloxifene, OH-tamoxifen, OH-toremifene, droloxifene, or idoxifene increased AP activity by 3.1-, 2.1-, 4.3-, 4.8-, 4.0- and 4.6-fold, respectively. The blockade of the stimulatory effect of all these compounds on AP activity by simultaneous exposure to EM-652-HCl well supports the suggestion that their stimulatory effect on this estrogen-sensitive parameter in human endometrial carcinoma is mediated through the estrogen receptor as previously reported [38].

The data obtained clearly demonstrate that the novel nonsteroidal antiestrogen EM-652 exerts pure antagonistic effects while being the most potent of the compounds tested on E_2 -induced alkaline phosphatase activity in human endometrial adenocarcinoma Ishikawa cells. OH-tamoxifen, OH-toremifene, droloxifene, idoxifene and raloxifene as well as its analog LY353381, in contrast to EM-652, exert a stimulatory effect on this estrogen-sensitive parameter, an effect which can be competitively blocked by simultaneous exposure to the antiestrogen EM-652, EM-652-HCl or EM-800, thus well supporting the suggestion that the stimulatory effect of these antiestrogens is mediated through activation of the estrogen receptor [38].

Prevention of bone loss and Inhibitory effect of serum cholesterol and triglycerides

The administration of 0.01 mg/kg of EM-800 already prevented by 52% the OVX-induced osteopenia while raloxifene had no detectable effect at the same dose [39]. Treatment with 1 mg/kg of EM-800 or raloxifene resulted in an approximately 75% prevention of the ovariectomy-induced osteopenia.

On the other hand, a 36% reduction of serum cholesterol was already observed with the lowest dose of EM-800 used, the serum cholesterol concentration being already decreased from 2.9 ± 0.18 mmol/L to

EFFECT OF EM-652.HCl, TAMOXIFEN OR THEIR COMBINATION ON THE GROWTH OF ZR-75-1 HUMAN MAMMARY CARCINOMA (XENOGRAPHS) IN OVARIECTOMIZED NUDE MICE

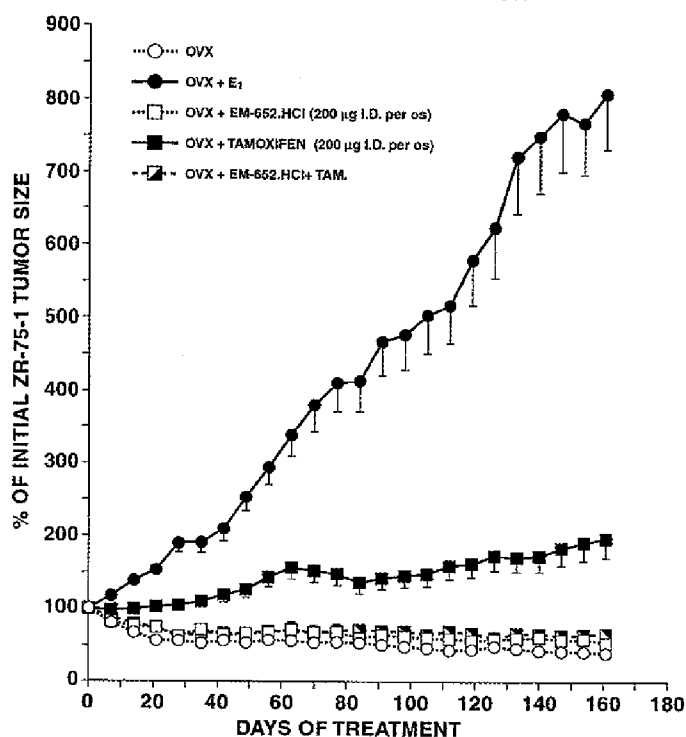


Fig. 4. Effect of daily administration of EM-652.HCl or tamoxifen alone or in combination for 161 days on the growth of human ZR-75-1 breast tumors (xenografts) in ovariectomized nude mice. The compounds were administered orally once daily at the dose of 200 µg per mouse. Mean tumor size in ovariectomized mice receiving the vehicle alone is shown for reference. Tumor size is expressed as percent of the pretreatment value (means \pm SEM of 18 to 30 tumors/group)

ISHIKAWA HUMAN ENDOMETRIAL ADENOCARCINOMA CELLS

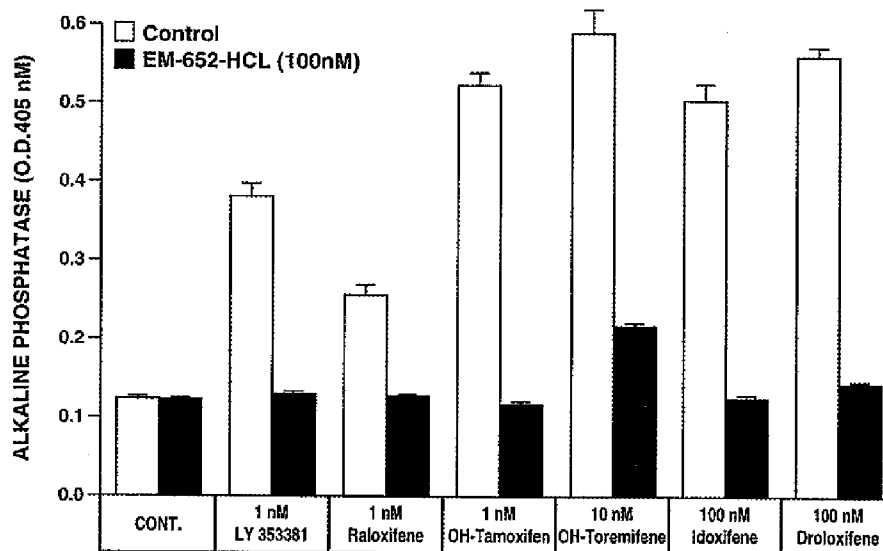


Fig. 5. Blockade by EM-652 of the stimulatory effect of LY353381, raloxifene, OH-tamoxifen, OH-toremifene, idoxifene and droloxifene on alkaline phosphatase activity in human Ishikawa carcinoma cells. Alkaline phosphatase activity was measured after a 5-day exposure to the indicated concentrations of the specified compounds in the presence or absence of 100 nM EM-652-HCl. The data are expressed as the means \pm SEM of four wells with the exception of the control groups where data are obtained from 8 wells

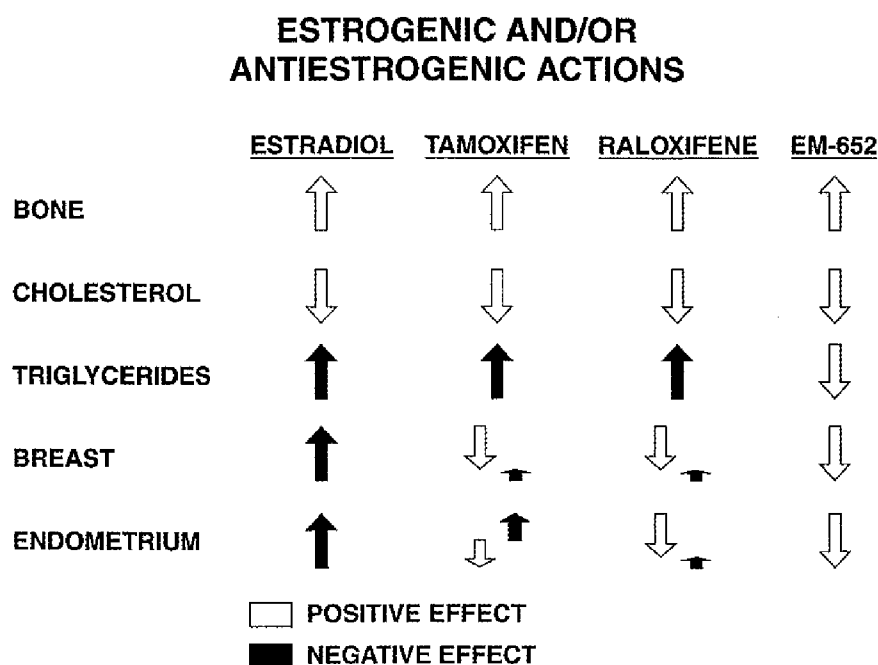


Fig. 6. Schematic representation of the estrogenic and/or antiestrogenic action of estradiol, tamoxifen, raloxifene and EM-652 on the main parameters important for women's health, namely the breast, endometrium, bone and serum lipids

1.8 ± 0.09 mmol/L at the daily 25 µg dose of EM-800 (p < 0.01). The daily 75 µg dose of EM-800 further decreased serum cholesterol to 1.6 ± 0.12 mmol/L (p < 0.01) while the 250 µg dose of EM-800 caused a maximal inhibition of 52% to a value of 1.4 ± 0.06 mmol/L (p < 0.01). The 250 µg dose had an inhibitory effect significantly (p < 0.01) more important than that of the 25 µg dose of EM-800, while the 75 µg dose had an intermediate inhibitory effect not significantly different from that of the 25 µg and 250 µg doses.

A similar inhibitory effect of EM-800 was observed on serum triglyceride levels. The daily administration of 25 µg of EM-800 for 9 months induced a near-maximal inhibition (69%) of serum triglyceride levels.

It is thus of particular interest to see that EM-800 reduces both serum cholesterol and triglyceride levels, thus indicating a potential more global beneficial effect of EM-800 on lipid metabolism. The other antiestrogens, such as tamoxifen [33, 40-42], droloxifene [43], and raloxifene [44], have been reported to elicit beneficial effects on the serum lipid profile, but they have not demonstrated such effect on serum triglycerides in the rat or in the human. The ability

to lower both serum cholesterol and triglyceride levels seems to be unique to EM-800.

Large range of activities of SERMS

Figure 6 summarizes schematically the activity characteristics of 17β-estradiol and of the three classes of antiestrogens so far available, namely tamoxifen (first generation SERM), raloxifene (second generation SERM) and EM-652 (pure SERM) on the best known parameters of women's health. As can be seen in this schematic figure, tamoxifen, while exerting beneficial effects on serum cholesterol, bone and breast cancer, possesses relatively strong estrogenic activity in the endometrium as well as some stimulatory effect on breast cancer proliferation. Raloxifene, on the other hand, represents an important improvement in terms of decrease of the stimulatory activity in the endometrium although some small estrogenic activity persists in the endometrium as well as on breast cancer cell proliferation. EM-652, on the other hand, is the only compound having pure antiestrogenic activity in the breast and endometrium while decreasing serum cholesterol and triglycerides, and protecting against bone loss.

References

- Greenlee RT, Murray T, Bolden S, Wingo PA (2000) Cancer statistics, 2000. *CA Cancer J Clin* 50: 7-33
- McGuire WL, Carbone PP, Seard ME, Esche GC (1975) Estrogen receptors in human breast cancer: an overview, in McGuire WL, Carbone PP and Vollmer EP, eds: *Estrogen receptors in human breast cancer*, New York, Raven Press, pp 1-7
- Asselin J, Labrie F (1978) Effects of estradiol and prolactin on steroid receptor levels in 7,12-dimethylbenz(a)anthracene-induced mammary tumors and uterus in the rat. *J Steroid Biochem* 9: 1079-82
- Davidson NE, Lippman ME (1989) The role of estrogens in growth regulation of breast cancer. *Crit Rev Oncog* 1: 89-111
- Dowssett M, Macaulay V, Gledhill J, Ryde C, Nicholls J, Ashworth A, McKinna JA, Smith IE (1993) Control of aromatase in breast cancer cells and its importance for tumor growth. *J Steroid Biochem Mol Biol* 44: 605-9
- Wakeling AE, Bowler J (1988) Biology and mode of action of pure antiestrogens. *J Steroid Biochem* 30: 141-7
- Dauvois S, Geng CS, Lévesque C, Mérand Y, Labrie F (1991) Additive inhibitory effects of an androgen and the antiestrogen EM-170 on estradiol-stimulated growth of human ZR-75-1 breast tumors in athymic mice. *Cancer Res* 51: 3131-5
- De Launoit Y, Dauvois S, Dufour M, Simard J, Labrie F (1991) Inhibition of cell cycle kinetics and proliferation by the androgen 5α-dihydrotestosterone and antiestrogen N, n-butyl-N-methyl-11-(16'α-chloro-3',17β-dihydroxy-estra-1',3',5'-(10')trien-7'α-yl) undecanamide in human breast cancer ZR-75-1 cells. *Cancer Res* 51: 2797-802
- Lévesque C, Mérand Y, Dufour JM, Labrie C, Labrie F (1991) Synthesis and biological activity of new halo-steroidal antiestrogens. *J Med Chem* 34: 1624-30
- Gronemeyer H, Benhamou B, Berry M, Bocquel MT, Gofflo D, Garcia T, Lerouge T, Metzger D, Meyer ME, Tora L, Vergezac A, Chambon P (1992) Mechanisms of antihormone action. *J Steroid Biochem Molec Biol* 41: 217-21
- Labrie C, Martel C, Dufour JM, Lévesque C, Mérand Y, Labrie F (1992) Novel compounds inhibit estrogen formation and action. *Cancer Res* 52: 610-5
- Labrie F, Li S, Labrie C, Lévesque C, Mérand Y (1995) Inhibitory effect of a steroidal antiestrogen (EM-170) on estrone-stimulated growth of 7,12 dimethylbenz(a)anthracene (DMBA)-induced mammary carcinoma in the rat. *Breast Cancer Res Treat* 33: 237-44
- Green S, Walter P, Kumar V, Krust V, Bornert JM, Argos P, Chambon P (1986) Human oestrogen receptor cDNA: sequence, expression and homology to *v-erb-A*. *Nature* 320: 134-9
- Kumar V, Chambon P (1988) The estrogen receptor binds tightly to its responsive element as a ligand-induced homodimer. *Cell* 55: 145-56

15. Fisher B, Costantino JP, Lawrence Wickerham D, Redmond CK, Kavanah M, Cronin WM, Vogel V, Robidoux A, Dimitrov N, Atkins J, Daly M, Wieand S, Tan-Chiu E, Ford L, Wolmark N (1998) Tamoxifen for prevention of breast cancer: report of the national surgical adjuvant breast and bowel project P-1 study. *J Natl Cancer Inst* 90: 1371-88
16. Bergman L, Beelen ML, Galtee MP, Hollema H, Benraadt J, van Leeuwen FE (2000) Risk and prognosis of endometrial cancer after tamoxifen for breast cancer. Comprehensive Cancer Centres' ALERT Group. Assessment of Liver and Endometrial cancer Risk following tamoxifen. *Lancet* 356: 881-7
17. Bunone G, Briand PA, Miksicek RJ, Picard D (1996) Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. *EMBO J* 15: 2174-83
18. Tremblay GB, Tremblay A, Copeland NG, Gilbert DJ, Jenkins NA, Labrie F, Giguere V (1997) Cloning, chromosomal localization and functional analysis of the murine estrogen receptor β . *Mol Endocrinol* 11: 353-65
19. Tremblay A, Tremblay GB, Labrie C, Labrie F, Giguere V (1998) EM-800, a novel antiestrogen, acts as a pure antagonist of the transcriptional functions of estrogen receptors α and β . *Endocrinology* 139: 111-8
20. Simard J, Labrie C, Bélanger A, Gauthier S, Singh SM, Mérand Y, Labrie F (1997a) Characterization of the effects of the novel non-steroidal antiestrogen EM-800 on basal and estrogen-induced proliferation of T-47D, ZR-75-1 and MCF-7 human breast cancer cells in vitro. *Int J Cancer* 73: 104-12
21. Katzenellenbogen BS, Kendra KL, Norman MJ, Berthois Y (1987) Proliferation, hormonal responsiveness, and estrogen receptor content of MCF-7 human breast cancer cells grown in the short-term and long-term absence of estrogens. *Cancer Res* 47: 4355-60
22. Gottardis MM, Robinson SP, Satyaswaroop PG, Jordan VC (1988) Contrasting actions of tamoxifen on endometrial and breast tumor growth in the athymic mouse. *Cancer Res* 48: 812-5
23. Poulin R, Mérand Y, Poirier D, Lévesque C, Dufour JM, Labrie F (1989) Antiestrogenic properties of keoxifene, trans-4-hydroxytamoxifen and ICI164384, a new steroidal antiestrogen, in ZR-75-1 human breast cancer cells. *Breast Cancer Res Treat* 14: 65-76
24. Wakeling AE, Newbould E, Peters SW (1989) Effects of antiestrogens on the proliferation of MCF-7 human breast cancer cells. *J Mol Endocrinol* 2: 225-34
25. Lykkesfeldt AE, Sorensen EK (1992) Effect of estrogens and antiestrogens on cell proliferation and synthesis of secreted proteins in the human breast cancer cell line MCF-7 and tamoxifen resistant variant subline. AL 1. *Acta Oncol* 31: 131-8
26. Osborne CK, Coronado Heinssohn EB, Hilsenbeck SG, McCue BL, Wakeling AE, McClelland RA, Manning DL, Nicholson RI (1995) Comparison of the effects of a pure steroidal antiestrogen with those of tamoxifen in a model of human breast cancer. *J Natl Cancer Inst* 87: 746-50
27. Pritchard KI, Thomson DB, Myers RE, Sutherland DJ, Mobbs BG, Meakin JW (1980) Tamoxifen therapy in premenopausal patients with metastatic breast cancer. *Cancer Treat Rep* 64: 787-96
28. Hoogstraten B, Gad el Mawla N, Maloney TR, Fletcher WS, Vaughn CB, Trantum BL, Athens JW, Costanzi JJ, Foukjes M (1984) Combined modality therapy for first recurrence of breast cancer. A Southwest Oncology Group study. *Cancer* 54: 2248-56
29. Carney PA, Griffiths T, Latief TN, Priestman TJ (1987) Clinical significance of tamoxifen withdrawal response. *Lancet* 1: 36
30. Howell A, Dodwell DJ, Laidlaw I, Anderson H, Anderson E (1990) Tamoxifen as an agonist for metastatic breast cancer, in Goldhirsch A, ed. *Endocrine therapy of breast cancer IV*, Berlin, New York, Springer-Verlag
31. Howell A, Dodwell DJ, Anderson H, Redford J (1992) Response after withdrawal of tamoxifen and progestogens in advanced breast cancer. *Ann Oncol* 3: 611-7
32. Wiebe VJ, Osborne CK, Fuqua SA, DeGregorio MW (1993) Tamoxifen resistance in breast cancer. *Crit Rev Oncol Hematol* 14: 173-88
33. Brunner N, Frandsen TL, Holst-Hansen C, Bei M, Thompson EW, Wakeling AE, Lippman ME, Clarke R (1993) MCF7/LCC2: a 4-hydroxytamoxifen resistant human breast cancer variant that retains sensitivity to the steroidal antiestrogen ICI 182,780. *Cancer Res* 53: 3229-32
34. Coopman P, Garcia M, Brunner N, Derocq D, Clarke R, Rochefort H (1994) Anti-proliferative and anti-estrogenic effects of ICI 164,384 and ICI 182,780 in 4-OH-tamoxifen-resistant human breast-cancer cells. *Int J Cancer* 56: 295-300
35. Lykkesfeldt AE, Madsen MW, Briand P (1994) Altered expression of estrogen-regulated genes in a tamoxifen-resistant and ICI 164,384 and ICI 182,780 sensitive human breast cancer cell line, MCF-7/TAMR-1. *Cancer Res* 54: 1587-95
36. Gottardis M, Jiang S, Jeng M, Jordan V (1989) Inhibition of tamoxifen-stimulated growth of an MCF-7 tumor variant in athymic mice by novel steroidal antiestrogens. *Cancer Res* 49: 4090-3
37. Osborne CK, Coronado EB, Allred DC, Wiebe V, DeGregorio M (1991) Acquired tamoxifen resistance: correlation with reduced breast tumor levels of tamoxifen and isomerization of trans-4-hydroxytamoxifen. *J Natl Cancer Inst* 83: 1477-82
38. Simard J, Sanchez R, Poirier D, Gauthier S, Singh SM, Mérand Y, Bélanger A, Labrie C, Labrie F (1997b) Blockade of the stimulatory effect of estrogens, OH-tamoxifen, OH-toremifene, droloxifene and raloxifene on alkaline phosphatase activity by the antiestrogen EM-800 in human endometrial adenocarcinoma Ishikawa cells. *Cancer Res* 57: 3494-7
39. Martel C, Picard S, Bélanger A, Labrie C, Labrie F (2000) Prevention of bone loss by EM-800 and Raloxifene in the ovariectomized rat. *J Steroid Biochem*, In press
40. Love RR, Newcomb PA, Wiebe DA, Surawicz TS, Jordan VC, Carbone PP, DeMets DL (1990) Effects of tamoxifen therapy on lipid and lipoprotein levels in postmenopausal patients with node-negative breast cancer. *J Natl Cancer Inst* 82: 1327-32
41. Love RR, Wiebe DA, Newcomb PA, Cameron L, Leventhal H, Jordan VC, Feysi J, DeMets DL (1991) Effects of tamoxifen on cardiovascular risk factors in postmenopausal women. *Ann Intern Med* 115: 860-4
42. Dipippo VA, Lindsay R, Powers CA (1995) Estradiol and tamoxifen interactions with thyroid hormone in the ovariectomized-thyroidectomized rat. *Endocrinology* 136: 1020-33
43. Ke HZ, Simmons HA, Pirie CM, Crawford TD, Thompson DD (1995) Droloxifene, a new estrogen antagonist/agonist, prevents bone loss in ovariectomized rats. *Endocrinology* 136: 2435-41
44. Black LJ, Sato M, Bowley ER, Magee DE, Bekele A, Williams DC, Cullinan GI, Bendete R, Kaufman RF, Bensch WR, Frolik CA, Termine JD, Bryant HU (1994) Raloxifene (LY139481 HCl) prevents bone loss and reduces serum cholesterol without causing uterine hypertrophy in ovariectomized rats. *J Clin Invest* 93: 63-9